
# ADVANCES IN PARASITOLOGY

Volume 7

Ben Dawes

*Advances in*

# PARASITOLOGY

VOLUME 7

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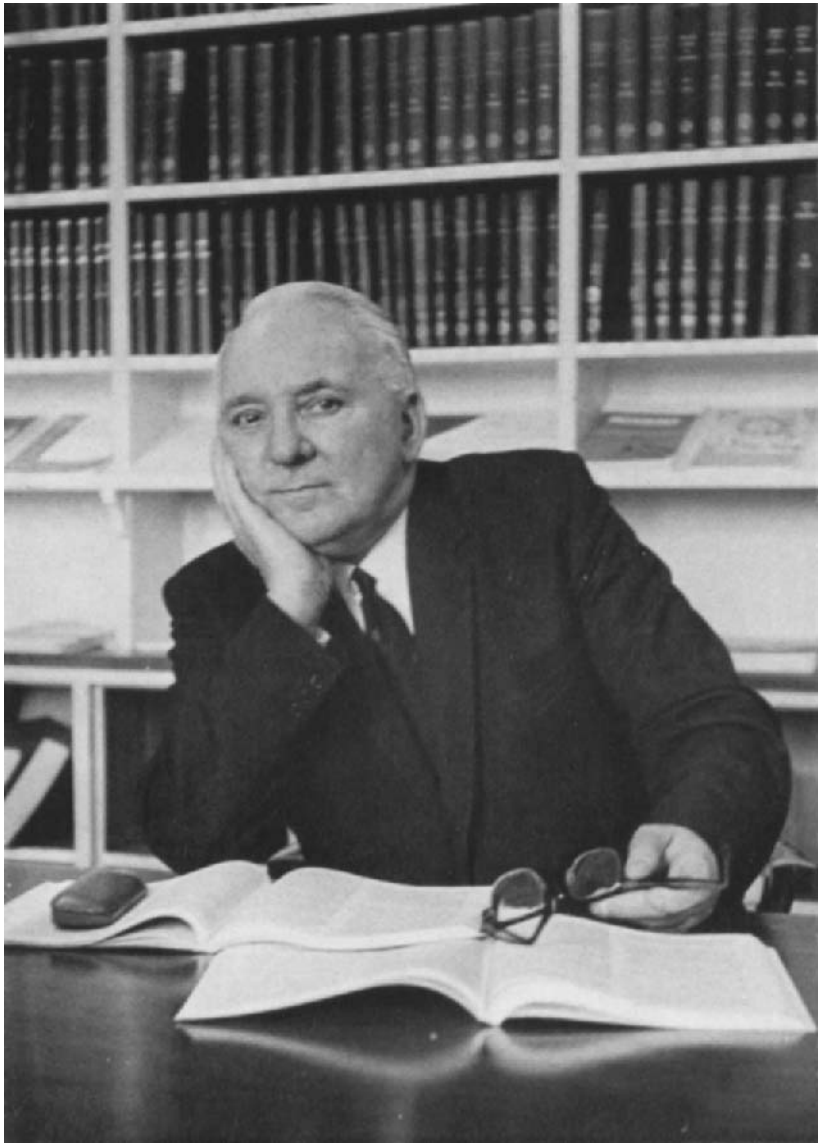

*Photo R. D. Reed*

Professor Ben Dawes, Editor of *Advances in Parasitology*

# *Advances in*
# PARASITOLOGY

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VOLUME 7

1969

ACADEMIC PRESS
London and New York

ACADEMIC PRESS INC. (LONDON) LTD.
Berkeley Square House
Berkeley Square
London, W1X 6BA

*U.S. Edition published by*
ACADEMIC PRESS INC.
111 Fifth Avenue
New York, New York 10003



Library of Congress Catalog Card Number: 62-22124

PRINTED IN GREAT BRITAIN BY ADLARD & SON LTD, DORKING

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# FOREWORD

I am uniquely qualified to write this foreword, as I was Professor Ben Dawes' first Ph.D. student and as such I can vouch from first-hand experience to the thoroughness with which he applies himself to any task. Subsequently, it has been my good fortune to have been his colleague and friend for over twenty years, and now he goes into compulsory retirement from the University of London King's College after 42 years of teaching and research. He will leave a void which will be difficult to fill, for as a group of recently qualified graduates remarked, he fired them with enthusiasm for parasitology and one could not help but learn from him.

Professor Dawes was trained at the Royal College of Science (Imperial College), was for 2–3 years Research Assistant at the Plymouth Laboratory of the Marine Biological Association, came to King's College 39 years ago and gained promotion to Lecturer in 1935, Reader in 1946 and Professor in 1963. During this period his teaching has been concerned with many groups of animals and various aspects of zoology to students of all levels, and he has instituted courses in such varied biological subjects as cytology, dental histology, experimental zoology, comparative physiology, and parasitology. For some years he was also Visiting Lecturer at what is now Sir John Cass College and organized its first B.Sc. course in Zoology. For more than 15 years he was Scientific Assistant (Zoology) to the University of London and was responsible for the conduct of practical examinations at local, provincial and colonial centres. He has had a long and varied experience of examining work at all levels and for 20 years he has been involved both here and abroad with the higher degrees of Ph.D. and D.Sc. candidates. He has served on many Boards, carried out much advisory work, made official visits to research institutions and attended many conferences. First and foremost, however, he has put himself at the disposal of students of many countries, and his files overflow with appeals for assistance and guidance in research and academic matters. It is a matter of regret to him that it is impossible even to answer all such appeals or to satisfy requests for reprints of his published work.

Professor Dawes' first piece of research was embryological and is published in *Phil. Trans. Roy. Soc. London.* The work carried out at Plymouth is published in several papers in *J. Mar. Biol. Assoc.* and in *Q.J.M.S.*, and research during a summer vacation at the Stazione Zoologica, Naples, gave two papers in *Roux' Archiv. f. Entwmech.* and one in *Nature.* However, his early duties in teaching allowed practically no time for research, although the courses he organized were in the nature of projects and he gained experience of the dynamic approach to teaching which excites and interests students and impels them to work and to learn.

During the Second Great War and at Bristol University he wrote his book "The Trematoda" (C.U.P.), which was published in 1946, reprinted in 1956, was out of print for several years and has been re-issued recently, 22 years after its first publication. He wrote also a Ray Society Monograph (1947) on

trematodes of fishes and a book on "A Hundred Years of Biology" (Duckworth, 1952). He has written many original papers, innumerable articles on zoological topics in encyclopaedic works and many reviews in scientific journals. His papers are individualistic, his research meticulous, and he has a fine collection of serialized sections on *Fasciola* and fascioliasis. He steadfastly refused to get on any research "band wagon" for the sake of publishing and is a perfectionist who sees much before writing anything. Many demands have been made of him as a sub-editor, and this is true of much incidental writing. Nevertheless, his work has engendered a keener interest in the study of trematodes, and "Advances in Parasitology" is achieving the same end for all parasites important in medical and veterinary sciences. He is intent on setting up a great fund of ideas and information based on modern methods of research in biological science and covering the entire field of parasitology. This project is global in outlook, and extensive travel during the past ten years has enabled him to contact and meet very many parasitologists.

As a young man Professor Dawes was keen on travel, visiting the continental countries during vacations and proceeding as far afield as Iceland, Jan Mayen and Spitzbergen. As an eminent and distinguished parasitologist Professor Dawes has been invited to and visited many cities in Canada, U.S.A., Mexico, Ghana, Nigeria, Australia, Tasmania and New Zealand, and also Kuala Lumpur, Singapore, Hong Kong, Tokyo, Honolulu, Alaska, and Fiji, visits sponsored by the Wellcome Trust, the Agricultural Research Council, the Australia C.S.I.R.O., the New Zealand Department of Agriculture and the Royal Society of London. More recently he was the guest of the British Council in Brazil, visiting Rio de Janeiro, Belo Horizonte, Brasilia, Saõ Paulo and Porto Alegre, and was further sponsored by the Royal Society of London to visit Buenos Aires, Santiago, Lima, Bogotá, Caracas and Barbados. Wherever he went he visited universities, research institutes and hospitals and gave lectures and seminars on parasitology at these institutions. In this way he has been an ambassador for parasitology, which is reflected in the contributors to "Advances in Parasitology". His Gargantuan efforts have made him many good friends, and he has been the grateful recipient of much kind hospitality. Not least pleasurable on such journeys have been surprise meetings with many former students, now doing useful academic work and/or experimental research in various parts of the world. He has also met estimable workers who have lived long with some of the great problems of parasitology and have gladly assisted in the production of this series of books. Despite his retirement the "Advances" project will continue under his keen Editorship, and he will consider any approach from worthy future contributors. From the end of September 1969, Professor Dawes will not be at King's College but can be reached at his new home at "Rodenhurst", 2 Meadow Close, Reedley Drive, Reedley, near Burnley, Lancashire, England.

And in conclusion all that I can say, as Head of the Department where we have worked together for so many years, is "Well done, thou good and faithful servant".

DON. R. ARTHUR

# PREFACE

In this volume interest is perhaps more varied than in the previous volume and the veterinarian is specially favoured. A wide range of topics includes host-parasite relationships of nematodes which infect plants, the immunology of schistosomiasis, experimental fascioliasis in respect of both snail and vertebrate hosts in Australia, the epidemiology and control of some nematode infections of grazing animals, meteorological factors and forecasts of helminthic disease in farm anaimals, and short reviews supplementing some previous contributions. One short review of this kind deals with the biology of hydatid organisms and another with veterinary anthelmintic medication. Two reviews published in Volume 2 cannot at present be supplemented profitably and one is withheld because it deals with fascioliasis. The sixth review of Volume 2 was the work of Saul Adler, whose death we mourn; his effort will be upheld by some worthy parasitologist in the future. A third supplemented review deals with *Paragonimus* and paragonimiasis, the original review belonging to Volume 3.

J. M. Webster writes about plant-parasitic nematodes, considering various features of the close relationship between host, parasite and external environment. Parasitism is established only when the proper combination of certain factors exists; the age of the nematode is important, and so are conditions of temperature, humidity and pH in the environment. There must be optimal orientation towards the correct host, and entry into the host is also an achievement. Such nematodes as are recognized as parasites of plants are concerned with the development of pathogenic symptoms in the host. Some plants are damaged by nematodes living in the foliage and others may be stunted by the activity of root parasites. Some plant parasites are so well adapted to their hosts as not to evoke any harmful reactions. Otherwise, diseases of plants may be due simply to one parasite only, but in other cases the primary nematode pathogen facilitates the entry of another pathogen, or it may even permit the entry of several secondary pathogens, thus creating a disease complex. Disease in parasitized plants may be caused by decreasing areas of photosynthesis, water absorption and nutriment uptake in malformed or malfunctioning organs, and the effects may be intensified by mineral deficiency, drought and other adverse conditions in the environment.

Dealing with types of host-parasite relationship, Webster first discusses pre-parasitic relations involving hatching stimuli due to root exudates, attractant stimulation by substances as yet chemically undefined, and specific regions of invasion and feeding. It is not possible here to consider the many examples that illuminate the text. Multiple associations may involve aggregates of nematodes of various species, or aggregates of bacteria or fungi or viruses with nematodes. In simple associations there may be ectoparasitism, although many nematodes are endoparasitic, can migrate through the host's tissues and may transfer from one host to another. More specialized forms

settle down at particular internal sites where feeding produces tissue breakdown, necrosis and abnormal growth. Such tissue responses are expressed in variety according to the species of host and parasite, and also the presence or absence of micro-organisms. The host response is considered in its relation to the structure and formation of giant cells, starting with the classical work of A. F. Bird, and continued in respect of mechanical details. The effect of the host on the parasite brings forth discussion on features of host specificity, biological races and nutrition, and there is a final note on areas of strength and weakness in host-parasite relationships and some consideration of future research hopes.

S. R. Smithers and R. J. Terry admit that recent years have brought great advances in our knowledge of the immunology of schistosomiasis, but the overall pattern remains obscure and both anomalies and lacunae remain. They have made a bold attempt to focus difficult problems and to define areas which still require urgent attention. Broadly, their review deals with innate immunity, acquired immunity and immunopathology, together with some final conclusions, but each section is subdivided into much varied and sometimes challenging information and ideas. The question of inate immunity brings up topics on host specificity and mechanisms of host susceptibility. Of the three common human schistosomes, *Schistosoma japonicum* is least host-specific, meeting slight resistance in dogs, goats, pigs, cattle and sheep, which serve as reservoir hosts. However, *S. mansoni* and *S. haematobium* have some non-human hosts, and the range of host specificity declines somewhat from the former to the latter. The degree of susceptibility of various hosts can be determined by the degrees of infection tolerated, which depends on various criteria, and intra-specific differences in susceptibility may make the problems of host specificity more complex. Mechanisms of host susceptibility call for consideration of the skin barrier to cercarial penetration, serum factors, the effect of the host's sex and sex hormones on infection, and factors associated with acquired immunity. Acquired immunity is discussed in respect of man and some experimental hosts which may terminate primary infections at different rates according to species, rats after about one month, some primates only incompletely even after some years. The question of immunity to reinfection has been studied most intensively in the rhesus monkey, which develops complete immunity after a period of unknown and controversial extent. The state of immunity that exists in the presence of the infectious agent, i.e. premunition, is referred to by the writers as "concomitant immunity", a term first used in connection with tumour transplantation. In this instance, it is evinced where in most rhesus monkeys which are resistant to challenge with *S. mansoni*, adult worms persist and produce eggs after worms of the challenge infection have died. Smithers and Terry believe that something like this occurs in Man, where in endemic areas children may be evacuating eggs from established adult worms but at the same time resisting reinfection, at least to some extent. Other aspects of acquired immunity discussed concern the stages of parasite that stimulate immunity, perhaps the eggs but notably schistosomulae, which come into contact with several tissues of the host and first provide antigenic stimulation of the host. Antigens are

considered separately, likewise antibodies and host antigens in schistosomiasis. The question then raised is how acquired immunity affects the parasite. After further considering that acquired immunity depends on specific allergic mechanisms and treating in much detail four modes of specific allergic reactivity, the writers find that none of them is involved unequivocably in immunity to reinfection with schistosomes, although none may be summarily dismissed as playing no part. Reasons are given, but I must go on to say that a section dealing with immunopathology contains equally stimulating findings about the nature and significance of the schistosome pseudotubercle, or pathological formation surrounding the imprisoned schistosome egg. The topics of splenomegaly, auto-immunity and serum protein changes also receive treatment, and final conclusions indicate where future research could most profitably be made.

Introducing experimental fascioliasis in Australia, J. C. Boray refers to enormous economic losses on a global scale and to about forty million sheep and five million cattle that graze potentially endemic pastures in Australia, where vigorous efforts have been made to elucidate the major problems of this disease. He is concerned with the larval stages of *Fasciola* in snail intermediate hosts and also with juvenile and adult flukes in sheep, cattle and other vertebrates. After considering the taxonomy of Australian Lymnaeidae and their distribution and environments, he concentrates on *Lymnaea tomentosa*, snail host of *F. hepatica* in the Antipodes, defining its habitats, reproduction, seasonal distribution, ability to survive under adverse conditions, and dispersal by migration. The breeding and maintenance of snails in the laboratory, which impressed me as a visitor to The McMaster Laboratory, is considered by special request. The susceptibility of snails to infection and the adaptation of species of *Fasciola* to various Lymnaeids leads to the point that in newly formed relationships between the parasite and unusual snail hosts, adaptation might occur rapidly as a result of passaging if snails have some degree of susceptibility in the adult stage, as various strains of *F. hepatica* in various races of *Lymnaea* spp. have in some geographical regions. A section on the biology of *Fasciola* deals with eggs and miracidia, environmental effects on development within the snail host, standard production of encysted metacercariae, including the selection of strains and many noteworthy matters of technique related to the assembly and storage of cysts, the testing of their viability, and their administration. The structure of the metacercarial cyst and both encystment and excystment are considered and there is much information on intermediary metabolism of *F. hepatica* in the adult state.

Attention is then transferred to juvenile and adult flukes in sheep and cattle in topics on growth, clinical symptoms and pathology, diagnosis of fascioliasis in various degrees, varying capacity of the parasite to produce eggs, acquired resistance, and the varying susceptibility and pathological changes seen in experimental hosts such as rats and mice, guinea pigs and rabbits, horses and donkeys, pigs and marsupials. Consideration of host–parasite relationships brings forth the conclusion that up to 1967 evidence showed that a common and important feature of heavy infection in sheep is retardation of fluke

development during tissue migration due to processes of fibrosis which does not eliminate flukes. The hosts of *Fasciola* are placed in three groups according to their resistance, although it is made clear that resistance in a purely immunological sense does not exist in *Fasciola* infections. In the Early Resistance Group, an early tissue reaction eliminates flukes and the hosts show relatively slight pathogenic effects. In the Delayed Resistance Group, a delayed host reaction controls the parasites during the later stage of their migration, and if flukes reach the bile ducts they are eliminated by a reaction-imposed barrier to feeding. The disease is self-limiting but there may be severe pathogenic lesions and mortality, especially in young or debilitated hosts. The third or Low Resistance Group has a severe early and delayed tissue reaction but there is not sufficient fibrous tissue and cellular reaction to immobilize and eliminate the flukes. Some overlapping between the three groups is attributed to the age of the host, its state of nutrition, and concurrent disease. We are told that there is a somewhat unbalanced host-parasite relationship between *Fasciola* and common domestic hosts and that it may be futile to ask which is the most successful system. In some hosts flukes will be eliminated, in others the parasites are destroyed along with the host, depending on the level of the infection and the physical condition of the host. Under natural circumstances, the most effective controlling factor in fascioliasis may be the death of heavily infected intermediate hosts or domesticated definitive hosts and the natural resistance and selective grazing habits of some domestic and wild animals. These important conclusions, I believe, should help readers puzzled by the complex pathological features of fascioliasis and sometimes mistakenly encouraged to simplify them into a background for "blood-sucking" habits of a fluke which can destroy by means of simple equipment the cells of practically any soft tissue in the host's body and which even in light infections destroys innumerable hepatic cells and evokes in the host local tissue reactions that subsequently yield enormous amounts of biliary epithelium and other tissues which did not exist prior to infection. In my opinion, the intensity of parasitism is important because related to the degree of anaemia produced in the host, and also because the survival of both host and parasite may depend on it. Survival and evolution for some millions of years has *not* characterized *Fasciola hepatica* as a purely haematophagous "killer".

To do justice to all areas of research on fascioliasis everywhere in a review such as this is just not possible and Boray has been obliged to concentrate on Australian efforts to an extent which may suggest falsely that research in the Northern Hemisphere has been of a more trivial nature. Many problems require much more research everywhere and the pathology of fascioliasis is one of them, because it ranges from a simple inflammatory reaction to malignancy bordering on cancer. Boray shows clearly, however, that a light infection of *F. hepatica* may persist in sheep permanently, without detrimental effect to either host or parasite. Apparently, there is no specific reaction in the host acting against the parasite, and survival of the host depends on the numbers of cysts ingested by the host. Moreover, previous repeated exposures to infection in the field may not prevent mortality from some future infection,

but adult sheep or sheep in full nutrition may tolerate reinfection better than lambs or sheep experiencing their first heavy infection or undernourished hosts. In cattle, there is epithelial hyperplasia, a dystrophic calcification and collagenous fibrosis proliferating into the deeper parts of the bile duct, and under such conditions adult flukes may be eliminated by "starvation". This cannot imply simply inability to suck blood, because the evidence of tissue feeding is confirmed by penetration into unusual organs such as the pancreas and lungs in acute and subacute fascioliasis, even into the spleen. That flukes can break down much tougher tissue elements than liver cells is shown by the penetration of the diaphragm, but there must be some truth in the idea of collagenous barriers in the wall of the bile duct, especially when calcification occurs as well. When we relate the textures of tissues encountered during migration or within the bile duct at different times during infection, we wonder less about the great size variability of flukes of the same infection and more about the poor prospect of obtaining a "necessary" diet of blood.

To pass to other matters in Boray's review, we note sections on chemotherapy, the control of snail intermediate hosts, and the epidemiology and control of fascioliasis. There is much useful information on drug screening techniques and the application of standardization before results are discussed. Drugs tested and considered suitable for use include carbon tetrachloride, Hetol, Hexachlorophene, Hilomid, Menichlopholan, Oxyclozanide, Disophenol, Nitroxynil, Chlioxanide and MK 990, and some other drugs are not included in the "suitable" category. Snail control can be achieved by proper drainage but chemical destruction with new compounds is also considered, and in respect of biological control new findings about larval Sciomyzids in Australia suggest their unimportance, although it is interesting to know that these larvae multiply when snails are plentiful and decline when snails are scarce. Boray considers also the possible antagonism between larval stages of *Fasciola* and echinostome larvae, and the value of *Chaetogaster* as a hunter of miracidia and predator of cercariae. The control of fascioliasis calls for "correct application of various curative and preventative measures, integrated into improved management", and Boray suggests that the warmer the climate the more difficult it will be to plan strategic control, emphasizing the effects of biological, climatic, topographical and human factors on the epidemiology of fascioliasis. He classifies the endemic areas of Australia into several defined Types (A–F), and mentions A–C as most extensive endemic areas in which the development of all larval stages of *F. hepatica* is very slow or negligible from May to September because of mean temperatures lower than 10°C. Efficient control of fascioliasis is said finally to depend on anthelmintic treatment, reduction in numbers of snail hosts by physical, chemical or biological means, and reduction of chances of infection by suitable farm management. In all the components of this review immense amounts of information and many ideas are communicated by means of tables and figures as well as by the text.

J. F. Michel draws attention to one special feature of the life cycle of nematode parasites of grazing animals; they do not multiply in the host's body. Eggs or larvae produced by adults fall on the herbage, have some free

period of development, become infective and are then able to embark on a parasitic mode of life. Each adult worm that develops has been picked up individually by the host, and every larva on the pasture arose from a single egg laid by an adult parasitic female. He then outlines by way of introduction the establishment of a population in terms of resistance and attempts to interpret the epidemiology of nematode infections in terms of host and external world together constituting a uniformly favourable environment. Free living forms in the life cycle assist in the colonization of new hosts but as they may perish before entering a suitable host they add a new hazard, so we are not surprised to find that parasitic nematodes are extremely prolific. In consequence, sudden increases in worm populations can and do occur, because a small decrease in environmental pressure may lead to disproportionate degrees of survival. Host resistance is considered in much detail under several headings—resistance to the establishment of worms, self-cure, the effects of resistance on fecundity, and inhibition of development. The free living stages of parasitic nematodes are considered in much detail in respect of development, the migration of infective forms, grazing behaviour, the survival of infective forms, and the relation of climate to free living stages.

In later sections of his review, Michel considers parasitic diseases such as gastro-enteritis in cattle and sheep, parasitic bronchitis in cattle, and nematodiriasis in cattle and sheep, and follows up with statements on husbandry and control of infections. The final conclusions reached must have great value, although my remarks utterly fail to do justice to the informative statements and well considered ideas expressed. The forms of diseases discussed arise as a result of exposing incompletely resistant grazing animals to pastures so heavily laden with nematode parasites that infective larvae are ingested at excessive rates. The infection which produces the disease is acquired during a short period of time, sometimes because the hosts soon become refractory to infection, or else because worm numbers are regulated in the host. Susceptible animals may encounter heavy infections on pastures contaminated by another group of animals (simple transmission) or when they build up an infestation on pasture without becoming resistant (autoinfection). Disease due to simple transmission can be avoided by keeping groups of susceptible animals from pastures infested by other groups. In autoinfestation, the number of generations involved is small, and the choice of control measures depends on how the number is limited. If it is due to a long phase of free living existence, the appearance of the disease-producing generation of larvae may be predictable, and stock can be removed from exposure to it. When rapid development of host resistance limits the number of generations of the parasite, vaccination may be a promising approach to control.

C. B. Ollerenshaw and L. P. Smith note that few reports dealing with helminthic diseases of livestock consider climate sufficiently to enable us by analysis to determine or predict the incidence of disease. Interactions between climate, soil and herbage provide a microcosmic environment of great variability in which parasitic worms undergo early development. Different stages in the life cycle may respond to changing weather in differing ways, and our problems are more complex because some parasitic diseases involve

different species of worms. A determined effort is made in this review to show what the possibilities are of forecasting the incidence of parasitic disease, with special reference to fascioliasis, parasitic gastro-enteritis, nematodiriasis and parasitic bronchitis. Laboratory observations, plot observations, and the experimental paddock are considered in turn and then, in making an approach to the problem of climate and disease incidence, a note is made that unusual and extreme variations occur in populations of helminths under the stress of extremes of weather. These are unlikely to recur within a period of three years, thus giving the most practical period for most investigators to repeat series of observations. It seems best to try to relate these two extremes without at once trying to understand the details of involved processes. First, we must collect data on the overall incidence of disease, particularly the years of extreme incidence, which indicate where research effort can best be expended. To try to summarize data here would be futile but the reader is well rewarded for his own efforts. At present, however, the relationships between climate and helminthic disease are unequally understood; much is known about fascioliasis, less about nematodiriasis, and least about parasitic forms of gastritis and bronchitis in livestock. In reviewing these major diseases, however, some common features are noted. The level of disease in any year may be influenced by the previous year's level, but disease levels may rise from low to high in one year and then fall from high to low in the next. Another important point concerns disease-free years, when the parasite population seems to decline to a harmless level. Normally, high reproductive potential in the parasite is controlled by a high environmental pressure, but epizootics arise when relaxation of environmental pressure causes loss of this control and allows a population explosion to take place.

It is claimed not only that the parasites under review can withstand adverse conditions but that they can also build up reservoirs of stages able to benefit from the relaxation of stringent environmental conditions. One feature which largely determines the seasonal incidence of disease is the development of free-living stages of the parasites in summer, but not in winter, in cool temperate climates. In Britain, much effort has been made to try to determine whether or not particular parasites survive in carrier animals or, instead, on the pastures. This is important, but in respect of climate emphasis comes elsewhere; if the parasite survives at a low level, tremendous fecundity ensures a high infection on the herbage by late summer, but possibly the parasites may overwinter on pasture in numbers sufficient to produce serious disease in a grazing stock, without further build-up in the populations. The development of eggs and larvae on the pastures is determined largely by temperature conditions, but development of the infective stage depends on available moisture, the factor of greatest importance in this matter of determining the incidence of disease. Extreme wetness facilitates the build-up of large populations of an infective stage on herbage, with resultant high level of disease. However, some eggs and larvae resist desiccation and parasitic gastro-enteritis shows an increased incidence after dry summer periods. Many other topics arise for consideration. For instance, the development of resistance to a worm may be influenced by long spells of dry weather, and the

lowest overall incidence of parasitic gastro-enteritis and bronchitis occurs in Britain in years when soil moisture conditions are neither "wet" nor "dry". Problems are considered by the reviewers in terms of practical economy with the farmer's needs in mind, but control tries to limit parasitism when climate fails to do so, and it must be used by the most efficacious means at the most advantageous times. The more we learn about climate and disease, the better recommendations for control will tend to be.

In the first of the short updated reviews, J. D. Smyth discusses research areas in which significant progress has been made in respect of the biology of hydatid organisms, drawing attention also to those areas which are still somewhat neglected. He does not have sufficient space to consider clinical, pathological and epidemiological aspects of his subject and he treats immunological and serological research briefly. However, he has provided references which will serve readers interested in these areas. He gives new information on speciation, taking definitive and intermediate hosts into account, and then passes on to the topic of establishment and development in the final host. A section on cytology and ultrastructure follows, with notes on the rostellar gland, cuticle, nervous system, cyst wall, eggs and hooks. Other sections deal with matters biochemical and immunological and there is a section on *in vitro* culture of adults, and the cystic stage and tissue culture of germinal cells. In all, new advances in many areas of research are indicated and valuable references provided.

T. E. Gibson notes that advances have been made in all the areas of research reviewed in 1963 and many new anthelmintics have appeared, most of which are less toxic, have a lower dose rate and a wider spectrum of activity than their predecessors. However, higher cost somewhat offsets greater efficiency. Gibson foresees a need for further proclamations of progress in the future. The main sections of his review are concerned with parasitic gastro-enteritis of cattle and sheep, parasitic bronchitis, fascioliasis and *Dicrocoelium*, tapeworms, ascariasis, equine strongyliasis, porcine oesophagostomiasis, hyostrongyliasis and strongyloidiasis, and with capillariasis in poultry and syngamiasis in other birds. The conclusions reached, sometimes after consideration of several types of drugs, will prove to be valuable to veterinarians. Several excellent agents are available for removing gastro-intestinal nematodes from sheep and cattle; thiabendazole is widely used in sheep, methyridine as well in cattle. In parasitic bronchitis cyanacethydrazine is no longer used and diethylcarbanazine widely used, though it acts mainly on immature worms. A statement is made about the comparative value of fasciolicides, bearing in mind the assays of Boray and colleagues. It is as well to state here that this short review arrived before it was known that the review by Boray would appear in the same volume, and what has been said by both of these friends of mine has been allowed to stand, and Gibson's conclusion will command attention. As anthelmintics increase in number, selection of an ideal compound for specific use becomes increasingly difficult, so that a final choice depends on personal preference or expense involved. In spite of great progress in anthelmintic medication, room for improvement exists in some areas, notably the toxicity of fasciolicides.

Muneo Yokogawa begins his short review by enumerating the species of *Paragonimus*. Of the 31 species proposed, some of which are probably not valid, 6 were made last century (1850–1883), 7 between 1908 and 1960, and no less than 18 since 1960, indicating the furiously accelerated pace of systematic work. The features of geographical distribution are considered in some detail for metacercariae as well as juveniles and adults, where information is available. Taxonomic features of these stages are considered also, and we must note how little value can be placed on isolated characters and how necessary it is when making specific allocations to consider groups of characters. Until recently, *P. westermani* has been regarded as the only species causing paragonimiasis, but recent evidence implicates some other species in China and West Africa. Recent research on clinical features of paragonimiasis and pathogenicity are outlined. We learn that although the lung is the primary site of *P. westermani* infection, there may be involvement of brain and spinal cord, subcutaneous tissues, abdomen, eyes and genitalia. Cerebral paragonimiasis occurs with greatest frequency in this connection. Radiological study of calcifications is also mentioned, along with serological studies in relation to epidemiological surveys, immuno-electrophoretic methods, and enzyme activity. The most recent work on treatment indicates that the drug of choice in paragonimiasis, bithionol, has been used in pulmonary cases with cure rates of 84–100 per cent, and also that the drug is effectual in subcutaneous forms of the disease.

I am glad once more to express my gratitude to friends and colleagues who have helped me in my aim to spread new ideas and information throughout the field of parasitology and richly deserve my thanks for their dedication to this worthy cause. I am happy to say thank you also to members of staff of Academic Press for faithful service rendered in the skilful production of another fine and useful book which will be much appreciated by many keen readers of "Advances in Parasitology" in various parts of the world.

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*March* 1969

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# CONTENTS

## The Epidemiology and Control of Some Nematode Infections of Grazing Animals

J. F. MICHEL



## Meteorological Factors and Forecasts of Helminthic Disease

C. B. OLLERENSHAW AND L. P. SMITH



# SHORT REVIEWS

Supplementing Contributions of Previous Volumes

## The Biology of the Hydatid Organisms

J. D. SMYTH

## Advances in Veterinary Anthelmintic Medication

T. E. GIBSON



## *Paragonimus* and Paragonimiasis

MUNEO YOKOGAWA

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# The Host-Parasite Relationships of Plant-Parasitic Nematodes

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## I. Introduction

Seinhorst (1961), Krusberg (1963a) and Mountain (1965) reviewed the literature on the host-parasite relationships of plant parasitic nematodes. Citations of recent advances will emphasize the influence of the environment and the host's physiology on the various components of the host-parasite association. Section II concerns the relationship between parasite and potential host prior to the establishment of a feeding site, and also the different types of nematode-micro-organism complexes which are associated with the host. We must consider these basic aspects of host-parasite relationship before examining more closely the form, mechanism of action and results of the intimate host-parasite relationship within the plant host.

## II. Types of Relationship

The association of host and parasite in parasitism can be studied meaningfully only in conjunction with the environment. The study of the host-parasite relationships of plant parasitic nematodes has shown that many features of host, parasite and environment facilitate this association. Thus, the phenomenon of parasitism is controlled not only by such factors as the age and

species of nematode parasite and plant host, but also by the temperature, humidity and hydrogen-ion concentration at the time of egg hatching, and the nematode's orientation towards, penetration of and development in a suitable host. It is only when the right combinations of these factors occur that there is growth and development of the nematode parasite. The morphological and physiological changes which ensue in the plant host determine the suitability of the plant as a host for the potential parasite, and the resulting symptoms are the basis for the diagnosis of the disease of the host. This draws our attention to the fact that although the terms parasitism and disease are not synonymous, those plant nematodes which we recognize as parasites are associated with pathogenic symptoms and so are regarded as disease-causing organisms. There are degrees of parasitism, and some plant nematodes may be so well adapted to their host that they do not induce a host response which is harmful to the host.

A particular group of symptoms may be the result not of nematode parasites alone but of a nematode with other pathogenic organisms to produce a disease complex. Hence, nematode diseases of plants may be the result of the nematodes acting as (a) the primary pathogen, (b) a pathogen and as a vector of one other pathogenic organism, or (c) a pathogen and as an organism which causes injury that facilitates the entry of many secondary pathogens resulting in a disease complex. When the nematode is the primary pathogen it may be as an ectoparasite, an endoparasite, or possibly its development combines both forms.

Stunted, malformed and discoloured foliage may be caused by nematode parasites in the foliage (e.g. *Ditylenchus dipsaci* on lucerne and *Aphelenchoides ritzemabosi* on chrysanthemums), but patches of stunted, slow growing plants are frequently also the first indication of nematode parasites on the roots (e.g. *Heterodera rostochiensis* on potatoes and *Meloidogyne hapla* on roses). Similarly, in tree crops *Pratylenchus penetrans* causes the peach replant problem and *Radopholus similis* the decline of citrus. More than one nematode species may produce similar above-ground symptoms, for example, *R. similis* (Loos, 1959) or *Helicotylenchus multicinctus* (Minz *et al.*, 1960) in the decline of banana. Similarly, one species of nematode can cause decline in different crops as does *R. similis* in pepper and citrus. Conversely, nematodes have been reported to stimulate host growth. Peters (1961) recorded an increase in yield of potatoes in the presence of a low population level of *H. rostochiensis*. The multiple crowns in sugar beet (Dunning, 1954) and increased tillering in oats (Webster, 1967c) caused by *Ditylenchus dipsaci* are an apparent growth stimulation although they can scarcely be regarded as increasing crop yield. Many nematode species probably cause an insidious reduction in crop yield because their above-ground symptoms are not obvious. This is particularly so with the hosts of various *Pratylenchus* species, and the several nematode species (e.g. *Helicotylenchus* sp. and *Tylenchorhynchus* sp.) attacking the roots of grasses.

Nematode parasites affect young seedlings rapidly, but mature trees may take several years to succumb to the pathogen. Most of the symptoms of the nematode infected plants are non-specific, and above-ground symptoms such

as leaf chlorosis and patchy, stunted growth, could indicate a chemical deficiency (e.g. nutrient) or a physical defect (e.g. pH) in the soil as well as a host response to a biological association (e.g. nematode parasite). Nevertheless, parasitism of the roots may modify their physiology and morphology so much that their normal function of water and nutrient absorption is inhibited and the above-ground symptoms then occur. Thus discoloration of foliage caused by nematode attack on the roots may be amended by spraying with ferrous sulphate, which suggests that the level of available iron in the soil was below that at which it was available to injured roots.

Basically, the parasitic nematodes are disease causing organisms because they decrease (a) the photosynthetic area, or (b) the water and nutrient absorbing area by the malformation and malfunction of the respective vital organs. The harmful effects are enhanced by adverse environmental conditions such as a mineral deficiency, lack of water or non-optimal pH.

## A. PRE-PARASITIC RELATIONSHIP

### 1. *Hatching stimuli*

The eggs of cyst (*Heterodera* spp.) and gall (*Meloidogyne* spp.) forming nematodes in the soil are stimulated to hatch by the exudates of some plant roots, and extensive work on the hatching of *Heterodera* spp. was reviewed by Shepherd (1962). The hatching response of some of these nematodes may be specific for a particular plant. *H. rostochiensis* is specific to exudates of its normal host, the potato, or tomato, although even in the absence in the soil of the host plant 50% of the eggs hatch each year. In laboratory *in vitro* tests only a small proportion of the eggs of *H. rostochiensis* hatch in tap water but the majority hatch rapidly in water containing potato root exudates. *H. schachtii* has a wide host range and the exudates of many plants stimulate the eggs to hatch, whereas *H. goettingiana* has a narrow host range and the eggs hatch only slightly in either water or in pea root exudate, but the hatch is satisfactory in soil in the presence of pea plants. The root exudate stimulates a rapid localized hatching which is supplementary to the basic water hatch. Wallace (1966) emphasized how environmental factors modify the hatching effect of root exudates on the eggs of *Meloidogyne javanica*. Thus soil at field capacity, at temperatures of 25–30°C and at a pH of 6·4–7·0, provide optimum conditions for egg hatch. The characteristics of root growth and the presence of soil micro-organisms in the rhizosphere are important biological factors influencing the stimulatory effect of the root exudate.

The hatching of larvae from eggs within the cyst is distinct from the emergence of the larvae from cysts, and Wallace (1963) suggests that the presence of free larvae within the cyst may inhibit further hatch. The rate of emergence of larvae *a priori* must be controlled by rate of hatch. There are two phases to the hatching of eggs: (1) trigger mechanism for increased larval activity and (2) the actual process of larval release from the egg. The composition and mechanism of action of root exudates has given rise to many theories on the mode of action of the exudates.

There is a delay in hatching of many *Heterodera* species and this is associated with periods of slow or no growth of the normal host plant (Oostenbrink, 1967). During this "facultative diapause" of the *Heterodera* cysts (Shepherd and Cox, 1967) it is not possible to hatch some species of *Heterodera* even in the presence of the appropriate exudate. Some animal parasitic nematodes have similar chemical hatching stimulants, e.g. ascarid eggs which respond to carbon dioxide (Rogers, 1962). Many plants which are resistant to the development of *Heterodera* spp. produce exudates which stimulate their hatch. Thus pea root exudate stimulates the larval emergence of *H. schachtii*, but these larvae do not feed or develop on pea roots. Some root exudates have a definite nematicidal action, e.g. *Tagetes* spp. and asparagus. The exudate from *Tagetes* does not have a nematicidal effect in *in vitro* hatching tests (Omidvar, 1961) but significantly decreases the soil population of *H. rostochiensis* (Omidvar, 1962). Exudates from raspberry canes are toxic to *Longidorus elongatus* and decrease the soil population of this nematode (Taylor and Murant, 1966).

In order to help ascertain the composition and method of action of the root exudate, the activity of several hundred compounds has been assayed and compared for efficiency as hatching agents. Substances such as sugars, amino acids, Krebs cycle acids and related compounds are generally inactive and so the hatching response does not seem to be associated with the availability of a common metabolite (Clarke, 1966). Many vitamins are active stimulants, and Viglierchio and Yu (1965) suggest that the vitamins may contribute to eliminating an enzymic block which prevents larval emergence. Most of the active inorganic hatching compounds are oxidizing agents, e.g. hydrogen peroxide, and the behavior of many of these compounds suggests that the hatching exudates might function as electron acceptors or oxidizing agents in the oxidative phosphorylation process. Ellenby (1957) suggested a connection between hatching activity and ion transport, while Nolte (1958) maintained that the exudates increase larval activity by making oxygen available. Certainly the larval metabolism changes and activity commences. Several inorganic salts were shown to stimulate the hatch of *H. schachtii* (Clarke and Shepherd, 1965), although less effectively than the beet root exudate, and the effective ions were oxidizing agents. Zinc ($Zn^{2+}$) and cadmium ($Cd^{2+}$) ions were very effective hatch stimulators for many *Heterodera* species and the hatch was greater than for water or the root exudate of the appropriate host plant. Many dyes were tried as artificial hatching agents (Shepherd, 1962), and showed a great range of activity from nil to better than root exudate. Ellenby and Smith (1967) reported very active hatch stimulation by water which had been in contact with the ion exchange resin "Amberlite IRA 400", which may explain some of the conflicting evidence on hatching stimulants. Dahlstrom *et al.* (1964) reported that the potato root exudate which stimulates hatch of *H. rostochiensis* also stimulates growth, acid production and respiration of *Aspergillus awamori*, and it is suggested that in *A. awamori* it stimulates the direct oxidation of glucose or glucose-6-phosphate. Clarke (1966) suggested two types of chemical that could induce immediate hatching: (a) the conjugated unsaturated organic compounds

which could bind on to molecules in the egg shell tissue to disrupt the tissues and make them more permeable; and (b) compounds that act as electron acceptors to enable an interaction of hatching agents in the electron transport system or oxidative phosphorylation.

Evidence to date indicates that potato root exudate is a monobasic carboxylic acid with an equivalent weight of 220 and a formula approximating to $C_{11}H_{16}O_4$ (Clarke, 1960). There is now support for the hypothesis that the hatching agents and the root exudates stimulate hatching by altering the structure and function of some binding material in the cyst and egg wall. Clarke and Shepherd (1968) suggest that two polarizable groups attach themselves to suitably spaced atoms such as proteins. The fact that the most effective hatching agents are known to be amine and amino acid precipitants is regarded with some significance by the authors.

2. *Attracting stimuli*

It would be beneficial to survival of the species if freshly hatched larvae of plant parasites were attracted to their host plant. Klingler (1965) reviewed the recent accumulation of evidence that confirms the hypothesis that plant-parasitic nematodes are capable of directed orientation, i.e. klinotaxis, towards a stimulus from the plant. Larvae aggregate at specific areas on the root, e.g. *Hemicycliophora* sp. at the root tip and *Meloidogyne* sp. at the region just behind the tip. The range of this attraction is about 4 cm for *Heterodera* spp. (Wallace, 1958) and 20 cm for *Hemicycliophora* spp. (Luc, 1961). Much of the earlier work had failed to distinguish klinotaxis from an orthokinetic reaction whereby nematodes aggregated in the vicinity of a stimulus and were "trapped" due to a decreased speed of movement. Wallace (1960) stated that *H. rostochiensis* orientates towards tomato roots and Blake (1962) observed *Ditylenchus dipsaci* moving almost directly towards oat seedlings.

The attraction of nematodes to a redox potential on the root surface has produced conflicting reports (Bird, 1959, 1960; Jones, 1960). The latter maintained that a potential difference of at least 20–30 mV/mm was required for orientation. Roots certainly produce this potential difference but the fall in potential between the root surface and the surrounding soil water is very rapid and is unlikely to extend beyond 1 mm (Klingler, 1965). The amino acids and carbon dioxide are the most attractive chemicals in the root secretions to nematodes. Both glutamic acid and aspartic acid tended to repel *D. dipsaci* at concentrations of 1 : 1000 and attract them at 1 : 100 000 (Jones, 1960). Initial experiments to test the attractiveness of carbon dioxide merely indicated aggregations, but Klingler (1963) finally demonstrated klinotactic orientation of *D. dipsaci* to carbon dioxide emerging from a capillary tube under a glass cover slip. Orientation of the nematode to the carbon dioxide source occurred from distances of up to 3 cm. Recently, Edmunds and Mai (1967) showed that *Pratylenchus penetrans* is preferentially attracted to the high carbon dioxide emission by fungal infected roots.

Thus, an increasing amount of evidence has demonstrated the attraction of nematodes to host plants, but there is little positive evidence on the specificity of the attractants.

### 3. *Invasion and feeding*

Nematodes feed normally in specific areas such as the foliage, the root meristem, the zone of root elongation and the zone of root maturation. However, under starvation conditions due to a poor root system, inter- or intra-specific competition, the nematode will feed wherever suitable cells are available (Kirkpatrick *et al.*, 1964). Nematodes are attracted to the root and accumulate around a specific region. They penetrate the superficial root cells by means of the mechanical thrusting action of the stylet. After puncturing the cell, the nematode may secrete enzymes and then, by means of the pumplike action of the muscular "oesophagus", withdraw the cell contents. The cell shrinks but a back flow from the nematode can cause a temporary return to the near-normal volume. This feeding action may be repeated several times before the nematode moves to another feeding site (Kirkpatrick *et al.*, 1964). McElroy and Van Gundy (1968) show that *Hemicycliophora arenaria* attaches to the plant root by means of an adhesive polysaccharide plug which may originate from nematode secretions, and that digestion of the cell contents may be partially or wholly extra-oral. Bird (1966a) demonstrated the presence of esterase in the amphidial pouch, which may thus serve in orienting the nematode towards a food source or towards a female. He speculates that the esterases may guide the feeding nematode when it changes its feeding site from one giant cell to the next. The number of *Meloidogyne incognita* penetrating into sterile cucumber seedlings increased with increasing concentrations of sucrose, micronutrients and iron chelate (McClure and Viglierchio, 1966a).

In a review, Kirkpatrick *et al.* (1964) outline the major nutritional problems involved in the host–parasite relationship. Those ectoparasitic nematodes which feed mainly on epidermal cells have little effect on plant host growth through nutritional competition, but may decrease water absorption in young seedlings. Extensive nematode feeding in the cortical parenchyma in the elongation and maturation zones retards root extention, and plant growth is seriously retarded due to poor uptake of nutrients and water. Some nematode parasites decrease the efficiency of the vascular tissues and so restrict plant development, and others feed in the root meristem and so inhibit root elongation and plant growth. Nematodes may injure host cells during feeding by mechanical damage and withdrawal of cell constituents, collapse of the cell wall, and chemical stimuli from nematode secretions. Any one or a combination of these factors may in conjunction with environmental characteristics retard or inhibit shoot or root growth. Nematodes thus modify the nutritional status of the plant directly and they may also do this by modifying the metabolic activity of host cells. The degree of harm to the host plant and benefit to the parasite reflects the degree of adaptation of the parasite to the host.

## B. MULTIPLE ASSOCIATIONS

### 1. *Nematodes of different species*

Ross (1959) reported interaction at various stages of development between *Heterodera glycines* and *Meloidogyne incognita* on soybean, which may reflect competition for a nutritional factor. Initially in plants infected with both species the population of *M. incognita* on the roots was greater than that of *H. glycines*, but the final population of *M. incognita* was decreased to about a third of that in the control and less than that of *H. glycines*. A special form of intraspecific competition was demonstrated when two races of *Ditylenchus dipsaci* were inoculated into one host plant. The final nematode population was significantly less when the tulip and red clover races were mixed than when the races occurred separately in the same species of host plant (Webster, 1967a). Probably the races hybridized and the reproductive potential of the hybrid is lower than that of either parent. This area of study needs attention, and the availability of *in vitro* culture techniques promises leads about the differential mechanisms of host response to nematodes.

### 2. *Nematodes and bacteria*

Pitcher (1965) emphasized the importance of nematodes in the transmission and transportation of other pathogens from plant to plant, and showed how little is known about the physiology of the host response to these multiple associations. The incidence of bacterial wilt caused by *Corynebacterium insidiosum* on lucerne is increased from 4% to 22% by the endoparasite *Ditylenchus dipsaci*, which carries the bacteria on its body surface (Hawn, 1963). The resistance of some, but not all, varieties of lucerne to wilt is broken by *D. dipsaci* acting as a vector for the bacterium (Hawn and Hanna, 1967). This suggests that we must explore the physiological associations of both the bacterium and the nematode in the plant. *Meloidogyne* spp. and *Helicotylenchus nannus* increase the rate of wilting of carnations by wounding the roots and facilitating entry of *Pseudomonas caryophylli* into the roots (Stewart and Schindler, 1956).

### 3. *Nematodes and fungi*

Mountain and McKeen (1962) showed that when the fungus *Verticillium dahliae* is added to soil heavily infested with *Pratylenchus penetrans* there is an increased reproductive rate of the nematode on the roots of eggplant and of tomato. There is an increase also in numbers of the ectoparasite *Tylenchorhynchus capitatus* on tomato roots in the presence of the fungus. *V. dahliae* utilizes the injured root surface to gain access to the cortex and vascular tissues of the plant, and the superficial injury provided by *P. penetrans* during its passage from the root surface to the stele or plant injury caused by transplanting, facilitates fungal entry into the root (Mountain and McKeen, 1965). Once established in the root, the fungus induces the host to produce a more suitable environment for nematode development. This could be regarded as a symbiotic relationship between the fungus and the nematode within the host. Edmunds and Mai (1966a) report the interaction of *P. penetrans* with

*Trichoderma viride*, a soil-borne fungus which is not normally pathogenic, in retarding the growth of celery and alfalfa roots. Greater numbers of *P penetrans* entered alfalfa roots treated with *T. viride* and *Fusarium oxysporum* than entered uninoculated roots (Edmunds and Mai, 1966b), and a series of experiments with sterile alfalfa seedlings on agar demonstrated that the nematodes were attracted towards the greater concentration of carbon dioxide being emitted from the fungal infected roots rather than towards exudates, which in fact tended to repel nematodes (Edmunds and Mai, 1967). Townshend (1964) stated that *Aphelenchus avenae* is more strongly attracted to *F. oxysporum* f. *lycopersici* than to roots of various seedlings. Varieties of plant exist which are resistant to *Fusarium* and also to some pathogenic nematodes. Smith and Dick (1960) describe the wilt resistance of Upland Cotton, *Gossypium hirsutum*, as being controlled by a dominant gene with modifying genes determining *Meloidogyne* resistance. *Meloidogyne* may lower the resistance of the host to wilt, so that some of the wilt resistance must be attributed to resistance to nematodes. Temperature influences the interaction between *F. solani* and *Tylenchulus semipenetrans* on citrus, and in soil at 30°C the growth reduction of rough lemon is greater when the soil is infested with both organisms than when either organism occurs alone (O'Bannon *et al.*, 1967).

Blake (1966) showed that *Radopholus similis* was the primary pathogen of bananas and that *Fusarium oxysporum* and *Rhizoctonia* sp. were non-pathogenic unless introduced into the root cortex by the nematode, when they acted as saprophytes on the newly damaged tissue. Both *Meloidogyne arenaria* and *M. javanica* enhance wilt (*F. oxysporum* f. *nicotianae*) development equally well in tobacco (Porter and Powell, 1967). When plants were inoculated with the fungus 2 or 4 weeks after nematode infection the wilt was much more severe than with simultaneous fungal and nematode inoculation or mechanical wounding. The authors suggest, therefore, that some change in the host plant physiology is necessary for fungal establishment. The gall tissues produced by the nematode on the wilt resistant and wilt susceptible tobacco were infected by *F. oxysporum* f. *nicotianae* with equal facility (Melendez and Powell, 1967). The fungal hyphae quickly infected the giant cells and the cytoplasm of the cell was removed.

Mycorrhizae are important for the growth and survival of many trees by improving the absorption of nutrients. Riffle (1967) reported agar-plate experiments in which the nematode *Aphelenchoides* sp. reproduced very rapidly and greatly restricted the growth of the mycorrhizal type fungi, *Suillus granulatus* and *Mycelium radicis atrovirens*. This suggests possible indirect pathogenic effect of the nematode on the trees. The feeding of *Aphelenchus avenae* on fungi prevented the formation of an ectotrophic mycorrhizal relationship between the red pine (*Pinus resinosa*) and *Suillus granulatus* (Sutherland and Fortin, 1968).

### 4. *Nematodes and viruses*

Since the demonstration by Hewitt *et al.* (1958) of transmission of fan leaf virus by *Xiphinema index*, there has been a succession of reports of virus

transmission between various plants by nematodes (van Hoof, 1964; van Hoof *et al.*, 1966). *X. americanum* transmits yellow bud mosaic (Frazier and Maggenti, 1962) and tobacco ring spot (Fulton, 1967). Sol and Seinhorst (1961) confirmed that *Trichodous pachydermus* is the vector of the rattle virus, and Harrison *et al.* (1961) found that *Longidorus elongatus* was associated with strawberry plants infected with tomato black ring virus. However, van Hoof (1966) found that not all populations of *L. elongatus* became infective when exposed to an infected plant. Both tomato ring spot virus and tobacco ring spot virus are transmitted by *X. americanum* and a single nematode may acquire both viruses from a doubly infected host or from two separate hosts. Similarly a single nematode can transmit one or both viruses (Fulton, 1967). Strawberry latent ring spot virus is transmitted to cucumber seedlings by adult male or female and by juvenile *X. diversicaudatum*, and nematodes kept without plants for 32 days after acquiring the virus later transmitted it (Harrison, 1967). Transmission of the virus may occur within 24 h. It was concluded by Raski and Hewitt (1960), when fanleaf virus was transmitted by *X. index* after 30 days, that the virus was not carried by the stylet but was intimately associated with the nematode's digestive system. Using serial transfers to fresh healthy plants every 2–4 days Harrison (1967) showed that one *X. diversicaudatum* transmitted the strawberry latent ring spot virus up to three times.

Roggen (1966) showed that there is an increased internal osmotic pressure of *X. index* when carrying the grape fanleaf virus (+V nematode) as compared with nematodes not carrying the virus (−V nematode). This may be due to the virus affecting the protein metabolism of its vector rather as it affects that of the host plant cell. In +V nematodes there is an increased nuclear activity in the lateral chord region which may be associated with increased protein metabolism. So far there is no evidence to prove or disprove virus multiplication in nematode vectors, although the fact that the +V nematode remains infective for long periods may suggest that the worm is not just a carrier.

### C. SIMPLE ASSOCIATIONS

Many nematodes are said to probe and browse on the root surface, and some of them appear to feed solely by probing the plant tissue with the stylet. This type of ectoparasitism is associated with the nematode's habit of moving from host to host throughout its life cycle, although any single nematode may feed at one site for several days. Some nematodes embed the anterior one-third of their body in the root tissue, and Seinhorst (1961) described this relationship as semi-endoparasitic. Many nematodes are endoparasitic during most or all of their development and migrate through the host tissues and from host to host. The more specialized endoparasites become sedentary at special feeding sites within the host plant. Two major types of tissue response occur as a result of nematode feeding, (a) tissue breakdown and necrosis and (b) abnormal growth. These tissue responses are manifested in different ways according to the species of host and parasite and the presence or absence of micro-organisms.

1. *Tissue breakdown and necrosis*

*Ditylenchus dipsaci* is an endoparasite of the stem and foliage of a wide range of plants. After penetrating the epidermis the worm moves freely through the mesenchyme cells causing cavitation and swelling of the tissue by dissolution of the middle lamella (Seinhorst, 1956) and cell hypertrophy (Blake, 1962). Cavities may form in the cortical parenchyma within 12 h of invasion (Krusberg, 1961). Swollen malformed apical meristems in lucerne, spikels on narcissus foliage, bent flower head in tulips and multiple swollen tillers of oats are typical manifestations of differential swelling. The spikels are a local area of loose hypertrophied cells which are produced when the nematode invades the foliage in the region of the active intercalary meristem before foliage elongation. The absence of the symptoms in some nematode infected narcissus foliage suggests a late infection that occurred after termination of meristem activity (Webster, 1964).

*Aphelenchoides ritzemabosi* migrates from plant to plant on the moist leaf and soil surface but develops within leaf tissues. After the nematode enters the leaf the first sign of infection is browning of the leaves (Wallace, 1961a), which is first confined to interveinal regions because the worm's migrations are restricted by the veins. In lucerne, and occasionally in chrysanthemums, *A. ritzemabosi* feeds on the epidermal cells of the leaves within the shoot apex, causing adjacent cortical cell walls to become thick and suberized, which isolates the outer few layers of cells and retards secondary bacterial and fungal infection. There is malformed shoot growth because of some inhibition of cell division, and some infected cells become enlarged and vacuolated.

*Rotylenchus uniformis* is an ectoparasite on roots. Initially it feeds on the root hairs and epidermal cells but later it penetrates deeper into the root to feed on the cortical parenchyma. Both epidermal and cortical cells are destroyed and the tissues turn first yellow and then brown. This necrosis extends beyond the cells upon which the nematodes feed and after a few days the partially embedded nematode is surrounded by brown necrotic tissue. The extent of the attack may be so severe as to initiate in the root cortex a secondary meristematic piliferous layer which produces a secondary hypodermis and root hairs and causes the original epidermis to slough. Thus, the *Rotylenchus* parasite can be isolated from the healthy root tissue (Goodey, 1951).

*Tylenchorhynchus* species feed primarily on the epidermal cells but the anterior portion of the body may penetrate to the cortex. Typically, the roots become stunted and discolored. *T. dubius* feeds on the root hairs of red clovers (Klinkenberg, 1963) and *T. claytoni* feeds on the epidermal cells between the root hairs in the region of root elongation of lucerne. Khera and Zuckerman (1963) and Sutherland and Adams (1964) reported that *T. claytoni* fed well on all parts of the root during *in vitro* studies but often concentrated at the root tip. The only root damage caused by the nematode on red pine seedlings in aseptic culture was a disarrangement of the root cap cells.

In general the Hoplolaiminae move freely from root to root feeding on the

superficial cortical cells and causing yellow-brown lesions. The number of cavities produced in the cortex may be so great that the outer layers may be sloughed. In pine seedlings infected with *Hoplolaimus tylenchiformis* the cortical cells collapse as the nematode migrates through the tissues (Ruehle and Sasser, 1960). Penetration into the vascular tissues may cause hyperplasia, the death of phloem parenchyma cells and blockage of the xylem vessels by tyloses.

*D. destructor* migrates through the potato roots and tubers causing cavities in the tissues, and the cells near the feeding area become necrotic. This host cell reaction to the nematode often extends beyond the feeding area (Faulkner and Darling, 1961). *Pratylenchus penetrans* causes extensive root necrosis in a wide range of crops. A heavy infestation may cause necrosis and sloughing of the root cortex but only minor aerial symptoms. *P. scribneri* causes extensive root proliferation in vines. Once the nematode has penetrated the cortical tissue it moves rapidly from one feeding site to the next causing cavities, and so the nematodes vacate the necrotic areas and reproduce in an optimum environment. Under aseptic conditions *P. penetrans* penetrated the root epidermis of tobacco within 10 h of inoculation and discoloration occurred 4–6 days later (Mountain, 1954). Similar discoloration occurred in the epidermal cells of apple roots 3 h after inoculation (Pitcher *et al.*, 1960) and in the roots of celery and strawberry seedlings 1 week after inoculation (Townshend, 1963a,b). Cells closest to the nematodes beneath the damaged endodermis of strawberry seedlings divide tangentially to form a layer of polyderm tissue.

The second stage larvae of *Tylenchulus semipenetrans* migrate from root to root in citrus and develop to the adult whilst feeding on the hypodermal and underlying cortical cells. Then the young females penetrate the root and migrate to the inner cortical cells where they initiate a permanent feeding site of cortical "nurse cells". There is no increase in cell size or number during feeding (Van Gundy and Kirkpatrick, 1964), and no damage of tissue along the path of nematode penetration. Cohn (1965) described severe necrosis in the cells adjacent to the nematode and along the penetration track of the nematode in field infected roots. This extensive necrosis may have been due in part to the reaction of the plant to secondary pathogens. *Rotylenchulus reniformis* is similar to *T. semipenetrans* in its feeding behavior except that it feeds on the phloem. The phloem cells near the head of the nematode and along the axis of the root turn darker (Birchfield, 1962). Necrosis occurs in field infections.

In citrus, *Radopholus similis* causes severe root damage but on necrosis. The nematode enters the root in the region of elongation and causes only slight discoloration. The nematode migrates through the cortex causing many cavities, and sometimes penetrates the endodermis where it produces similar damage in the stele. There is hypertrophy of the pericycle cells, hyperplasia in other cells, and in these tumors the nematode burrows and feeds. The tumors may coalesce and girdle the stele with an expanding pericycle which ruptures the endodermis. Under non-sterile conditions *R. similis* causes cortical necrosis in banana roots (Blake, 1961). In the area ahead of the

necrosis the cells undergo hyperplasia and hypertrophy, which are optimum conditions for nematode reproduction.

*Belonolaimus, Dolichodorus* and *Longidorus* cause stunted root systems and various degrees of necrosis. There are no true galls but root tips attacked by *Belonolaimus* may become swollen as the stubbed roots try to initiate new roots which themselves remain stunted. Paracer *et al.* (1967) ascertained by monaxenic culture techniques that *D. heterocephalus* caused hyperplasia and hypertrophy and that they both contributed to gall formation.

2. *Abnormal growth*

In contrast to tissue breakdown where the enzymes destroy the cell tissues, many tissues react by localized increased growth producing galls of various types. *Anquina tritici* larvae live in the moisture layer between the young leaves of wheat, but when the inflorescence develops the nematodes enter, migrate through the tissues, destroy the plant's ovaries and develop into adults. The larval progeny hatch but remain in the "galled" seed until the following year. The second stage larvae of *D. radicicola* invade the roots of grasses and stimulate the cortex to produce galls on which the nematode feeds. Both the epidermal and cortical cells enlarge and the cytoplasm of the cortical cells becomes granular and contains several nuclei.

The types of root-gall caused by *Hemicycliophora arenaria* feeding on the apical meristems vary greatly; the galls on celery are large and multibranched, whereas those on tomato and citrus are small and simple. This root malformation is a result of the inhibition of cell elongation and an increase in cell division behind the root tip (Van Gundy and Rackham, 1961). *H. similis* feeds distal to the root hairs and causes asymmetrical root galls in cranberry because elongation of the cells behind the root tip is inhibited only on the side of the root upon which the nematode is feeding (Zuckerman, 1961). Although *H. similis* inhibited root elongation in *in vitro* studies, it did not produce the characteristic galls of a field infection, and Khera and Zuckerman (1963) suggest that their absence may be due to a nutrient deficiency of the agar.

*Xiphinema* species cause swelling and stubby, branching roots with varying degrees of necrosis and surface lesions. The curved galls caused by *X. diversicaudatum* on root tips are a result of unilateral cell proliferation (Davis and Jenkins, 1960). In the galls giant cells are produced which are two or three times larger than the surrounding cortical cells and have granular cytoplasm. These giant cells differ from those caused by *Meloidogyne* in that there are only two or three slightly enlarged nuclei. *X. index* initiates similar cell hypertrophy and the formation of multinucleate, undifferentiated cortical cells near the feeding site. Schindler (1957) stated that the water in which *X. diversicaudatum* had been stored caused a growth response when applied to plants, which may suggest that a growth promoting substance diffused into the water from the nematode.

*Trichodorus viruliferous* feed as massive aggregations at the root tips but cause little or no necrosis (Pitcher, 1967). The initiation of flattened, enlarged

roots may be partially due to the stimulation of cell division in the pericycle by the nematode (Standifer and Perry, 1960).

The two genera of nematodes, *Heterodera* and *Meloidogyne*, have developed a special relationship with the host by causing production of giant cells. The second-stage larvae of both these species penetrate the host-plant just behind the root tip, and migrate to the stele by intra- and intercellular means (Dropkin and Nelson, 1960). The larvae of *Heterodera* species often cause necrosis during this migration, whereas those of *Meloidogyne* rarely do. The head of the larva may become embedded in the endodermis or pericycle but the rest of the body remains in the cortex (Mankau and Linford, 1960). Occasionally developing larvae of *Meloidogyne* have been found in the leaves, stems and seed cotyledons. Once the nematode has established a site at the endodermis it induces the formation of giant cells or syncytia in the vascular parenchyma. The close proximity of these giant cells to the vascular tissue seems to be a prerequisite for optimum nematode development. Up to 12 giant cells have been recorded around the head of one nematode. *Meloidodera* sp. may induce giant cell formation in pine root tips associated with mycorrhiza, but *Nacobbus*, another endogenous root parasite, does not, and only causes the formation of a spindle-shaped area of enlarged thin-walled multinucleate cells. Only the developing females of *N. serendipiticus* produced galls containing the spindle of cells (Clark, 1967). Most infections by *Meloidogyne* species are associated with the production of galled root tissue but this normally does not result from a *Heterodera* infection; although *H. rostochiensis* does cause small galls on tomato. Giant cell formation is essential for development of these nematode genera, but gall production is not. Galls are produced by cell hypertrophy and may be produced (e.g. on the leaf) without nematode entry into the tissue (Loewenberg *et al.*, 1960). Also galls can be produced by resistant plants without nematode development.

## III. Host Response to Parasite

### A. Structure and formation of giant cells

Considering the large research effort on the mechanism of the tissue response of both resistant and susceptible plants, surprisingly little study has been done on the fine structure of these plant tissue responses. Bird's (1961) paper on the ultrastructure of the giant cell is now a classic and the only detailed study of ultrastructure in a host's reaction to a plant nematode parasite. The giant cell deserves special consideration as the most elaborate plant response to nematodes.

The establishment of a successful host-parasite relationship by *Heterodera* spp. and *Meloidogyne* spp. depends on the formation of giant cells associated with the host's vascular tissue. The root cells are punctured and torn open as the infective second stage larvae force their way towards the stele, and the resulting necrosis of broken cell walls and fragmented cytoplasm is often partially isolated by cork deposition in the damaged or adjacent cells. Hyper-

trophy of the cortical cells occurs within 24 h of larval entry into the root and constitutes the beginnings of the characteristic gall. Nematode entry into the root does not always cause these symptoms and, conversely, they may occur as a response to a nematode's surface feeding without its subsequent entry (Loewenberg *et al.*, 1960). On reaching the stele the larva penetrates the endodermis and becomes stationary. The initiation of a feeding site is important for a sedentary nematode so that it obtains sufficient food to mature and produce several hundred eggs. Modification of the cells surrounding the developing nematode forms two groups of cells: (1) the giant cells, sometimes called syncytia, may be produced by the plant in response to penetration of the nematode's stylet and feeding, and (2) the adjacent cells which the nematode does not use as food but which because of secretions or excretions from the nematode produce cell hyperplasia and hypertrophy. It is now generally agreed that gall formation and giant cell production are separate but often associated phenomena. The giant cells are similar in nutritional function but not in form to the "nurse cells" caused by developing *Tylenchulus semipenetrans* in citrus roots (Van Gundy and Kirkpatrick, 1964). These cells stain darkly with haematoxylin and the nucleus and nucleolus enlarge. The reaction to *T. semipenetrans* is confined to those cells upon which the nematode feeds, and unlike the response to *Meloidogyne* there is no cell enlargement. Young female worms become embedded in the cortex and establish a feeding site of six to ten cortical "nurse cells" surrounding a cavity in which the head is situated. In each modified cell the cytoplasm gradually occludes the cell vacuole, while the nucleus and nucleolus enlarge. Later in its development the cell wall becomes thickened, the cytoplasm very dense and the nucleus amoeboid.

Giant cells are initiated by *M. incognita* larvae within a few hours in sweet potato roots (Krusberg and Nielsen, 1958) or within a few days in tomato roots (Bird, 1961), but the period varies with environmental conditions such as moisture, temperature and host species (Wallace, 1966). The giant cells may occasionally be situated in the cortex (Davis and Jenkins, 1960) but more usually in the vascular stele (Dropkin and Nelson, 1960). In laboratory experiments giant cells have been induced in leaves by *Heterodera* larvae but these were associated with the vascular tissues of the leaf veins, which suggests that the siting of giant cells in plant tissues is influenced by the close proximity of vascular tissue towards which the nematode seems to be attracted.

Within 2 days of inoculation of *M. incognita* on to susceptible tomato seedlings there is an increase in quantity and the electron density of the cytoplasm and a decrease in size of the central vacuole (Paulson and Webster, 1969) of the hypertrophied cells adjacent to the nematode's anterior end. This early giant cell then increases rapidly in size. Endo (1962) maintained that the giant cell enlarges by dissolution of the walls of the hypertrophied cells outwards from the area in the immediate vicinity of the nematode, and the large multinucleate syncytia produced by the incorporation of the cytoplasm and nuclei of adjacent cells (Krusberg, 1963a). The enlargement of giant cells is partially due to turgor pressure (Owens and Novotny, 1960), and Lee (1965) suggests that the increased turgor pressure may be partially

due to the hydrolysis of starch by amylase in the saliva secretion of the nematode. (See Fig. 1.)

Despite observations by Endo (1962) of fragments of broken cell wall associated with the newly enlarged giant cells, knowledge of the mechanism of cell wall dissolution has been little advanced since Kostoff and Kendall (1930) noted in giant cell formation that cell wall dissolution often proceeds in one direction from the nematode's head. During the development of the giant cell the walls become progressively and irregularly thickened (Krusberg and Nielsen, 1958). Electron microscopy shows that the cell walls may be many times thicker in some areas than they were originally (Bird, 1961), and that they are associated with microtubules (Paulson and Webster, 1969). Later in the development of the giant cell some cytoplasmic cell wall protuberances occur which may be equivalent to the "fragments" described by Endo (1962). Dropkin and Nelson (1960) described inner and outer layers in the giant cell wall, the inner containing bifringent cellulose, the outer cellulose-free. The wall lacks lignin (Bird, 1961) and suberin (Dropkin and Nelson, 1960). (See Figs 3 and 4.)

In the developing giant cell the large central vacuole breaks down to form many small vacuoles in cytoplasm which becomes granular (Crittenden, 1958; Piegat and Wilski, 1965). This cytoplasmic granulation first occurs near the nematode's lips (Birchfield, 1965) and it may be due to an increase of plastids and mitochondria reported by Bird (1961). The shape of giant cell nuclei is greatly modified and their number in the cell increases, Owens and Novotny (1960) noting 30–40 in young syncytia. Dropkin and Nelson (1960) account for this increase in number by the incorporation of the nuclei of adjacent cells into the syncytium and by rare nuclear division, but Owens and Specht (1964) attribute multinucleation in the early giant cell solely to syncytium formation and not to nuclear division. Nemec (1910) claimed that mitosis occurs occasionally throughout the life of the giant cell. Bird (1961) noted synchronous mitotic division in a giant cell although other giant cells in cytoplasmic continuity were not undergoing nuclear division. Dropkin and Nelson (1960) described two types of giant cell nuclei, one small and pyriform, and the other large, ovoid and having "clumps of matter", possibly the Feulgen-positive bodies described by Bird (1961) and associated with a ruptured nuclear membrane in tomato giant cells. Birchfield (1965) stated that nuclei derived from cells which had undergone cell wall dissolution assembled within the syncytium. The nucleoli were greatly enlarged in the area of the nematode feeding site, and an electron dense outer zone surrounded a much lighter core (Bird, 1961). The hypertrophy of the nuclei and nucleoli occurs early in giant cell development, while the nematode is a second stage larva (Owens and Specht, 1964). Huang and Maggenti (1969) observed nuclear division and polyploidy in the enlarged nuclei of giant cells. (See Fig. 2.)

The double nuclear membrane becomes indistinct in the older giant cells, and also in the region close to the stylet (Owens and Specht, 1964). The highly irregular shape of the nuclei and the indistinct nuclear membrane may account for the reports of very large numbers of apparently separate nuclei in giant cells (Paulson, private communication).

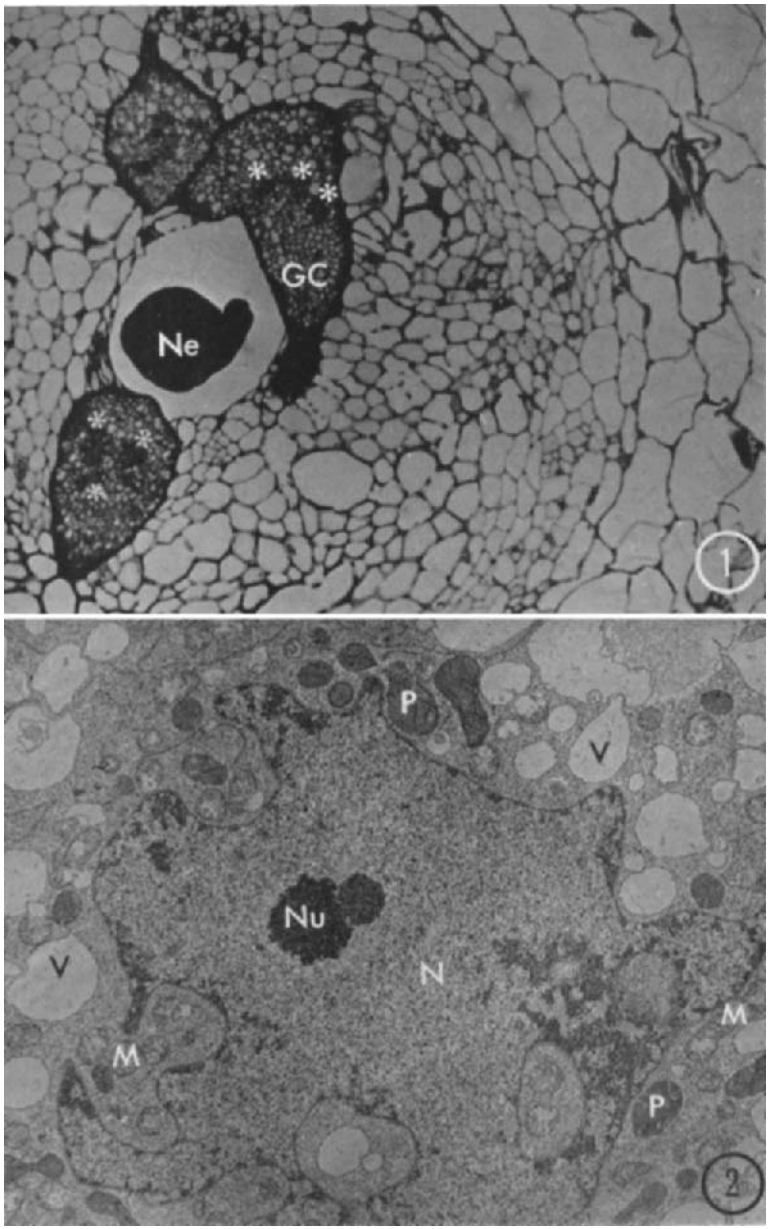
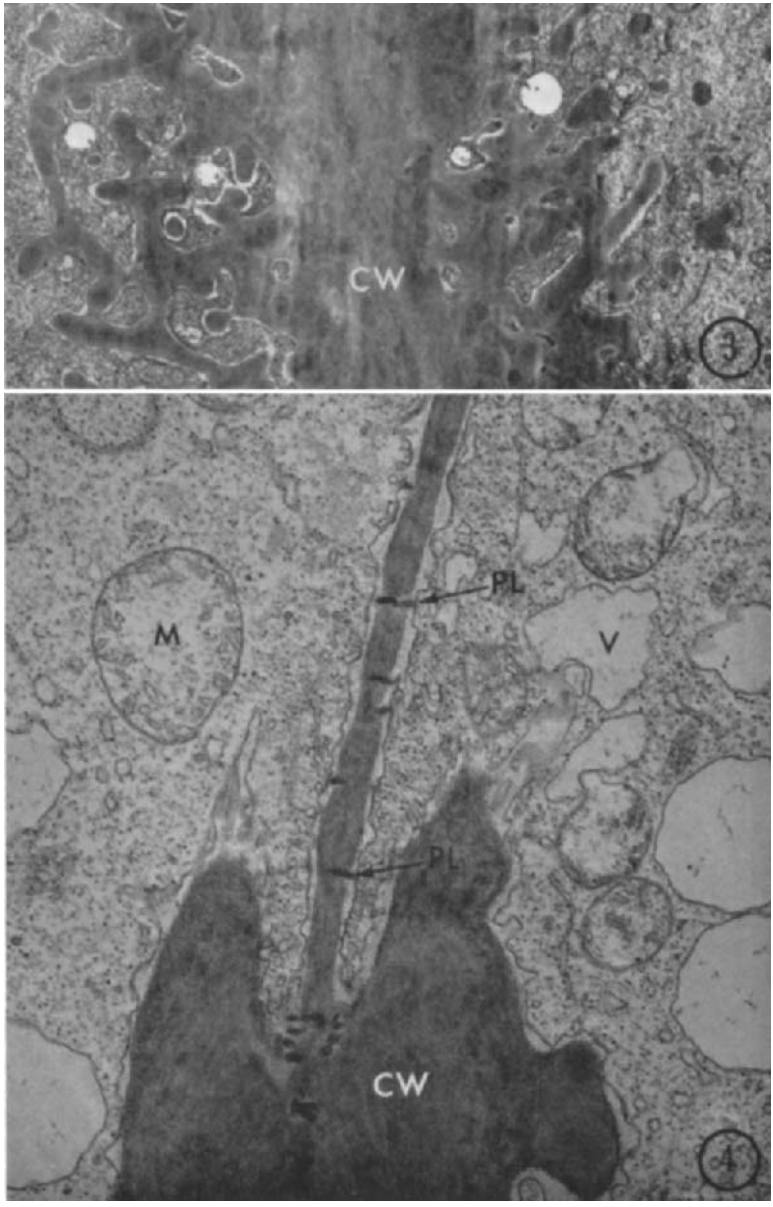



FIG. 1. Light micrograph of a cross-section of a tomato root showing three giant cells (**GC**) and a nematode (**Ne**) within the vascular tissue. Giant cells are filled with cytoplasm and small vacuoles. Asterisks denote nuclei. 14-day-old infection. (Magnification × 670.) By courtesy of R. E. Paulson.

FIG. 2. Electron micrograph of part of a giant cell showing the neucleus (**N**), nucleoli (**Nu**), and numerous plastids (**P**), mitochondria (**M**) and vacuoles (**V**). Nucleus is highly lobed. 35-day-old infection. (Magnification × 14 500.) By courtesy of R. E. Paulson.




FIGS 3 and 4. Regions of the cell wall (**CW**) between adjacent giant cells; Fig. 3 shows extreme irregularity of wall outline and Fig. 4 shows plasmodesmata (**PL**) connecting giant cells in region of wall that has not become thickened. 35-day-old infection. (Magnification Fig. 3 × 11 000, Fig. 4 × 22 400.) By courtesy of R. E. Paulson.

Rough and smooth endoplasmic reticulum proliferates during giant cell development and it is very extensive in mature giant cells (Bird, 1961; Paulson and Webster, 1969). There is also a great numerical increase of ribosomes but only a few appear bound to the endoplasmic reticulum. The mitochondria are numerous in the giant cells of tomato infected with *Meloidogyne* (Bird, 1961) and are said to be swollen and vesiculate in the giant cells of potato infected with *Heterodera* (Piegat and Wilski, 1965), and in the giant cells of tomato infected with *Meloidogyne* (Paulson and Webster, 1969). Dart and Mercer (1964) reported some similar ultrastructure changes in the development of bacterial nodules on clover where there was proliferation of endoplasmic reticulum into parallel layers and an increase in the number of ribosomes, plastids and mitochondria.

During the third larval stage of *M. incognita* var. *acrita* in cucumber roots the cell nuclei in the giant cells showed synchronous mitosis, and during the final developmental stages of the nematode thickenings appeared adjacent to the giant cell walls. They stain deeply with fast-green and haematoxylin, possibly containing protein and polysaccharide. Owens and Specht (1964) claim that nematode feeding is concentrated on one giant cell towards the end of its development, and that adjacent giant cells begin to degenerate.

The roots of tomato seedlings resistant to *M. incognita* do not produce galls or giant cells. The cells of the stele adjacent to the nematode's anterior end do not become modified except for accumulations of electron dense material around the margins of the vacuole. Only traces of this electron dense material are visible in the cells of the uninfected resistant plant or in the infected or uninfected cells of susceptible plants (Paulson, private communication).

In a few hours, therefore, a stimulus from the nematode apparently causes the cells to undergo significant morphological change, much of which is indicative of increased cell activity. This stimulus may be mechanical or chemical. However, persistence of the giant cell depends on continuous stimulation by the nematode, and if the worm is removed or killed the giant cell breaks down and is overwhelmed by the activity of the normal cells (Bird, 1962).

### B. MECHANISM OF HOST RESPONSE

The feeding of a nematode on a plant root induces certain host responses which are often specific, and the ensuing development of the nematode and degree of damage to the host plant depends largely on the nature of this tissue change. Therefore, the susceptibility or resistance of a plant to a nematode species is controlled primarily by the production of the appropriate host tissue response and secondarily by the presence or absence of egg hatching stimuli, or nematode larval attractants. An initial tissue response may occur immediately after invasion, and the tissue reaction may isolate the nematode in the surface cortical layers or even in the epidermis. The production of a toxic or unpalatable chemical by the plant may deter nematode feeding. Some nematodes burrow into the tissues towards a potential

feeding site, and produce non-specific necrosis during migration. Finally, the specific tissue response occurs and the form it takes varies with the species of host and parasite, but it may be modified by the presence of secondary pathogens.

Kostoff and Kendall (1930) suggested that salivary secretions from *Meloidogyne* induced giant cell formation, and Goodey (1948) was the first to suggest that plant growth substances (i.e. hormones) might be the cause of nematode induced galls. However, it is a truism to state that plant growth substances are associated with gall and giant cell formation as, like the animal hormones, they are intimately involved in the function of every living cell. Nevertheless, let us consider the recent advances in this approach.

Nematodes are known to exude substances from the mouth, excretory pore and anus, and the cuticular body surface has antigenic properties. Thus some or all of the host tissues could respond to one or all of these stimuli at the same or at different times. Presumably, the nematode initiates giant cells in plants by injecting a substance which does one of three things, namely, behaves as an auxin, acts as an inhibitor to the IAA oxidase, or triggers the plant to produce an excess of its own plant growth substances. Sandstedt and Schuster (1963) found that callus was formed when *Meloidogyne incognita* larvae invaded carrot slices on a sterile nutrient agar medium. This callus was similar in form to that induced by 2,4-dichlorophenoxyacetic acid (2,4-D). It is known that 2,4-D prevents the normal action of the naturally occurring plant growth substances, possibly by releasing inhibitors; nematode secretions may act similarly but only locally where the nematode is feeding. When red clover seedlings were grown on nutrient agar containing extracts of *D. dipsaci* there was cell proliferation resulting in small areas of callus and, associated with this, an increase in nematode reproduction greater than that which occurred on red clover seedlings on unsupplemented agar. Neither callus production nor nematode reproduction was as great on the nematode-extract supplemented medium as with the 2,4-D and kinetin supplemented media (Webster and Lowe, 1966). This suggests that a substance from the macerated nematode was functioning weakly as a plant growth substance.

Auxins increase the plasticity and permeability of cell walls and result in cell enlargement and elongation. When *D. dipsaci* enters a stem apex stunting occurs which could be a result of inactivation of the auxin and a consequent upsetting of the balance (Viglierchio and Yu, 1965). These authors maintain that the stunting and galling produced by *D. dipsaci* are a result of an auxin inactivating system of nematode origin which competes with the auxin system of the plant. Hence, the galls produced by *M. hapla* on tomato probably are pinhead size because inactivation is caused only by *M. hapla*; but on brussels sprouts the auxin inactivation system of the nematode is supplemented by that of the host plant and the resultant galls are almost invisible.

Sandstedt and Schuster (1963, 1965) induced callus formation on aseptic slices of carrot tap root tissue by inoculating the egg sacs of *M. incognita*, and the larval nematodes developed to maturity in the giant cells they induced. *M. incognita* produced giant cells and developed beyond the second larval stage on excised *Nicotiana tabacum* pith, provided there was a balance of

supplementary IAA and kinetin (Sandstedt and Schuster, 1966). They maintained that (1) both exogenous IAA and cytokinins are necessary for tissue growth in this experiment, (2) nematode secretions do not contain auxins or cytokinins and yet induce giant cells in actively growing tissue, and (3) the nematodes develop and produce more secretions which act synergistically with the auxin and cytokinins of the host to induce further tissue growth. Bird (1966b) was unable to detect auxins, kinins or gibberellins in the exudates of *M. javanica*. Neither was he able to detect the enzymes amylase, pepsin and cellulase, and there was no evidence of IAA oxidase in the exudation of *M. hapla* larvae. He suggests that in the *Heteroderidae* neither enzymes nor plant growth substances are produced by the nematode larva until it receives a stimulus from the plant. Nevertheless, substances have been seen passing down the salivary duct and secreted into plant cells, and Bird (1964) and Webster and Hooper (1968) showed that substances secreted from the nematode's mouth were antigenic and therefore probably contained a protein or polysaccharide.

Hydrolytic enzymes are probably the most important group in the salivary secretions. Once the nematode has penetrated the plant, saliva is extruded through the hollow stylet into the cell where the enzymes partially hydrolyze the cell contents prior to nematode ingestion. Amylase, invertase and protease were reported from *D. destructor*, *D. dipsaci* and *Meloidogyne* (Myuge, 1957; Zinoviev, 1957), while Goffart and Heiling (1962) found that some *Ditylenchus* spp. and *Heterodera* spp. secreted amylase, invertase and pectinase into water. Cellulase has been found in many plant parasitic nematodes and in *D. myceliophagus* but not in the bacterial feeders *Turbatrix aceti* and *Panagrellus redivivus* (Krusberg, 1960; Dropkin *et al.*, 1962), and polygalacturonase is present in *P. penetrans*, *H. trifolii* and *D. dipsaci* but not in *D. myceliophagus* or *D. triformis* (Krusberg, 1963a). These cellulolytic and pectinolytic (e.g. pectinmethylesterase in *Ditylenchus* spp.) enzymes are probably secreted in the saliva and aid the stylet in penetrating plant cell and fungal walls. Only those nematodes able to feed on higher plants contain polygalacturonase (Krusberg, 1963a). There is considerably more polygalacturonase and cellulase in *P. penetrans* than *H. trifolii* and their great hydrolytic properties may produce an environment more suitable for invasion by secondary pathogens and result in the severe lesions of *P. penetrans* infected tissues. Tomato root tissues galled by *M. incognita* var. *acrita* contain a greater proportion of polyhexuronates and less cellulase when compared with healthy roots.

The host response to the nematodes may in part be due to the parasite's excretory wastes, which are known to have antigenic activity. The second stage larvae of several *Heterodera* spp. produce precipitates around the excretory pore when placed in their homologous antisera (Webster and Hooper, 1968). Myers and Krusberg (1965) determined the identity of some of the organic substances discharged by *Ditylenchus* spp. and *Meloidogyne* spp. A considerable quantity of nitrogen was discharged as ammonia and amino acids during a 24 h period but this was less than for animal parasitic nematodes such as *Ascaris* or *Nematodirus* (Fairbairn, 1960). When *Meloi-*

*dogyne* spp. were incubated with acetate $-C^{14}$ and tryptophane $-C^{14}$, both *D. triformis* and *Meloidogyne* spp. discharged several labelled amino acids. The quantity and quality of amino acids in the excretory wastes may aggravate the wound and be one of the causative agents for the necrotic and/or the more general host response such as gall formation, rather than being the initiator of the specific response of giant cell formation.

The cuticle of *H. rostochiensis* larvae and egg shells contains collagen (Clarke *et al.*, 1967), which is released during maceration and is probably serologically active. The lack of a specific precipitate on the cuticle of living nematodes when exposed to *H. rostochiensis* antiserum may be because this collagen layer is covered by a lipid layer, as in *Ascaris lumbricoides* (Lee, 1965).

Setty and Wheeler (1968) obtained separate extracts from the larval saliva and excretions, body contents and walls of *Meloidogyne*. They maintain that the nematode larvae contain too little auxin to account for the extra found in galled roots, so it is possible that the extra comes from bound auxin or auxin precursor in plant cells. Sayre, quoted by Mountain (1960), suggested that after the larvae penetrate the root tissues they secrete proteolytic enzymes which hydrolyze IAA-protein complexes and release IAA and tryptophane. The latter reacts with the phenolic acids to give auxins. McComb (1961) stated that gibberellins were bound to proteins and Webster (1966) reported that gibberellic acid increased the number of nematodes in lucerne callus culture. Hence, it is suggested that the proteolytic enzymes secreted by the nematodes (Krusberg, 1963a) may release the gibberellins and growth substances which then stimulate the plant tissue response. It is known that tryptophane is converted to IAA via indole-3-acetalaldehyde and that excess IAA is removed by IAA oxidase. The action of gibberellic acid is believed to act in three ways: (1) it promotes conversion of tryptophane to IAA (Sastry and Muir, 1965); (2) it functions as a growth-promoting substance (Brian and Hemming, 1957; Sirois and Parups, 1965); (3) it increases the activity of hydrolytic enzymes (Paleg, 1961). Both gibberellic acid and IAA increase the quantities of amino acids and sugars in the tissue, and increase cell wall permeability, cell respiration, cell division and cell hypertrophy (Digby *et al.*, 1964). Plant parasitic nematodes develop and multiply most rapidly under those conditions which also occur during active plant growth, and are similar to those in the developing giant cell (Bird, 1962). This may explain why young, actively growing seedlings and rapidly elongating root tips support large plant nematode populations. Gibberellic acid may increase nematode development not solely by providing an optimum plant tissue environment but also by directly stimulating growth and moulting. Gibberellic acid also increases the rate of development of locusts (Carlisle *et al.*, 1963).

Maleic hydrazide sprayed on tomato or tobacco seedlings infected with *M. incognita* or *M. javanica* decreased the rate of development and changed the sex ratio of the nematodes. The larvae freely entered the roots during the 24 h following maleic hydrazide application, but progressively fewer larvae entered the roots after longer periods. The larvae were not prevented from developing normally by previous immersion in a 10% solution of maleic

hydrazide (Davide and Triantaphyllou, 1968). Peacock (1960) emphasizes that this is due not to nematicidal effect of maleic hydrazide, but to inhibiting the action of IAA. Webster (1967b) decreased the reproduction of *A. ritzemabosi* on callus culture by adding an IAA inhibitor 7,azo-indole, or a tryptophane inhibitor 2-hydroxy-5-nitrobenzyle bromide, or the gibberellic acid inhibitor (2-chloroethyl) trimethylammonium chloride. This emphasizes the role plant growth substances play in providing an adequate tissue response but does not fully explain their role.

Nucleic acid inhibitors, such as 6-azauracil, suppress callus tissue growth by inhibiting RNA synthesis. Endo and Schaeffer (1967) used this substance to inhibit *Heterodera trifolii* development in red clover at the third larval stage and its effects were partially reversed by adding uridine and uracil. Difficulties occurred in separating the general effects of azauracil on the host nutrition and translocation from the specific local effect on giant cells. Bird and McGuire (1966) inhibited the development of *M. javanica* in tomato roots by adding the metabolic inhibitors 6-Azauridine and 5-Bromo-2′-Deoxycytidine (5BrCdR) to the nutrient solution in which the plants were growing. The 5BrCdR was probably converted to deoxyuridine and competed with thymidine for incorporation into DNA. This would minimize the quantity of DNA in the multinucleate giant cells and so decrease the metabolic activity of the cell.

Howell and Krusberg (1966) suggested that the rapid increase of free amino acids in *D. dipsaci* infected lucerne plants as compared with healthy ones may occur in four ways: (1) greater translocation of the amino acids into the galled area, (2) increased rates of synthesis, (3) decreased rates of translocation out of the gall, and (4) decreased rates of breakdown. An increase in free amino acids and also, as we have seen, a considerable increase in bound amino acids conflicts with the theory that hydrolytic enzymes from the nematode break down the plant protein to produce large quantities of free amino acids (Myuge, 1956b). There is also an increase in the leaves and a 40% reduction in the roots of total organic acid content of grapefruit seedlings infected with *Rhadopholus similis* (Hanks and Feldman, 1968), but Owens and Specht (1964) found that the concentrations of individual organic acids differed, e.g. succinic decreased 31% and maleic increased 101% in galled compared with healthy tissues. Nematodes discharge a considerable variety of amino acids into water (Myers and Krusberg, 1965) and probably, therefore, into plant tissues.

If extracts are made of the tissues of healthy and nematode infected plants, generally there seems to be a greater activity of plant growth substances in the diseased material. Nolte and Köhler (1952) extracted the tissues of healthy plants and those infected with *D. dipsaci*, *P. penetrans* and *H. schachtii*. The extracts of rye plants infected with *D. dipsaci* significantly increased the length of *Helianthus* seedlings when applied to the decapitated stocks of *Helianthus* seedlings in an assay. They concluded that the substance, probably a plant growth substance, which induced increase in stock length also caused galling of plants. Recently Setty and Wheeler (1968) examined for auxin content healthy tomato roots and tomato roots infected with *Meloidogyne*

spp. They found, as did Bird (1962) and Yu and Viglierchio (1964), that the infected galled roots contained more auxin than ungalled roots, but that as the galled roots were larger they contained the same concentration of auxin as did the healthy roots. The auxins were extracted from roots with ethyl acetate and were soluble in 5% sodium hydrogen carbonate, which suggested the presence of organic acids. When the auxinlike substance was chromatogrammed it showed considerable variation although the Rf. value implicated indoleacetic acid (IAA). The variation may have been due to the IAA being conjugated with amino acids, e.g. indolyl-3-acetyl-aspartic acid (Andreae and Good, 1955), or with sugars, e.g. indolyl-3-acetyl-D-glucose (Zenk, 1961). Yu and Viglierchio (1964) showed that the IAA salt present in tomato galls and parasitizing nematodes often varied with species. Thus with *M. hapla* it was indoleacetonitrile, indoleacetic acid ethyl ester and IAA itself, and with *M. incognita* only indolebutyric acid. These substances were found not only in the galled tissue but also in the second stage larvae, egg sacs and mature females.

Balasubramanian and Rangaswami (1962) extracted substances from *Abelinoschus esculentus* roots galled by *M. javanica* and this appeared similar to IAA on the chromatograms and under ultraviolet light. Bird (1962) found that chromatograms of extracts from galled tomato roots contained a substance which promoted the growth of wheat coleoptile. However, the substance did not fluoresce under ultraviolet light, and did not react with color reagents. Similarly, Myuge (1956a) could not show auxins in extracts of galled tomato roots by histochemical or bioassay techniques, but gelatin containing an alcohol extract from galled tomato roots caused root galls on beans when applied to the plant (Myuge, 1956b).

Plant nematode infected tissues contain a plant growth promoting substance which is similar to IAA in chemical and biological assays. This substance is in greater concentrations in infected plants than in healthy ones and is probably a product of the plant rather than of the nematode. The substance which causes the specific response is probably secreted from the nematode's oral aperture and functions either as a trigger or an inhibitor of one of the plant enzyme systems. Other physical and chemical stimuli from the nematode induce the less specific plant responses.

## IV. Effects of Host on the Parasite

### A. Host specificity

The degree of plant resistance to nematodes varies with host and parasite species and with the environmental conditions. The number of nematodes in a plant may not indicate the degree of resistance because low populations could indicate either resistance or, if the nematodes cannot survive in heavily injured tissues, extreme susceptibility (Rohde, 1960). Dropkin and Nelson (1960) divided the effect of host tissue reactions on the invading parasite into four main groups, namely tolerant, intolerant, resistant and susceptible. Susceptible plants grow poorly due to damage but carry a large worm population. Resistant plants grow vigorously with little host damage and contain relatively few maturing nematodes. Tolerant plants grow well

without excessive host tissue damage and carry a large nematode population. The term "hypersensitivity" is commonly used and must be regarded as being a mild form of "necrosis".

Hypersensitivity of the plant tissues may be the factor determining the resistance of chrysanthemums to *A. ritzemabosi* (Wallace, 1961b). When the nematodes enter the leaves of resistant varieties the leaves brown rapidly and so the isolated nematode fails to spread or reproduce. The leaves of susceptible varieties brown more slowly and this enables the nematodes to spread throughout the plant, and they reproduce rapidly. However, the rate of browning is proportional to the number of damaged cells, and a developing female nematode in a resistant plant moves around puncturing many cells in an attempt to find one suitable for a temporary feeding site; whereas in a susceptible plant she pierces fewer cells in order to find nutrients for her needs (maturation). The polyphenols and the enzyme polyphenol oxidase are present separately in healthy leaves but during nematode feeding or mechanical damage the polyphenols and enzyme meet, and oxidation and polymerization produce the brown leaf pigment. The disappearance of chloroplasts and the oxidation of free tyrosine by polyphenol oxidase also may cause browning of the tissues (Krusberg, 1961). Wallace (1961b) suggests that the resistance is due to the absence of a nutritional factor in resistant plants, as there is no difference in the concentration of polyphenols or polyphenol oxidase, in the resistant and susceptible plants. Such a rapid hypersensitive reaction which isolated the nematode may confer on the plant resistance to nematode attack providing the plant can survive this reaction. This form of plant resistance is most effective in older plants, as young seedlings often do not survive the reaction.

*Pratylenchus penetrans* induced necrotic lesions in the roots of peach seedlings under aseptic conditions (Mountain and Patrick, 1959). Within 90 min of the nematode contacting the root surface discoloration was visible, and after 9 h the nematode was partly buried in the tissue, the area had developed a tan colour, and the cells were necrotic with sunken, granular cytoplasm. The discoloration was caused by the hydrolysis of the glycoside, amygdalin, and the release of benzaldyhyde and hydrogen cyanide. The susceptibility or tolerance of a plant to a nematode may be due to the presence or absence of certain glycosides, the breakdown products of which are phytotoxic.

Initially it was thought that the breakdown of the middle lamella resulted in the susceptibility of the plant to *Ditylenchus dipsaci* (see Seinhorst, 1957), but this is probably only part of the susceptible response. Huisingh and Sherwood (1968) varied the availability of calcium to resistant and susceptible varieties of lucerne and inoculated the seedlings with *D. dipsaci*. They found that calcium, which is a component of the middle lamella, was not essential for plant resistance, but that nematode reproduction increased in susceptible plants as the calcium concentration in the nutrient media decreased.

The first proof of a specific method of inheritance of resistance in plants to nematode parasites was that of Toxopeus and Huijsman (1953). They showed that a single dominant gene controlled the resistance of potato (a

subspecies *Solanum tuberosum andigenum*) to *Heterodera rostochiensis*, but then Ellenby (1954a) found that some *Solanum* species were resistant to populations of *H. rostochiensis* and others were not. Jones (1966) reviewed the work leading to our present understanding of the genetic interrelationships of the potato host and *H. rostochiensis*. The ability of the nematode to reproduce depends on the ability of the larva to inject saliva, which stimulates the plant root to produce giant cells on which the larva may feed and develop. When this occurs larvae are able to develop, mature into females, and produce eggs. An inability to produce the giant cell results in the larvae dying or becoming males. After a series of experiments Jones and Parrott (1965) showed that in Britain there were at least four pathotypes all of which were capable of reproducing on the normal commercial varieties but behaved differently on a series of resistant potato varieties, carrying different genes for resistance.

*Relationship between pathotype and host plants*
*(based on Jones and Parrott,* 1965)

| | Susceptible | | Resistant | |
|---|---|---|---|---|
| | *Solanum tuberosum* | *S. tuberosum* ssp. *andigena* | *S. multi-dissectum* | ex-*andigena* × ex-*multidissectum* |
| Pathotypes | *ab* | *Ab* | *aB* | *AB* |
| 0 | + | − | − | − |
| 1 | + | + | − | − |
| 2 | + | − | + | − |
| 1, 2 | + | + | + | + |

+ indicates ability of larvae to become female.

The hypothesis they proposed was that the major genes for resistance to *H. rostochiensis* in potatoes bred from *S. tuberosum* ssp. *andigena* (*Ab*), from *S. multidissectum* (*aB*) and from both (*AB*), are matched by homozygous recessive genes in the female nematodes able to overcome resistance. The pathotypes are designated 0, 1, 2 and 1,2 depending on their ability to reproduce on different potatoes. They assumed that males could have any genetical constitution and so were able to develop on any type of plant, whereas only double recessive females would mature on resistant plants. Thus, the genetic constitution of the nematode pathotypes would appear as below (Jones and Parrott, 1965):

| | *Pathotypes* | | | |
|---|---|---|---|---|
| | 0 | 1 | 2 | 1, 2 |
| *Genetic constitution* | AABB<br>AABb<br>AaBB<br>AaBb | aaBB<br>aaBb | AAbb<br>Aabb | aabb |

The effect of growing ex-*andigena* plants is to eliminate progeny of genetic constitution *AABB*, *AABb* and *AAbb* because *aa* females cannot have *AA* progeny. Thus, the proportion of pathotypes 0 and 2 decreases and that of pathotypes 1 and 1,2 tends to increase. Much experimentation has provided considerable supporting evidence for this hypothesis (Jones *et al.*, 1967; Trudgill *et al.*, 1967). The hypothesis is similar to the gene-for-gene theory which explains the inheritance of the ability of certain rust fungi to overcome the resistance of the wheat host. With *H. rostochiensis* and possibly *Meloidogyne* spp. account has to be taken of the ability of the larvae to become either male or female according to circumstances. Trudgill *et al.* (1967) found that infective larvae of *H. rostochiensis* entered several non-host solanaceous plants and they either degenerated without further development or developed into males. Endo (1964) states that the syncytia associated with developing males of *H. glycines* in soybean roots began to degenerate after 9 days but that they remained healthy where they were associated with developing females. He suggests that the syncytia start to degenerate when the nematode stops feeding, but presumably the developing males must continue to feed even if they do not require a syncytium. There is much circumstantial evidence, but there is need for more precise investigation into the relationship between resistance of the host and the developmental requirements of male and female *Heteroderidae*.

Resistance, therefore, of plants to some *Heteroderidae* and also to *Ditylenchus dipsaci* is known to be genetically controlled, but the biochemical system that the gene controls is not known. In the light of our discussions in the previous section it seems probable that a particular gene in the plant cell could control the type of protein bound to a plant growth substance or control the occurrence of a particular enzyme in the plant. Similarly, the genes in the nematodes presumably affect the nature of the salivary secretion. Wilski and Giebel (1966) propose that the resistance of some potato varieties to *H. rostochiensis* is due to the presence in the root cells of phenolic glucosides which are hydrolysed by $\beta$-glucosidase present in the larvae of resistant breaking pathotypes. The resulting free polyphenols cause necrosis. They found a high level of the enzyme activity in pathotype 2 and only a low level in pathotype 1.

The resistance of citrus to *Tylenchulus semipenetrans* is based on chemical and morphological factors (Van Gundy and Kirkpatrick, 1964). The wound periderm formation in the cortex and the necrotic or hypersensitive cell reaction to the young larval feeding stages are especially effective forms of plant resistance. In resistant hosts the cytoplasm of hypodermal cells collapses and becomes necrotic with increased deposits of suberin-like material. In susceptible hypodermal cells the cytoplasm swells to fill the whole cell. The toxicity of root juices, which probably reflects the toxicity of *Citrus* spp. to nematodes, varies with species and may be the reason for the very high degree of resistance of some species, e.g. *Severinia buxifolia*. This toxicity is seasonal, and the temperature optimum for nematode development occurs during the period when root juice toxicity of *Citrus Jambhiri* is least and when the toxicity of *S. buxifolia* spp. is highest. Second stage larvae of *M. javanica*

penetrate the roots of *Nicotiana repanda* but are unable to develop owing to the hypersensitive reaction of the roots (Milne *et al.*, 1965). *N. repanda* has a higher chlorogenic acid and chlorogenic acid-oxidase activity than *N. tabacum*, and the authors suggest that the oxidation products of chlorogenic acid may have a role in the hypersensitive reaction of the more resistant *N. repanda* to nematode attack.

We know some of the effects of plant growth substances on the nematode host-parasite relationships, and so it may be possible in the future to spray plants in order to increase their resistance to nematode attack. Certainly the gibberellin inhibitor [(2-chloroethyl) trimethyl-ammonium chloride or CCC] decreases the rate of nematode development on plant callus tissue (Webster, 1967b), the number of *D. dipsaci* on oats (Webster, 1967d) and *Verticillium* on tomato (Sinha and Wood, 1967). The reverse is possible also because spraying 2,4-D solution (140 mg/100 ml/yd$^2$) on oat varieties resistant and susceptible to *D. dipsaci* increases the number of nematodes per plant compared with the untreated control (Webster, 1967c). This treatment, which decreased the resistance of oats but not of barley to nematodes, caused plant cell hypertrophy and proliferation which is normally a necessary response for favorable nematode development. These effects are probably peculiar to the 2,4-D type of herbicide because Courtney *et al.* (1962) found that other types (e.g. dalapon) inhibited nematode development in the bentgrass *Anguina agrostis*.

### B. BIOLOGICAL RACES

Regardless of the morphology of a nematode, its ability to parasitize a certain host depends on the physiological and ecological aspects of the host–parasite relationship, which is controlled by the genetical constitution of the host and the parasite. In several species of plant nematodes physiological and ecological data have resulted in species being subdivided into "biological races", "pathotypes" or strains.

Seinhorst (1957) listed 11 biological races of *Ditylenchus dipsaci*, and Sturhan (1964) suggested that there are as many as 20. Hesling (1966) in a review of the subject stated that races are probably being selected all the time, concurrently with the spread of selections already made and perpetuated with minimal genetical change. Their host range is the basis of differentiation into races because no morphological differences have been found despite intensive statistical analysis of many body measurements (Barraclough and Blackith, 1962). Several workers have recently examined the effect of race hybridization on the host range. These explorations were done using plant callus tissue (Webster, 1964; Eriksson, 1965) and plant seedlings (Sturhan, 1964; Webster, 1967a) as a common host to the two parental races of a hybrid. The authors used stock cultures of each race from which to obtain the male and female parents for hybridization experiments. These stock cultures do not reflect the natural population because they are themselves the inbred progeny of a single gravid female. Such inbreeding may increase the frequency of genes controlling abnormal characters and this may explain the tail abnormalities in the hybrid crosses described by Sturhan (1964). The host range of a natural

population is the mean response of all the individuals and it is possible, though unlikely, that a single female extracted to initiate a stock culture may be an extreme form. Onion was used as the common host when producing ten fertile hybrids (Webster, 1967a). On average the hybrids multiply less rapidly than, and the host range is similar to, that of their parent races. These observations conflict with those of Eriksson (1965), who claimed that hybrids were more polyphagous than their parent races. Webster (1967a) inoculated a nematode suspension of each of two races separately and together into tulip, which was a normal host of one race (tulip race) and resistant to the other race (red clover race). The two races together produced significantly fewer nematodes than did an equivalent inoculum of the tulip race alone, and more than the red clover race alone. The poor multiplication of the two races together suggests the production of the hybrid with low fecundity. Mixed populations of races together with their hybrids and back-crosses must occur in nature where repeated back-crossing with parental types would obscure the nature of hybrid populations. The occurrence of back-crossing, together with some infertility and low fecundity, may explain the slight variation in host range of the known races and the absence of dominant new races.

The fact that several races of *D. dipsaci* reproduce with almost equal facility on lucerne callus (Webster and Lowe, 1966) suggests that the factor for race separation is the compatability of the plant's physiology with the nematode's salivary secretions. The antigenic specificity of these races and of their oral secretions was tested by Gibbins and Grandison (1968) and Webster and Hooper (1968), using the agar gel diffusion technique. Although they found serological differences between saline extracts of macerated *D. dipsaci*, *D destructor* and *D. myceliophagus*, they were unable to separate the races of *D. dipsaci* by serological means. It was not possible to detect serological differences between the oral precipitates of *Ditylenchus* species or of *D. dipsaci* races.

Considerable advances have been made in the elucidation of the genetic and ecological factors controlling the occurrence of pathotypes of *H. rostochiensis* and *H. avenae*. Ellenby (1952) first reported some potatoes to be resistant to *H. rostochiensis* and, as was seen in the previous section, Jones and Parrott (1965) have identified four pathotypes of *H. rostochiensis* on the basis of the gene-for-gene theory of resistance-breaking, but this number is certain to increase. Recently a morphological character has been associated with this physiological separation of pathotypes, as Guile (1966, and *in litt.*) observed a correlation between the cyst wall color and the pathotype of *H. rostochiensis*. The cyst changes color over several weeks from white, through intermediate phases of a color characteristic of a particular pathotype, to the tanned brown. Norton (1967) describes an intensely yellow intermediate phase in the color change of *H. trifolii* cysts on certain varieties of *Trifolium pratense* and *T. repens*, and he gives this as an example of the effect of the host on the parasite.

Andersen (1959) in Denmark and Cotten (1963) in Britain have found that populations of *H. avenae* from various districts have different varieties of oats and barley as hosts. Although wheat varieties in Europe are relatively

unaffected by *H. avenae* those in Australia are often seriously attacked by this nematode, which gives rise to speculation as to whether these are geographical races or even a separate species and, if so, are they separated physiologically or only ecologically. Further evidence that *H. avenae* occurs as races or pathotypes is that in Australia and Canada this nematode is affected by different environmental factors which influence egg hatch. Fushtey and Johnson (1966) showed that populations of *H. avenae* from Ontario require a minimum period of 8 weeks at 0°–7°C to stimulate optimum hatch. Such temperatures do not occur in the wheat growing area of Australia.

### C. NUTRITION

The development of the nematode parasite is intimately related to the development of the host and is influenced greatly by the physiological and nutritional status of the plant cell environment. The ambient environment may effect the host and in turn this may be beneficial or harmful to the parasite. Krusberg (1967) reviews this general theme.

Ellenby (1954b), using *H. rostochiensis* on potato, was the first to show with certainty that the sex ratio of plant parasitic nematodes is influenced by the density of larval attack of the host. More recently the age of the plant and the nutritional status of the plant in association with the density of larval attack has been shown to affect the development, reproduction and sex of *Meloidogyne* (see Triantaphyllou, 1960) and *Heterodera* (see Trudgill, 1967). Triantaphyllou (1960) showed that the direction of post infection development of the second stage larvae of *M. incognita* is controlled largely by the prevailing environmental conditions. Favorable conditions for the nematode's development promote the differentiation of the majority of the larvae into females. Unfavorable conditions, which can be induced by decapitation of the plant and non-optimal root incubation, favor differentiation of a larger proportion of the larvae into males. Young, developing female larvae may change their course of sexual differentiation and develop into adult males but with two testes instead of the normal single testis. Trudgill (1967) suggests that the larvae do not develop into females unless they are able to induce a giant cell of sufficient size to provide adequate nutrition. Increasing the larval density, the age of the plant, or decapitation of the host plant appear proportionately to decrease the ability to produce giant cells and so to increase the male to female ratio of the nematode parasite. Trudgill maintains that the decrease in carbohydrate status of the host roots is the significant factor in environmental sex determination of the nematode larvae.

By using *M. incognita* on excised cucumber roots McClure and Viglierchio (1966b) found that retarded development is explicable because crowding causes a reduction in the amount of food available to the nematode, and/or crowding results in increasing nematode interactions such as toxic effects of excretory products or competition for sites suitable for giant cell formation. When *M. incognita* was cultured on excised roots on nutrient media with different additives they found that the sex ratio was greatest and most uniform

when there was a sucrose deficiency. This is consistent with the tests of Triantaphyllou (1960) with *M. incognita* and those of Trudgill (1967) with *H. rostochiensis* on tomato where decapitation, which also depletes sucrose supply to the roots, increased the number of developing males. In a series of experiments Davide and Triantaphyllou (1967a,b) showed that the effect of high larval density on the sex ratio of *M. incognita* was increased by low temperatures (15°C). They suggest that most larvae are genetically determined as females, and that certain adverse environmental conditions induce masculinization. Temperature changes the sex ratio of *Nacobbus serendipiticus* developing on excised tomato roots (Prasad and Webster, 1967a). The male to female ratio was 2·33, 0·14, 1·00 and 7·33 at 15°, 20°, 25° and 30°C respectively. The temperature may be affecting nematode development by restricting the availability of nutrients to the worm. Ion concentration of various elements (K, P, Mg) are known to effect sex determination, and in *H. schachtii* a nitrogen deficiency increased the proportion of males (Kämpfe and Kerstan, 1964). The authors also reported a seasonal variation in sex ratio, which probably reflects the seasonal changes in the plant's physiology and hence the availability of nutrients to the developing nematode. However, Bird (1960) observed an increased rate of development of *Meloidogyne javanica* on plants deficient in potassium, iron, nitrogen and magnesium. The production of a metabolically active giant cell in response to some members of the *Heteroderidae* implies that the nematodes require a considerable quantity of amino acids for their development. There is an increase in protein and nucleic acids in the giant cells (Owens and Novotny, 1960). By adding L-amino acids it was possible to restrict the availability of the normal D amino acids and retard nematode development. Overman and Woltz (1962) and Prasad and Webster (1967b) tested the effect of several soil-applied amino acid antimetabolites on species of plant-parasitic nematodes. Seven of these antimetabolites, including DL methyl glutamic acid and D allyl glycine, decreased the amount of galling caused by *M. incognita acrita*, which may indicate that the D form of these amino acids blocked the availability of L forms in the nematode's metabolism. Both α alanine and methionine sulphoxide are discharged by *D. dipsaci* (see Myers and Krusberg, 1965), and neither of these applied exogenously in the DL form retarded the development of *D. dipsaci* or its host plant. On average the soil-applied amino acid antimetabolites decreased the number of root parasites (e.g. *Nacobbus* and *Heterodera*) more readily than the foliage parasites (e.g. *Ditylenchus* and *Aphelenchoides*), which suggests that these antimetabolites were more readily available to the root parasites than were the normal L forms produced in the leaves.

Goodey (1952) showed that the average body dimensions of *D. destructor* are changed as a result of transferring the nematodes from one host to another. Thus females from iris are significantly longer than those from potato. When transferred from iris to potato the average length of the population is significantly shorter, but the length does not increase significantly when they are being transferred from potato to iris. The males of the species show a trend diametrically different from that of the females. This is

further evidence to support the findings in relation to the *Heteroderidae*, that the development of nematodes is strongly influenced by nutritional factors of the host, also that the males and females of a species require different nutrient factors.

## V. The Future

A review of the host-parasite relationships inevitably points to areas of strength and weakness in this association. Dropkin and Webb (1967) pointed to two major sites where this association can fail in plant-parasitic nematodes. First, secretions from the potential host may fail to stimulate the nematode or they may actually be toxic to the nematode. Second, the host plant tissues may respond to produce an isolating periderm or may fail to produce the required feeding site. It is this last response where there is/is not the greatest interplay between host and parasite. It is my opinion that this area has the most exciting research prospects. By manipulation of the host-physiology we shall perhaps be able to inhibit the production of nematode feeding sites, prevent the maturation of female parasites, encourage the production of low fecundity races of nematodes, or even infect the plant with two potential parasites which are incompatible in the modified physiological environment. Hopefully the host will survive!

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# The Immunology of Schistosomiasis

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## I. Introduction

For more than 50 years, and since the pioneering observations of Fujinami (1917) on acquired immunity to *Schistosoma japonicum*, the problems of immunity to schistosomes have puzzled parasitologists. Research has been intensified since the Second World War and many groups of workers throughout the world have explored the field. At times progress has seemed to be rapid, the solution to some major problems close at hand. But in spite of all the information that has been gathered, the overall pattern of immunity in schistosomiasis remains obscure, full of apparent anomalies and unanswered questions. Various aspects of the subject were reviewed recently (Smithers, 1962a; Stirewalt, 1963; Kagan, 1966; and Smithers and Terry, 1969); and in this review we have tried to define more clearly those areas where work is urgently needed to explain these anomalies and to answer these vital questions. We hope the review will help progress in this fascinating field of research by focusing attention on these particular problems.

## II. Innate Immunity

### A. Host specificity

Consideration of innate immunity to schistosomes must begin with host specificity shown by the parasitic species. It is now well recognized that of the three common schistosomes of man, *S. japonicum* is the least host-specific. This species meets little innate resistance in a variety of vertebrate animals, especially dogs, goats, sheep, pigs and cattle, which as domestic animals gain importance as reservoir hosts (Ho Yi-hsun, 1963). *S. haematobium* is not so well adapted to non-human hosts, but a variety of rodents and primates are susceptible (Stirewalt, 1963; Capron *et al.*, 1965a; Gear *et al.*, 1966; Jordan *et al.*, 1967) and a baboon, a guenon and a chimpanzee have been found naturally infected (Nelson, 1960; De Paoli, 1965). The range of host-specificity of *S. mansoni* lies somewhere between those of the other two species; natural or experimental infections occur in primates (Sadun *et al.*, 1966a), rodents and insectivores (Loos, 1964; Andrade, 1964a; Barretto *et al.*, 1964), marsupials (Stirewalt, 1963) and cattle (Barbosa *et al.*, 1962), and a tribe of wild baboons in Tanzania is sustaining the life cycle of *S. mansoni* without any human intervention (Fenwick, 1969).

Although a variety of mammals have been implicated as hosts of the schistosomes of man, the degree of infection tolerated by each host species varies considerably. The degree of susceptibility of a host can be determined by criteria such as the percentage of parasites developing, the growth and structural development of the worms, the ability of the worms to produce eggs, the viability of the eggs recovered from the faeces, intestine and liver, the infectivity of miracidia for suitable snails, the sex ratio and location of the worms in the host, the length of the prepatent period and the pathological manifestations of infection (Bruce *et al.*, 1961). However, only those animals which become infected with large numbers of worms and emit numerous viable eggs will have epidemiological significance as potential reservoirs of infection.

Host specificity is complicated further by other factors, e.g. intra-specific differences in host susceptibility. Man and some primates are innately resistant to the "Formosan" strain of *S. japonicum* (Hsü and Hsü, 1956); the resistance is complete in that the cercariae penetrate, but adult worms do not develop. Other strains of *S. japonicum* vary in virulence in mice and in egg distribution in the viscera of hamsters and mice (Hsü and Hsü, 1960b, c). The prepatent period, the number of worms developing, the distribution of eggs in the host's tissues and the pathology differ with varying strains of *S. mansoni* (Saoud, 1966; Warren, 1967); and differences in the distribution of eggs in host tissues occur also in two strains of *S. haematobium* (see Wright and Bennett, 1967).

In addition to differences in susceptibility to schistosomes between host species, minor intra-specific variations of infection with *S. douthitti* and *S mansoni* exist amongst inbred strains of mice (Kagan *et al.*, 1954; Stirewalt *et al.*, 1965). Added to these difficulties, the recognition of mammalian schistosome species presents problems as yet unsolved. Because of the morphological variation of schistosomes in a particular host and the possible hybridization

of related species, an inadequacy in establishing species identity remains, which masks the precise nature of host specificity (Najarian, 1966).

Thus the analysis and rationalization of data on host specificity is complicated by variations in the balance of the host-parasite relationship and the perpetual genetic variations of parasite and host from geographically isolated areas. It is clear that many biological problems are involved. Certainly the specificity of *S. mansoni* and *S. haematobium* is not as narrow as was once believed, and although potential reservoir hosts may be insignificant because of epidemiological factors, such factors could change rapidly, unexpectedly leading to a prominent role of animal hosts in the zoonosis of schistosomiasis.

## B. MECHANISMS OF HOST SUSCEPTIBILITY

The poor susceptibility of certain hosts to infection with schistosomes is generally assumed to be a manifestation of innate immunity. However, innate immunity is taken to mean the resistance displayed by an animal that has never experienced the particular pathogenic organism either as a pathogen or as a related non-pathogenic variant (Humphrey and White, 1966). In acquired immunity, the parasite will be destroyed or adversely affected by a humoral or cell-mediated response resulting from prior sensitization of the host. Innate immunity may play a large part in determining the resistance of the pigeon to *S. mansoni*, the cercariae of which are killed in the epidermis of the previously unsensitized host (Coutinho-Abath and Jampolsky, 1957). In animals more closely related to the true host, resistance may be expressed as a poor worm yield or a stunting of the parasites rather than an absolute resistance, and mechanisms other than those responsible for innate immunity may be involved. The poor susceptibility of the albino rat to *S. mansoni* compared with the mouse, for instance, can be explained partly by an innate mechanism and partly by the development of a more effective acquired immunity.

### 1. *The skin barrier*

The first defensive barrier of the host to schistosome infection is the skin, and most information on innate resistance is related to this organ. Apparently there is little or no selection by the cercariae in penetrating the epidermis of mammals or birds. *S. mansoni* cercariae can pierce the epidermis of the pigeon (Coutinho-Abath and Jampolsky, 1957) and the numbers of cercariae penetrating the skin of the hamster, mouse, guinea-pig, rabbit and rat are almost identical, although these rodents show ranging susceptibilities to infection (Warren and Peters, 1967).

The penetration of cercariae through the skin of the host is dependent to a large degree upon an enzymatic mechanism (Lewert, 1958). One effect of the cercarial enzymes is on the acellular non-fibrillar constituents of connective tissue, namely, the ground substance of the dermis and the subepithelial basement membrane. Histochemistry shows that cercarial secretions cause these largely glycoprotein structures to depolymerize and soften. Lewert and Mandlowitz (1963) believed that the ability of the cercariae to penetrate

depends on the ease with which these acellular glycoproteins can be altered by enzymes. The physical or physiological state of host glycoprotein may thus be of great significance to the relative success of the invading parasite. The density or polymerization of glycoprotein varies from one species to another and also within a species according to the physiological state of the animal. Host specificity or specificity within a species may therefore be related to the ability of the cercarial enzymes to break down the acellular glycoprotein barriers of the skin.

It has been demonstrated clearly that mice less than 1 month old are more susceptible to *S. mansoni* than are older mice (Lewert and Mandlowitz, 1963; Da Motta *et al.*, 1965; Purnell, 1966). Lewert and Mandlowitz believed this to be due to the acellular barriers of the skin of older mice being more highly polymerized and therefore more resistant to enzyme attack. This explanation was strengthened by the demonstration that old mice of the LAF strain, which are characteristically slow ageing, are as susceptible as CF mice less than 1 month old. The connective tissues of old LAF mice have the characteristics of a young animal with regard to the density of the ground substance and the distribution of water. Further evidence for this hypothesis comes from studies on scorbutic mice and cortisone-treated mice. The acellular elements of the skin in animals deficient in ascorbic acid are looser and contain more water, an alteration in the direction of reduced resistance. Lewert and Mandlowitz (1963) were able to show that scorbutic mice are more susceptible than normal mice of the same age. On the other hand, cortisone treatment increases the density of the basement membrane so that resistance to cercariae is enhanced in cortisone-treated animals (Lewert and Mandlowitz, 1963; Coker, 1957).

Clegg and Smithers (1968) showed that the skin of some laboratory hosts is a major barrier to invading cercariae of *S. mansoni*. They recovered schistosomula from the skin of animals within a short time of cercarial penetration and examined the larvae by a dye-exclusion test, showing that up to one half the cercariae which enter the skin of rats die within 10 min of penetration, whereas only about 30% die in mouse skin, and only 10% in hamster skin. The higher recoveries of adult worms from hamsters than from mice in their experiments were attributed to the lower proportion of cercariae which died in the skin of hamsters; only 20–26% of the cercariae which entered the skin could not be accounted for by adding the percentage loss in the skin and the percentage recovery of adult worms. The lower recovery of adult worms from rats was not entirely due to the higher proportion of cercariae which died in the skin of rats; apparently losses occurred at some other stage in the parasite's life cycle.

The deaths in the skin occur within 10 min of penetration, when the cercariae are still within the epidermis. After 15 min many of the cercariae have entered the dermis. The cause of death of the cercariae was not determined. It is not related to developing sensitivity to water which accompanies the transition from cercaria to schistosomulum. It may be due to exhaustion of some reserve material, as Rai and Clegg (1968) suggested from their studies on the penetration of bird skin by *Austrobilharzia terrigalensis*. Another possibility is

that a toxic substance may be present in the epidermis. The thickness of the stratum corneum seems to be of little importance; it is about the same in the abdominal skin of rats and hamsters, hosts with the broadest difference in proportional cercarial deaths within this layer of the skin. Cercariae die as they are penetrating the Malpighian layer of the skin, which suggests that the variation between host species may be due to differences in the susceptibility of the cells of the Malpighian layer to lysis by enzymes, or, as Lewert and Mandlowitz (1963) have suggested, to the density of the sub-epithelial basement membrane. Few deaths occur once the cercariae have entered the dermis, so that the density of the ground substance is unlikely to play a role in the skin barrier to penetration.

2. *Serum factors*

Kagan and Levine (1956) suggested a correlation between the cercaricidal activity of normal unheated serum and the susceptibility of the host to infection. For instance, serum from the guinea-pig, the albino rat or the dog, poor hosts for *S. mansoni*, is cercaricidal for *S. mansoni* cercariae, whereas sera from man, mouse, and monkey (good hosts for *S. mansoni*) are not cercaricidal. However, it is unlikely that a true correlation exists. Standen (1952) examined 502 samples of normal unheated human sera and found a cercaricidal factor present in 74%. Furthermore, the rat and the guinea-pig are not as resistant to *S. mansoni* as was once believed; 38% of cercariae used in exposure can be recovered as worms in the guinea-pig, and 20% in rats, 4 weeks after exposure (Warren and Peters, 1967). The extrapolation of results from *in vitro* studies to situations *in vivo* requires caution. During skin penetration, the cercaria is rapidly transformed into a schistosomulum, changing in the nature of the larval surface and the development of sensitivity to water (Stirewalt, 1966). Clearly, therefore, the schistosomulum is physiologically very different from the cercaria and factors which affect one do not necessarily affect the other. D. L. H. Robinson (1960) found a negative correlation between *in vitro* and *in vivo* studies when he reported that adult *S. mansoni* laid greater numbers of eggs *in vitro* when maintained in serum from animals which are poor hosts. Thus, sera from some hosts which support schistosomes manifest cercaricidal properties, but there is no evidence that these properties affect the parasite *in vivo*.

3. *Effect of host's sex*

Attempts have been made to define the effect on infection of the sex of the host, or of sex hormones. Purnell (1966) found male mice and male hamsters more susceptible to infection with *S. mansoni* than female animals; and male hamsters are more susceptible to *S. haematobium* than females. This has also been shown by other workers. The length of male *S. haematobium* worms from male hamsters is greater than those from female hamsters and Purnell suggests that the sex of the host may influence the sexual balance of the worm population. Berg (1957) found that castration of mice, or injection of testosterone, lowers the survival rate of male *S. mansoni* and that testosterone injections,

but not castration, lower the survival rate of female worms. E. J. Robinson (1959) carried out similar studies and found that combined castration and testosterone treatment did reduce the number of male worms, but he was not able to confirm Berg's other findings. In a later paper, E. J. Robinson (1960) demonstrated adverse effects on *S. mansoni* in mice receiving massive doses of stilboestrol. These effects included a delay in maturation of both sexes; the development of a high proportion of males with accessory gonadial tissue, probably ovarian; and a reduction in the length of male worms compared to worms from untreated hosts. It could not be decided, however, whether these effects were due to the direct action of stilboestrol, or due to an indirect cause from the complex disturbances induced in mice by the great excess of hormone. The possibility exists of altering the survival rate of either sex in hormonally imbalanced hosts, but the conditions required for predictable specific alterations still remain unknown.

4. *Factors associated with acquired immunity*

Lichtenberg *et al.* (1962) were impressed by the close similarity between the cellular reaction to schistosome invasion observed in abnormal hosts and that observed in immunized laboratory animals. This prompted them to suggest that a cellular mechanism is one way in which the abnormal host prevents the migration and development of schistosomes. Presumably, by "cellular mechanism" the authors are referring to the non-specific inflammatory response and not to a specific cell-mediated immunity. Inflammation is the normal response of the animal to a foreign body and it is not surprising that this response is seen around dead or dying parasites in both abnormal or normal immune animals. Inflammation consists essentially of dilatation and increased permeability of the capillaries and the mobilization of phagocytic cells at the site of parasitism. Numerous bacteria can be removed rapidly from the circulation by the phagocytic process, but whether or not the much larger schistosomulum can be as effectively dealt with in this way is doubtful. If, however, the migration of the parasites is slowed down in an abnormal host, they are then more likely to be trapped in the inflammatory foci where death and phagocytosis could occur. However, we are no nearer to identifying the factor responsible for the slow migration or the parasite's death.

Lichtenberg *et al.* (1962) accepted the possibility that in abnormal hosts, specific antibody may be formed more rapidly in response to infection. This appears to be likely. Albino rats develop a similar degree of protection to *S. mansoni* whether they are exposed once to normal cercariae or once to irradiated cercariae (Smithers and Terry, 1965c). On the other hand, the rhesus monkey develops no resistance after one exposure to irradiated cercariae (Sadun *et al.*, 1964). There is thus a difference, perhaps qualitative, perhaps quantitative, in the protective response to infection by the rat and the rhesus monkey. Rats can also eliminate most of their worm burden between the 4th and 5th week after infection and thereafter are highly resistant to reinfection. This performance cannot be approached by the mouse or hamster.

The relatively poor susceptibility of the rat to *S. mansoni* can therefore be

explained in at least two ways. First, there is an enhanced skin barrier to cercarial penetration; larger numbers of cercariae die in the epidermis of the rat than in the epidermis of the mouse or hamster (Clegg and Smithers, 1968). Possibly, this is due to the nature of the glycoprotein of the sub-epithelial basement membrane (Lewert and Mandlowitz, 1963). Secondly, there is a rapid and effective development of acquired resistance by the rat as a result of exposure to infection, a response which is far superior to that of the mouse, hamster or monkey exposed to the same stimulus (Smithers and Terry, 1965c). Why the rat is able to respond to schistosome antigens by the development of a high level of protection whilst other hosts cannot do so, is surely the fundamental question with regard to host susceptibility.

## III. Acquired Immunity

### A. The nature of acquired immunity

Undoubtedly man and animals acquire immunity to schistosomes; and we believe that this immunity is essentially similar to that found in other infectious diseases. This aspect is discussed fully in a later section, but we must realize at the outset that although the immunological mechanisms active against schistosomes are probably the same as in other infections, their expression differs considerably from that seen in the classical type of antibacterial immunity. In schistosomiasis we do not see the classical situation of an immunological crisis occurring during the primary infection, with a massive destruction of the invading organisms and the development of a strong immunity to subsequent re-infection. Instead, resistance to schistosomes appears to develop only gradually, often taking several years to become absolute. We must appreciate also that an immunological state exists in some animals, and perhaps in man, where although the host is immune to re-infection it is unable to kill off the established population of worms from the primary infection. We have termed this state "Concomitant Immunity" (Smithers and Terry, 1969); a possible explanation for its existence will be discussed later.

It has perhaps not been always sufficiently appreciated in the past, how widely host species differ in their ability to acquire immunity to schistosomes. This is a different problem from that of innate resistance or host susceptibility. In order to illustrate its complexities, the acquisition of immunity in man, and in some of the more commonly used experimental animals, will be discussed.

#### 1. *Acquired immunity in man*

For obvious reasons, there has been little direct experimentation on man and only three reports exist of human volunteers being exposed to cercariae and subsequently examined for eggs and other symptoms associated with schistosome infections. In an area endemic for *S. intercalatum*, Fisher (1934) exposed six volunteer African adults to cercariae of this species and all appeared to resist this challenge. In Rhodesia, Clarke (1966a) exposed two volunteer adults to cercariae of what was probably *S. matthei*. These volunteers had lived in an area endemic for this species all their lives. Although there was

some dermatitis and eosinophilia following exposure, neither volunteer passed eggs or showed any other symptoms of infection. It may be argued that *S. intercalatum* and *S. matthei* are not primarily human parasites. Man may be able to develop resistance against these species but not against the species which more commonly infect him. The only direct experiment on a well recognized parasite of man is that reported by Gothe (1963). Gothe exposed himself to cercariae of *S. haematobium* which had been treated with D.D.T. and finally with untreated cercariae of this species. No eggs or any other symptoms of the disease were observed. However, the infectivity of Gothe's challenge cercariae was not controlled, and the validity of this experiment is therefore uncertain.

The greatest difficulty of direct trials on man is the impossibility of controlling the experiments adequately. Perhaps a better approach to the problem would be the follow-up of patients in endemic areas after drug treatment. Very little work of this kind has been reported. In Tanzania, Jordan (1968) obtained evidence that approximately 75% of children infected with *S. haematobium* and treated with stibocaptate did not become reinfected within 2 years of treatment. Kloetzel (1967a) treated children in Brazil with Astiban; although reinfection with *S. mansoni* did occur, the median egg count 4 years later was still only about one third of the count before treatment. Evidently, at least some degree of resistance to reinfection had developed. Studies of this kind, including a quantitative estimate of egg output, are greatly to be encouraged, for they may well provide the most useful information on the development of resistance in populations.

A mass of evidence gathered from epidemiological studies indicates that man acquires immunity to schistosomes, and many workers have found that in endemic areas schistosomiasis is mainly a disease of the young, and that with advancing age there is a decreased passage of eggs and a lessening of the associated symptoms. This has been reported for *S. japonicum* (Pesigan *et al.*, 1958), *S. haematobium* (see Gerber, 1952; Gothe, 1963), *S. mansoni* (see Bassenes and Pantoja, 1947) and *S. intercalatum* (see Fisher, 1934). Most of these authors conclude that their findings support the idea that man acquires resistance to reinfection, although other explanations of their findings, such as reduced exposure to infection in older people, cannot always be excluded.

Clarke (1966b) provided the most vigorous exposition of human acquired resistance to schistosomes. A detailed study carried out in Rhodesia showed that the prevalence of infection with *S. mansoni* and *S. haematobium* increased with age and reached a peak between 7–15 years. The prevalence then decreased, sometimes dramatically, until in adults a low level of detectable incidence was reached. Clarke reasoned that this pattern of infection can be explained satisfactorily only by the development of acquired resistance. He rejected, with some justification, alternative explanations such as reduced exposure of adults, or a hindered passage of eggs by fibrotic lesions of the bladder and intestinal wall, and he suggested that after the peak of prevalence, the number of infections overcome because of acquired resistance becomes progressively greater than the number of reinfections, which explains the decreasing prevalence with age.

These results could be explained equally well by postulating the existence of an age resistance, rather than an acquired resistance; but Kloetzel and Rodrigues da Silva (1967) strongly suggested that age resistance has no role in the epidemiology of schistosomiasis, after studying a population which was first exposed to *S. mansoni* as adults, and finding that resistance to reinfection appeared to depend upon the duration of infection and not directly upon age.

Clarke (1966b) also showed that the age at which the peak prevalence occurred was related to the pattern of transmission. In areas of high incidence the peak occurs earlier than in areas of low incidence. Maximum resistance seems to develop when a moderate initial exposure is followed, after an appreciable period, by regular re-exposures, a conclusion also reached by Gerber (1952). If infections are very heavy or very light, then there is a poorer development of resistance in the population as a whole.

Despite this evidence that man can acquire resistance to schistosomes, the details of the process are not clear. It appears that immunity develops gradually, taking several years to become pronounced. In the early stages of infection immunity may be only partial, but nevertheless it is of vital importance to the host in limiting infection. It is of great interest and importance to determine whether anything resembling the concomitant immunity shown by the rhesus monkey, and described in the next section, exists in man.

### 2. *Acquired immunity in experimental animals*

In contrast to the few direct experiments on acquired resistance in man, there have been numerous studies on a variety of experimental animals. The earlier work has been well reviewed by Stirewalt (1963) and Kagan (1966). It is clear that many experimental host species can develop some degree of resistance against reinfection, although there is considerable variation in the level of immunity and the speed with which it is acquired. Recent work has shown a growing awareness of a need to define the criteria of acquired immunity more clearly. In particular there is now a more general recognition that acquired immunity may act in two phases, against the adult parasites of a primary infection and against the immature stages of a second infection. It is these aspects in particular that will now be examined in several of the more commonly used experimental schistosome hosts.

(a) *Self-cure in experimental animals.* The rhesus monkey, *Macaca mulatta*, is the experimental host which has been investigated most extensively from the standpoint of acquired immunity. The rhesus is very readily infected with several species of schistosomes and allows these infections to mature fully. In a series of infections analysed by Smithers and Terry (1965a), a mean of $49{\cdot}1 \pm 4{\cdot}3\,\%$ of the cercariae of a Puerto Rican strain of *S. mansoni* in the infecting dose matured into adult worms. The female worms produce viable eggs which, in the case of *S. mansoni*, first appear in the faeces about 6 weeks after exposure to cercariae. There seems to be general agreement that in heavily infected animals the egg output thereafter rises rapidly to a peak, 8–12 weeks after infection; there follows a more or less precipitous fall to a low level (Naimark *et al.*, 1960; Meisenhelder and Thompson, 1963; Smithers and

Terry, 1965b; McMullen *et al.*, 1967). This low level of egg production may continue for upwards of a year (Naimark *et al.*, 1960) and is then often terminated. McMullen *et al.* (1967) found that lightly infected monkeys continue egg production at a low level for relatively longer periods.

Most workers believe that the steep fall in egg production is brought about by the immune defences of the host. If this were so, it would parallel the "self-cure" phenomenon often reported in *Nippostronglyus brasiliensis* infections (Mulligan *et al.*, 1965). This seems to be the most likely explanation, but it has by no means been proved. Proof might be obtained by demonstrating prolonged egg output at a high level in monkeys treated by immunosuppressive procedures or with immunosuppressive agents, in the same way as has been done with *N. brasiliensis* (Ogilvie, 1965; Ogilvie and Jones, 1967). Bruce *et al.* (1966) and Cheevers and Powers (in preparation) have reported that splenectomy of rhesus monkeys results in the production of rather more eggs for a somewhat longer period, but other and more convincing demonstrations are required before an immunological explanation of this fall in egg output can be accepted unreservedly.

The innate resistance of the laboratory rat to infection with *S. mansoni* has already been considered. A markedly lower percentage of the infecting cercariae develops into young adults, than in many other laboratory hosts, and the worms never mature fully. In addition, the majority of the worms which do reach the liver are eliminated between the 4th and the 8th week (Ritchie *et al.*, 1963). Smithers and Terry (1965c) have shown that this elimination takes place in two phases. There is a phase of rapid elimination between days 28 and 37 where the biological half-life of the worms is only about 6 days. Following day 37, the rate of elimination is much slower with the half-life of the worms at least 30 days. It is tempting to assume that this elimination of worms is due to an acquired immune response on the part of the host, but this has not been proven. Alternative explanations are available; the rapid elimination could be due to a failure of this rather unsuitable host to supply an essential nutrient or to cater for some other physiological need of the parasite.

Egg production following initial infection in mice has been intensively studied by Kloetzel (1967b), who found that faecal egg output reached an early peak on day 60 and thereafter declined rapidly. However, he was able to show that there was a steady rise in the number of eggs retained in the gut and liver, and he calculated that oviposition is fairly constant during the first 100 days, so that the sharp fall in the faecal egg count after day 60 must have some other explanation. Kloetzel suggested that it is due to a host immune mechanism directed against the enzymes of the eggs, preventing eggs from escaping into the lumen of the gut. Further work is required before the existence of this immunological mechanism can be admitted. The effect of cortisone on *S. mansoni* infections in mice has been studied by Coker (1957) and Weinmann and Hunter (1959): these workers agree that this treatment tends to decrease rather than increase the worm burden in mice. It is suggested that cortisone may have an unfavourable direct effect on the penetration of the cercariae (Lewert and Mandlowitz, 1963).

Other experimental hosts have been less studied. The primates such as *Papio*

*hamadryas* (see Newsome, 1956), *Cercopithecus aethiops* (see Meisenhelder and Thompson, 1963; Ritchie *et al.*, 1964; Jordan and Goatly, 1966) and the chimpanzee (see Sadun *et al.*, 1966b), do not show the dramatic fall in egg output that is characteristic of *S. mansoni* infections in the rhesus. In this, they more nearly resemble man where egg output may continue for years following a primary infection. On the other hand, primary infections in guinea-pigs and rabbits are terminated fairly quickly although more moderately than in the rat (Warren and Peters, 1967).

In summary, primary schistosome infections are terminated at widely different rates in different host species, ranging from the rat which loses the majority of its worms after 4 or 5 weeks to some primates where a large element of an initial infection may persist for some years. Most workers have assumed that the termination of a primary infection is brought about by the immune mechanisms of the host, but much further work is needed before this is established conclusively. It appears to us that this particular aspect of immunity to schistosomes requires more critical investigation than it has received hitherto.

(b) *Resistance to reinfection in experimental animals.* The phenomenon of immunity to reinfection in experimental hosts is in some respects a separate problem. Again, this has been studied most intensively in the rhesus monkey. Vogel and Minning (1953) and Naimark *et al.* (1960) demonstrated that the rhesus became completely resistant to reinfection after one previous infection. Their work suggested, however, that this resistance developed slowly, taking more than one year to become complete. In contrast, Smithers and Terry (1965b) reported that eight rhesus monkeys, initially exposed to 100–1600 *S. mansoni* cercariae, all completely resisted challenge with 2000 cercariae 16 or 21 weeks later. A partial resistance to challenge was demonstrated even when the interval between primary infection and challenge was only 2 or 4 weeks. Naimark *et al.* (1960) and Ritchie *et al.* (1966) suggested that acquisition of resistance was delayed when the primary infections were light; but Smithers and Terry (1967) found that as few as 25 cercariae in the initial exposure afforded protection against a challenge of 1800 cercariae 17 weeks later, a challenge which proved lethal to the control monkeys. Smithers and Terry (1969) summarize their findings in this field as follows: "We now feel confident that, with our strain of *S. mansoni*, most monkeys will develop solid immunity if exposed to about 100 cercariae and challenged 16 weeks afterwards. If either the number of cercariae or the time interval is reduced we cannot predict the outcome".

Both groups of workers agree, then, that the rhesus does develop complete immunity to reinfection; they differ on the time taken for this immunity to develop. The discrepancies in the two sets of results cannot be explained fully at present, but different criteria in judging immunity (Smithers and Terry, 1967) and differences in strains of both host and parasite (McMullen *et al.*, 1967) may be involved.

All agree, however, on one point of the greatest practical and theoretical importance. In most rhesus monkeys resistant to challenge with *S. mansoni*, egg production from established adult worms persists during and after the

destruction of the challenge infection. Clearly, whatever the nature of the immune response which prevents the schistosomula of the challenge from maturing, this response does not at the same time necessarily destroy established adult worms, or prevent them from producing eggs. The existence of a state of immunity in the presence of the infectious agent is usually referred to as premunition. Smithers and Terry (1969), in discussing this phenomenon, suggest it may be more profitable to consider this immunity in the presence of living worms as an example of concomitant immunity rather than premunition. The term concomitant immunity was invented by Bashford *et al.* (1908) to describe a phenomenon of tumour transplantation and was discussed recently by Gershon *et al.* (1967). Animals bearing one tumour were sometimes resistant to a second graft of the same tumour; the new tumour was not accepted even though the first tumour continued to grow progressively. By analogy, invading schistosomula are destroyed by the immune response of the host, while the adult worms are unaffected.

Regarding the explanation of this phenomenon, Hsü and Hsü (1963) have suggested the existence of two types of immunity, one directed against the schistosomula and one directed against the adult worms. This idea was supported by Smithers and Terry (1965b) and McMullen *et al.* (1967), but it has had to be reappraised since the findings of Smithers and Terry (1967) that, in the *S. mansoni*-rhesus system, the adult worm provides the major stimulus to immunity. This finding will be discussed in more detail in a later section, but it will be appreciated that a strange situation arises with regard to hypotheses of schistosome immunity. If the adult worms stimulate an immunity which destroys schistosomula, then these stages must share antigens. These antigens in the adult worm stimulate the immunity, and in the schistosomula act as the targets for the response. How, then, does the adult worm evade the immune response which it itself engenders? A possible mechanism is discussed in the later section dealing with host antigens in schistosomiasis.

Whatever the mechanism of concomitant immunity, we are attracted to the idea that something like it exists in man. Could it be that many children in endemic areas, although excreting eggs from established adult worms, are at the same time resisting reinfection at least partially? It would explain the long persistence of infections with the relative rarity of the intolerable worm burden which might be expected in those continually exposed to infection.

The acquisition of immunity to reinfection with schistosomes is less well marked in other experimental hosts than it is in the rhesus monkey. Nevertheless, among primates it has been reported to occur against *S. mansoni* in *Pithecus mordax* (Meleney and Moore, 1954) and in the baboons *Papio hamadryas* (Newsome, 1956) and *Papio anubis* (McMahon, 1967); and it occurs against *S. japonicum* in *Macaca irus* (Sadun and Lin, 1959). Acquired resistance against *S. japonicum* has also been reported to occur in mice and hamsters (Lin *et al.*, 1954; Sadun *et al.*, 1961) and in rabbits (Sadun and Lin, 1959). More variable results have been encountered with *S. mansoni* in mice and hamsters, but positive findings based on various criteria have been reported by Stirewalt (1953), Olivier and Schneidermann (1953), Thompson (1954), Lurie and De Meillon (1957), Ritchie *et al.* (1962), Hunter *et al.* (1962)

and Frick *et al.* (1965). In these reports, however, there is a disagreement on the level of resistance attained and on the number of cercariae and exposures necessary to induce resistance. Stirewalt (1963) has appealed for greater standardization of experimentation in this work and also for an extension of the criteria examined in the search for evidence of acquired resistance. We would echo this appeal!

### B. THE STAGES OF THE PARASITE WHICH STIMULATE ACQUIRED IMMUNITY

Accepting that some species of animals certainly, and man almost certainly, acquire immunity to reinfection with schistosomes, it is pertinent to enquire into the nature of the stimulus that provokes this immunity. Progress in this field has been disappointingly slow, mainly because of a general failure to provoke immunity by means of non-living homogenates and secretions of the parasite. This will be discussed in more detail in a later section, but it may be appreciated that, because of this failure, attempts to isolate the functional antigens responsible for resistance have been delayed; studies on resistance have perforce had to be carried out with the living parasite.

All stages in the life cycle of schistosomes which occur in the vertebrate host, schistosomulum, adult worm and egg, are exposed to recognition by host leucocytes as foreign tissue. Thus, all stages are potentially immunogenic. Most workers, however, have been attracted to the idea that one or other of these stages provides a greater stimulus to immunity than the others; eggs, schistosomula and adult worms have all been put forward as candidates for this role.

#### 1. *Eggs*

The most convincing demonstration that schistosome eggs supply a major stimulus to immunity was provided by Kagan (1952), working with *S. douthitti.* Kagan found that infection of mice with worms of both sexes conferred a substantial protection against reinfection. This was also true of infections with female worms only (the females of *S. douthitti* produce eggs parthenogenetically) but no resistance was conferred by an infection of male worms only. The conclusion drawn was that the egg is a necessary stimulus for the development of acquired immunity in this host-parasite system. Crandall and Hunter (1961) and Hunter *et al.* (1962) studied the time taken for immunity against *S. mansoni* to develop in the mouse. Their results led them to conclude that substantial egg production by the worms is necessary before this is achieved.

There is also indirect evidence that eggs are involved in the immune response. The increase in immunoglobulin level which occurs in infected animals only becomes apparent at the onset of egg production (Evans and Stirewalt, 1957; Sadun and Walton, 1958; Smithers and Walker, 1961). The appearance of various precipitating antibodies also coincides with the beginning of egg deposition (Senterfit, 1958). More recently it has been shown that

reagin-like antibodies do not appear in the blood of monkeys until this time. (Ogilvie *et al.*, 1966; Edwards *et al.*, 1967.)

There is, however, even stronger evidence that eggs are not essential to the development of acquired immunity against the major schistosome species. Unisexual infections of *S. mansoni* and *S. japonicum* do not lead to egg production. Nevertheless Vogel and Minning (1953) and Vogel (1958) found that rhesus monkeys became partially and in some cases completely immune to challenge following infection with cercariae giving rise to male worms only. Smithers (1962a) demonstrated a similar resistance following infection with either male worms only or female worms only. Olivier and Schneidermann (1953) and Lin *et al.* (1954) reported similar findings in mice following unisexual infections of *S. mansoni* and *S. japonicum* respectively.

More recently, Smithers and Terry (unpublished work) transferred either all-male or all-female worms directly into the portal systems of normal monkeys. These monkeys showed a high degree of resistance to a subsequent cercarial challenge, almost as great as in monkeys receiving worms of both sexes. Smithers and Terry (1967) also transferred worms which had been cut in two, by this procedure of portal inoculation. The anterior ends of these worms survived for several weeks but the females produced no eggs. Nevertheless, those monkeys which received the "half-worms" showed resistance to subsequent cercarial challenge.

Further evidence against the role of eggs in inducing resistance has been obtained from experiments with irradiated cercariae. These cercariae do not give rise to mature egg-producing worms and yet, as described in the next section, they are able to induce a degree of immunity against infection with normal cercariae.

Finally, the injection of viable eggs into the circulation has failed to stimulate resistance to *S. mansoni* in mice (Lichtenberg *et al.*, 1963; Moore *et al.*, 1963) and in monkeys (Smithers, 1962b). This was found to be true even when as many as half a million eggs were injected directly into the mesenteric veins of rhesus monkeys (Smithers and Terry, 1967).

In summary, it seems that eggs provide a considerable antigenic stimulus to the host, leading to the production of circulating antibodies, and possibly a condition of delayed hypersensitivity (Lichtenberg *et al.*, 1962; Warren *et al.*, 1967). Nevertheless, the balance of evidence favours the view that, with the possible exception of *S. douthitti*, the presence of eggs is not essential for the development of acquired resistance to schistosomes.

### 2. *The schistosomulum*

The finding that eggs are not essential to the acquisition of immunity to schistosomes is encouraging from the standpoint of possible vaccination. It is the eggs that cause the major pathological lesions in schistosomiasis; if they are not essential to immunity, it is theoretically possible to develop a non-pathogenic living vaccine against this parasite. Workers in this field have felt that the right way to achieve this is through the agency of the immature migrating schistosomulum. During its migration from the skin to the liver,

this form comes into intimate contact with a variety of host tissues and certainly provides the first antigenic stimulus to the host. In order to minimize damage to the host it is essential that the schistosomulum shall not develop into a mature adult, producing large numbers of pathogenic eggs. This has been achieved in two ways. Some workers have employed strains or species of schistosomes which are not pathogenic for man but which stimulate a degree of cross-immunity to the pathogenic species. Others have used pathogenic species but have prevented the schistosomula from maturing by exposing them to irradiation. It must be admitted that, although both approaches have met with a degree of success in experimental systems, neither has proved sufficiently potent or reliable for use in man.

Immunization with strains of bacteria and viruses non-pathogenic for man has proved very valuable in immuno-prophylaxis. The discovery of a Formosan strain of *S. japonicum* which did not develop into the adult stage in man (Hsü and Hsü, 1956) was accordingly of great interest. It was later shown that this zoophilic strain did not reach maturity in the rhesus monkey (Hsü and Hsü, 1960a), so that immunizing experiments could be carried out in this experimental host. Histopathological studies (Hsü *et al.*, 1962a) demonstrated that, in a primary infection with this strain, most schistosomula were able to reach the liver and were destroyed there; very few schistosomula were destroyed in the skin and lungs. With repeated attempts at infection with this strain, there was an intensified tissue reaction in the skin and lungs, which resulted in more schistosomula being destroyed in these organs (Hsü *et al.*, 1965a). Hsü and Hsü (1961, 1963) investigated the protective effect of the Formosan strain in rhesus monkeys against a Japanese strain of *S. japonicum*, pathogenic to rhesus monkeys and man. The level of immunity was judged by comparing the egg output in the immunized monkeys with that of the control monkeys after challenge. Measured in this way, the effect of immunization was quite good with two immunizing inoculations and still better with three or four inoculations. Further inoculations did not bring any additional improvement. The acquired immunity was shown to persist for at least 717 days.

Over a period of years considerable evidence has been gathered to show that cross-immunity exists between different species of schistosomes. That most extensively studied has been the relationship between the parasite of cattle, *S. bovis*, and the parasites of man, *S. haematobium* and *S. mansoni*. In Sardinia, Corsica and Sicily, where *S. bovis* is endemic, infections of *S. haematobium* in man have never been observed, even though a suitable vector for this parasite is present on the islands. Le Roux (1961) put forward the hypothesis, based on these findings, that the cercariae of *S. bovis* may immunize man against *S. haematobium*. This idea gained some support from studies carried out in East Africa. Nelson *et al.* (1962) suggested that constant exposure to *S. bovis* cercariae may modify the course of *S. haematobium* in man and may be helpful in avoiding complications such as cancer of the bladder. Webbe and Jordan (1966) disagree with these opinions, however; in the area in which they were working they could find no evidence of cross-immunity between these species and concluded that any immunizing effect of animal schistosomes must be minimal.

More recently, direct experimental evidence of cross-immunity has been obtained. Since Brumpt (1936) showed that the cercariae of *S. bovis* could not develop into adult worms in the rhesus monkey, and since *S. haematobium* and *S. mansoni* will develop to maturity in this animal, the rhesus has been the host of choice. Hsü *et al.* (1966) immunized six monkeys with three to six inoculations of *S. bovis*; the total number of *S. bovis* cercariae employed was 13 000–19 500. Eggs were not found in the stools during the immunization procedure, which lasted 333–575 days. These monkeys, together with three control monkeys, were then challenged, each with 1000 cercariae of *S. haematobium*. At post mortem 127–148 days after challenge, 231–484 adult *S. haematobium* worms were found in the controls, and 11–53 worms in the immunized monkeys. Evidently, the exposure to *S. bovis* cercariae had induced a considerable protection against infection with *S. haematobium*. Nelson *et al.* (1967) claimed that a single previous exposure to *S. bovis* results in a marked decrease in the expected number of *S. mansoni* eggs deposited in the tissues of mice and monkeys subsequently challenged with cercariae of this species. They also reported heterologous immunity between *S. rodhaini* and *S. mattheei* and *S. mansoni*. Hsü *et al.* (1964) have also shown that the inoculation of rhesus monkeys with the cercariae of *S. douthitti* produces a partial protective effect against *S. japonicum* but this is not as marked as in the case of *S. bovis* and *S. haematobium*.

The immunizing effect of irradiated cercariae has been studied for both *S. mansoni* and *S. japonicum* in a variety of experimental hosts. There is general agreement that doses of irradiation greater than about 2000 r prevent the majority of the worms from developing into mature egg-laying adults. Villella and Weinbren (1965) reported that gamma radiation from Cobalt-60 in the range 2000–2500 had produced the following general changes in *S. mansoni*: a decrease in body length, parenchymal vacuolation, cuticular swelling and malformation of the reproductive structures, and the adults mainly sterile. Hsü *et al.* (1963a) found that when the cercariae were exposed to 1700–6000 r, the resulting schistosomula were destroyed mainly in the liver. As the dose of irradiation was increased, eventually to 48 000 r, the site of destruction of the schistosomula moved back along the migration pathway to the lungs and the skin. Hsü *et al.* see an analogy with the destruction of normal cercariae in an immune host. Lichtenberg and Sadun (1963) obtained substantially similar results with irradiated *S. mansoni* in mice and were also impressed by the way in which host tissue reactions to irradiated cercariae resembled those observed in the attempted reinfection of an immune host.

Agreement is less general on the immunogenic effect of these irradiated cercariae. This is in part due to different groups of workers studying different parasites in different hosts. The most encouraging results have been obtained with *S. japonicum* in the rhesus monkey (Hsü *et al.*, 1962b; 1963b; 1965b). Monkeys immunized with three or four inoculations of cercariae irradiated at 1700–3000 r and subsequently challenged with normal cercariae, showed a powerful immunity as judged by egg output and worm burdens. Hsü *et al.* (1965b) suggest that a period of about 6 months is necessary, between the last immunizing inoculation and challenge, in order to obtain the highest degree of

immunity in this system. In contrast with their results in the rhesus, Hsü *et al.* (1965c) were unable to demonstrate that two exposures to irradiated cercariae of *S. japonicum* induced any immunity in albino mice.

The situation with regard to the stimulation of immunity in mice by means of irradiated cercariae of *S. mansoni* is confused. For example, Villella *et al.* (1961), Erickson and Caldwell (1965) and Radke and Sadun (1963) all agree that exposure to irradiated cercariae induced resistance in mice. The first two groups find, however, that the mice show a better degree of immunity with repeated exposures, whereas Radke and Sadun reported a strong resistance whether there was one or three exposures, or whether as few as 200 or as many as 15 000 cercariae were used in the immunizing infection. Some writers do not agree that exposure to irradiated cercariae induces a true immunity in mice (Perlowagora-Szumlewicz and Olivier, 1963; and Perlowagora-Szumlewicz, 1964a). Their findings suggest that the principal effect of irradiated cercariae is to slow down the migration and development of the worms of a challenge infection, but these worms nevertheless mature eventually. Although previous exposure to irradiated cercariae does not protect the actively immunized mouse, it nevertheless stimulates humoral antibody which does have a protective effect on passive transfer to other mice (Perlowagora-Szumlewicz, 1964b). It is difficult to draw any really firm conclusions from all this work in mice, except that the mouse is probably an unsatisfactory experimental host for this type of investigation.

The situation is clearer in the rhesus monkey. Smithers (1962c) was able to stimulate immunity to *S. mansoni* in the rhesus monkey but found that repeated exposure to large numbers of cercariae was necessary to achieve this end. Sadun *et al.* (1964) obtained substantially similar results; a particularly significant finding was that whereas five exposures, each to 5000 irradiated cercariae, stimulated a useful degree of immunity, a single exposure to 25 000 irradiated cercariae did not.

Smithers and Terry (1965c) and Erickson and Caldwell (1965) have demonstrated that irradiated cercariae stimulate a useful degree of resistance in the rat. Erickson and Caldwell claimed that irradiated cercariae are superior to normal cercariae in this respect. If these results are recalculated according to the method of Smithers and Terry (1965c), however, there is no difference in the immunogenicity of irradiated and normal cercariae. It is doubtful if experiments in this rather unsuitable host have much relevance to what occurs in more susceptible hosts, including man.

In summary, good immunity to reinfection with schistosomes has been induced by non-pathogenic strains and species and by irradiated cercariae. This has been particularly striking with *S. japonicum* in the rhesus monkey. In neither procedure do mature worms result from the immunizing exposures; it seems reasonable to assume, therefore, that the schistosomulum is of paramount importance in stimulating immunity. Whilst admitting that these procedures can be made to be effective, Smithers and Terry (1965b) drew attention to the vastly greater immunogenicity of normal cercariae of *S. mansoni* in the rhesus monkey. Thus a single exposure to as few as 100 or 200 normal cercariae stimulates a powerful and almost complete immunity; a

single exposure to 20 000 cercariae irradiated at 2000 r induced only a 30% resistance as judged by worm burden. They concluded that, at least in this host-parasite system, something more than a short-lived schistosomulum is required in order to produce a rapid and highly effective resistance.

### 3. *The adult worm*

Smithers and Terry (1965b) considered the adult *S. mansoni* as a major stimulus to immunity in the rhesus monkey. In order to confirm this, monkeys had to be exposed to adult worms, but not at the same time to cercariae or migrating schistosomula. This was achieved by directly transferring established adult worms from donor animals by means of a cannula, into the portal system of normal monkeys (Smithers and Terry, 1967).

Five out of 11 monkeys treated in this way, with about 80 pairs of transferred worms, were almost completely resistant to challenge with 2000 cercariae, 8–14 weeks later. This challenge proved lethal for the majority of the control monkeys. None of the remaining immunized monkeys suffered as severely as the controls; they showed reduced worm burdens and egg output and none became ill or lost weight as did the control monkeys. Worms from monkey, hamster and mouse donors all induced resistance. There was no correlation between the egg output of the transferred worms and the degree of resistance induced. As stated earlier, worms cut in two immediately before transfer induced resistance. Worms killed by snap freezing immediately before transfer did not induce immunity and neither did large numbers of viable eggs injected into the portal system. It was concluded from these results that, at least in the *S. mansoni*–rhesus monkey system, the major stimulus to immunity is from antigens associated with the living adult worm, but not with egg production. It seems that the schistosomulum provides a much poorer stimulus but that if this is prolonged, as in multiple exposures to large numbers of irradiated cercariae, then a fairly high grade immunity may be induced.

It is important that attempts should be made to confirm these findings on the importance of the adult worm and in particular in the *S. japonicum*–rhesus monkey system. It should be noted that the findings will not apply, however, to all host–parasite systems. For example, it is known that irradiated cercariae are as immunogenic as normal cercariae in the rat (Smithers and Terry, 1965c). This is to be expected because even normal cercariae will not mature fully in this rather unsatisfactory host and the migrating schistosomulum must therefore act as the major stimulus for any acquired immunity.

## C. ANTIGENS

The study of schistosome antigens has usually been undertaken with one of three long-term aims in mind. One type of study has been aimed at isolating and characterizing those antigens which invoke acquired immunity in the vertebrate host, another at isolating and characterizing antigens which would yield unequivocal results in immunodiagnosis, and a few studies on the biological role of schistosome antigens have attempted to reveal the molecular architecture of the parasite. This has stimulated research on schistosome

antigens and many studies have been reported. Because of the different aims of the investigators and widely different techniques used, these researches have produced a heterogeneous assemblage of factual results. The only clear finding is that schistosomes are antigenically very complex! A greater collaborative effort is needed by laboratories engaged in this work, and an agreed nomenclature for schistosome antigens based on their physicochemical behaviour. This would help progress and ease the task of future reviewers. At present the subject is difficult to review in any logical order, but we shall consider some of the techniques used in the antigenic analysis and the results obtained, and then discuss how far the aims noted above have been met.

The techniques for separation and analysis of schistosome antigens have been derived largely from those found useful in other systems, and both chemical and immunochemical methods have been employed. In particular, the antigenic components active in the complement fixation test and in the intradermal test for schistosomiasis have been extensively studied by chemical means. Sleeman (1960) obtained an active antigen by extracting adult worms with sodium desoxycholate followed by ethanol fractionation. The resulting antigen was free from carbohydrate and nucleic acids, and contained protein and lipid. Rieber *et al.* (1961) fractionated adult worms into lipid, carbohydrate and protein moieties. The active antigen was contained in an acid-insoluble protein fraction which (using the techniques of the time) was electrophoretically homogeneous. Pellegrino *et al.* (1956) concluded that chemical components other than carbohydrates were important in the diagnostic skin test; Kagan and Goodchild (1963) studied a series of antigens active in the intradermal test and found that the carbohydrate content did not correlate with activity. Gazzinelli *et al.* (1965) fractionated cercarial extract on DEAE-Sephadex A-50, and found that the fraction most active in the intradermal skin test was free from polysaccharide. Edwards (personal communication) has attempted to isolate allergens which react with the reaginic antibodies induced in rhesus monkeys by schistosome infections. Using gel filtration on Sephadex, he obtained two active fractions from cercarial extract; one has a molecular weight of about 10000 and the other, which may be a complex of the first, is considerably larger. Only the larger fraction has been isolated from adult worm extract. Both fractions, which have a significant carbohydrate content, are far from homogeneous, and electrophoresis in polyacrylamide gel and electrofocusing (Vesterberg and Svensson, 1966) are being carried out on these antigens in an attempt to purify them further.

Other chemical separations of Smithers and Williamson (1961) and Williamson *et al.* (1965) were of an antigenic polysaccharide extracted from eggs and cercariae of *S. mansoni*. Extensive analysis indicated that this was a glucan polysaccharide with glycogen-like properties. Kronman (1965) subjected cercarial extract to gradient elution chromatography on DEAE cellulose. Nine protein antigens were found, some of them glycoproteins and lipoproteins. Sodeman (1967) applied disc electrophoresis in polyacrylamide gel to the separation of schistosome components, a sensitive technique which demonstrated 22 protein components; differential staining showed that nine of the components contained lipid, three polysaccharide and ten DNA.

The use of immunochemical techniques, especially immunodiffusion in agar gel and immunoelectrophoresis, has revealed the great complexity of the antigenic structure of schistosomes. As techniques for separation improve so the apparent complexity increases, and a few recent analyses reviewed here must serve as examples. Using water soluble extracts, and by immunoelectrophoresis, N. H. Kent (1963) detected ten protein systems in the adult and eight in the cercariae. One antigen cross-reacted with *Trichinella spiralis*. Kagan and Norman (1963) demonstrated 25 antigenic components by immunodiffusion, some shared and some specific to the various stages. By immunoelectrophoresis they demonstrated the heterogeneity of earlier "antigens", an extract prepared according to the method of Melcher (1943) containing 11 components. Perhaps the most intensive and successful researches are those summarized by Capron *et al.* (1965b). Using prolonged immunization procedures in order to obtain powerful anti-sera, they were able to demonstrate 21 antigens in extracts of *S. mansoni* adults; 11 were shared by adult and egg, 14 by adult and cercariae, and 12 by adult and the excretions and secretions of the worm. Of these 21 antigens, 19 were common to *S. haematobium* and ten to *S. japonicum*. Various glycoproteins and lipoproteins were identified by immunochemical means. One very interesting finding was the detection of a specific and major antigenic fraction very early in the course of immunization. Antigens common to the parasite and its vertebrate or invertebrate host were also demonstrated; a similar finding was that of Damian (1967). The significance of the presence of these host antigens is such that we have devoted the whole of a later section to its consideration. The use of radio-labelled antigens in immunodiffusion studies (Dusanic and Lewert, 1966) may prove helpful in unravelling the complexities of these antigens.

With this impression of the techniques employed and results obtained in the study of schistosome antigens, we can enquire how far the objectives have been met. As regards the first objective, that of isolating and characterizing those antigens which evoke acquired immunity, we must confess to failure. There is, however, a good reason for this. The isolation and characterization of any molecule showing biological activity, be it antigen, enzyme or hormone, usually follow a common pathway. Activity is first detected in the crude extract which is then fractionated and the fractions purified more and more. At each step the fractions are tested to determine if the activity has been retained; an increase in specific activity at each stage indicates that the procedure is successful. In the case of schistosome antigens (and indeed in most helminth antigens) this process is blocked at the first stage, as it has generally proved impossible to evoke an unequivocal immunity to infection by the injection of extracts of schistosome homogenates.

Early work (Ozawa, 1930; Kawamura, 1932) indicated that it was possible to vaccinate dogs and rabbits against *S. japonicum* by injections of dead adults and cercariae, but Vogel and Minning (1953) failed to protect rhesus monkeys against this parasite by injecting homogenized adult worms over a long period of time. Lin *et al.* (1954) were successful in partially protecting mice against *S. japonicum* using a whole worm antigen, as were Sadun and Lin (1959); however, cercarial antigen did not induce protection.

Watts (1949) detected a slight protection against *S. mansoni* in mice by injection of adult worm extract, and prolongation of life was observed by Levine and Kagan (1960) in mice which had been injected with antigen from the fluid in which cercariae and adults of *S. mansoni* had been incubated. By contrast, Thompson (1954), Ritchie *et al.* (1962), Moore *et al.* (1963) and Sadun (1963) all failed to induce protection against *S. mansoni* using extracts of eggs, cercariae and adult worms. Ritchie *et al.* (1962) injected cercarial antigens, adult worm antigens and egg antigens in sequence, so as to expose the animal to the whole range of antigens encountered during an infection. Sadun and Bruce (1964) had some success with whole worm homogenates of *S. mansoni* in rats, rather unnatural hosts, and in any case animals immunized with bovine serum albumin were also protected, so protection probably had a non-specific basis. In the more suitable host, the rhesus monkey, protection was not induced by cercarial, egg and adult worm antigens (Smithers, 1962b). Smithers and Terry (1967) showed that whereas living worms injected directly into the portal system of monkeys induced a protective immunity, worms killed by snap-freezing immediately before injection did not; denaturation of the antigens of these killed worms must have been minimal.

This failure to induce more than marginal protective immunity with non-living material is not peculiar to schistosomes; it is true of helminths in general (Terry, 1968). It does, however, seriously compromise the search for the functional antigens, which surely must exist and induce protective immunity. It will take great courage and resources for any group to search among the 20–30 antigens identified already in schistosomes for perhaps the one antigen which is important, when the starting material is so uncertain in its effects. Great resources, because besides the biochemical and immunological expertise required, the work will probably have to be carried out in rhesus monkeys where there is unequivocal acquired immunity. Great courage, because failure may result. There is perhaps a case here for collaboration between several laboratories.

The situation is better in regard to immunodiagnosis, and the problems in the field have been well defined by Kagan and Pellegrino (1961) and Haley *et al.* (1963). The antigenic complexity of crude extracts of parasites is now well established and the degree of cross-reaction with other helminth parasites has been revealed. The results summarized by Capron *et al.* (1968) suggest that it should not prove too difficult to distinguish schistosome infections from other helminth diseases by immunodiagnosis. Schistosomes share relatively few antigens with other helminths, and it should be possible to discard these whilst retaining antigens of greater specificity. Distinguishing between the various species of schistosomes will be more difficult; there is extensive sharing of antigens, particularly between *S. mansoni* and *S. haematobium*. Nevertheless, the existence of "personalized" antigens gives some encouragement, but whether or not these can be isolated in pure form remains to be seen. At least, the assay of antigens for immunodiagnosis is much swifter than for protective immunity.

Immunological and immunochemical techniques have been applied intensively in mammalian and bacterial biochemistry and physiology, yielding

much useful information on the nature, occurrence and synthesis of biologically active macromolecules. An idea of the interest and activity in this field may be obtained from Cinader (1967). Unfortunately, studies of this type on schistosomes, or on helminths generally, are little developed, although there is information on which antigens are shared by the various stages in the life cycle of the parasite and which are stage specific, but little idea of the biological role of these antigens. Tran van Ky *et al.* (1967) have provided elegant demonstrations of enzymatic activity in extracts of *S. mansoni* separated into antigenic components by immunoelectrophoresis, showing by this technique 16 antigen–antibody complexes possessing different enzymatic specificities including four different dehydrogenases.

The aim must be to locate all these antigens within the living worm, a difficult task requiring the raising of specific antisera against the separate antigens obtained by fractionation of the homogenates. This work will be tedious in the initial stages, but once specific antisera have been raised rich rewards may accrue. Immunofluorescence and immunological markers for electron microscopy may then be used to analyse the cellular or sub-cellular localization and synthesis of the biologically active macromolecules of the parasite. Such studies, combined with the sub-cellular fractionation of worm homogenates (Smithers *et al.*, 1965), might also lead to the identification of the functional antigens important in protective immunity, more rapidly than could other more empirical studies.

Some success in locating the position of antigens within the parasite has already been achieved. The demonstration of "host" antigens in schistosomes was important, and as antisera specific for these are easily raised, their situation in the worm has been defined, as later discussion shows.

This work may be hampered by the want of an agreed nomenclature for schistosome antigens, but once this has been gained and it is standard practice for the various laboratories engaged in this work to exchange specific antigens and antisera, rapid progress may be made.

## D. ANTIBODIES

### 1. *The nature of the antibodies found in schistosomiasis*

Separating a section on antibodies associated with schistosome infections from one on antigens may seem to be an artificial procedure. Besides a certain circularity in the definition of these two types of active molecule, one is usually required in order to demonstrate the presence of the other; for each antigen there is at least one antibody. Nevertheless, the subject is complex and there may be greater clarity if antibodies are considered separately, especially when, as in this section, antibodies are considered largely in relation to their reactions with the living parasite.

A raised immunoglobulin level is characteristic of schistosome infections in man and experimental animals. Good evidence suggests that much of this increase may be due to non-specific stimulation and tissue damage by the parasite. Accordingly, the general rise in immunoglobulins is discussed in Section IV.

Many techniques have been derived for detecting antibodies specifically directed against schistosome antigens. Soluble antigens have been used to detect antibodies active in intradermal tests, precipitation reactions of various kinds, complement fixation reactions, and the agglutination and flocculation of antigen-sensitized cholesterol-lecithin complexes, bentonite, latex and charcoal particles and erythrocytes. The detection of these antibodies was carried out in the interests of immunodiagnosis and the whole subject has been reviewed by Kagan and Pellegrino (1961) and by J. F. Kent (1963).

Antibodies also react with whole parasites and various workers have shown that antibodies take part in the cercarienhüllenreaktion (CHR), the cercarial agglutination (CA), the miracidial immobilization (MI), the circumoval precipitin (COP), and fluorescent antibody (FA) reactions. Again, the stimulus to effort has been immunodiagnosis and these reactions were reviewed by Anderson (1963), who listed the original references.

Attempts have been made to characterize antibodies in terms of their physicochemical behaviour, particularly with regard to their electrophoretic mobility (Stirewalt and Evans, 1955; Evans *et al.*, 1955; Evans and Stirewalt, 1958, 1959; Cancio *et al.*, 1959; Lee and Lewert, 1960). Unfortunately most of this work was carried out before the essential unity of the immunoglobulins was appreciated and their main classes defined (W.H.O., 1964, 1966, 1968). More recently, it has been shown that the COP antibody is probably an IgG (Toro-Goyco, 1964); the antibody which takes part in the fluorescent antibody test with cercariae is probably an IgM (Del Rey Calero *et al.*, 1967); and the reaginic antibody active in the intradermal test may well be an IgE (Edwards *et al.*, 1967). More studies of this type are required, particularly on the serum antibodies of infected patients. Useful reagents in the shape of anti-sera specific to human immunoglobulin classes are now available and their use might provide much valuable information.

Most of these antibodies appear in quantity in the serum of infected hosts at the time the parasite matures and eggs are deposited in the tissues (Naimark *et al.*, 1957; Smithers, 1960; Sadun *et al.*, 1965). There seems to be a sudden increase in the antigen liberated by the parasite at this stage. Other antibodies, involved for example in the fluorescent antibody test, can be detected earlier (Jaimes and Lichtenberg, 1965; Magalhães Filho *et al.*, 1965). This may be related to the earlier appearance of antibodies associated rather with the IgM fraction than with IgG (Del Rey Calero *et al.*, 1967).

Unfortunately, none of these antibodies which were demonstrated in human and experimental schistosomiasis may be concerned with acquired resistance. Thus, the CHR, COP, complement-fixing antibody, fluorescent antibody titres, and the various precipitins show no relation to protection (Vogel and Minning, 1953; Smithers, 1962b; Jachowski *et al.*, 1963). Preliminary investigations a few years ago suggested that reaginic antibodies, those associated with immediate hypersensitivity reactions, might be involved in protective immunity (Ogilvie, 1964; Smithers and Terry, 1964). There was evidence that the production of this class of antibodies was stimulated in monkeys which had experienced an infection and were immune, but not in monkeys which had been vaccinated with worm homogenates and were not immune. There was

also some evidence that causing cercariae to migrate through the skin of rats saturated with reagins, reduced the resulting worm burden. These reaginic antibodies have been extensively investigated in recent years (Ogilvie *et al.*, 1966; Hsü and Hsü, 1966; Sadun *et al.*, 1966b; Edwards *et al.*, 1967; Zwaifler *et al.*, 1967; Schoenbechler and Sadun, 1968). Although there was some passive transfer of resistance to *S. mansoni* in the rat, this could not be detected in the rhesus monkey, a more normal host, either in *S. mansoni* or *S. japonicum* infections. Although reaginic antibodies do not seem to be directly concerned with acquired immunity, their presence still prompts many questions. Why are reagins produced so readily in helminth infections but not in responses to other parasites? Why are they mainly induced only by the living infection and not by the injection of helminth antigens? Is there a link between their presence in helminth infections and also in allergic diseases such as hay-fever or asthma? Have they a significant role in the host-parasite relationship or are they merely the result of a misdirected function of the immunological mechanisms? Answers to these questions would be of great interest to allergists as well as parasitologists.

2. *Passive transfer of immunity to schistosomes*

The role of antibodies in acquired immunity is usually demonstrated by the passive transfer of resistance from a resistant to a susceptible animal by means of injections of serum. Most attempts to achieve this in schistosome infections have failed (Stirewalt and Evans, 1953; Vogel and Minning, 1953; Kagan, 1958; Levine and Kagan, 1960; Meisenhelder *et al.*, 1960; Weinmann and Hunter, 1961). Weinmann (1960) found no significant difference between the susceptibility of neonatal mice born of mothers infected with *S. mansoni* and those born of normal mothers, demonstrating that if there had been any passive transfer of immunity *in utero* or in the mother's milk, then this had no effect on the infection. Hunter and Moore (1964) and Hunter *et al.* (1967) reported that immunity to *S. mansoni* could not be transferred by parabiotic union between infected and normal mice.

There have been a few reports of successful transfer of resistance by means of serum. Sadun and Lin (1959) found a slight but significant resistance to *S. japonicum* in mice injected with immune rabbit serum, and Bruce and Sadun (1964) reported that they had obtained increased resistance to *S. mansoni* in rats by injecting them with serum from monkeys immunized with irradiated cercariae. It will be noted that both these successes were achieved with heterologous sera. Perlowagora-Szumlewicz (1964b) reported some resistance conferred on normal mice receiving serum from donors previously immunized with irradiated cercariae, although these donors could not be considered as truly immune to reinfection. Ogilvie *et al.* (1966) showed a significant protection to *S. mansoni* in rats injected with serum containing reagins, but this serum had to be injected intradermally into the direct path of the invading cercariae; much larger volumes of the same serum failed to confer any protection after intraperitoneal injection. Protection could not be demonstrated in rhesus monkeys by this technique.

These failures and uncertainties in the passive transfer of resistance have led several workers to doubt whether antibodies play any role in immunity to schistosomes. This problem will be discussed fully later but here we can enquire if there is any evidence that schistosomes may be seriously injured by antibodies. The answer to this is a decided yes—but only in a rather artificial situation (see Section III E). Briefly, Smithers *et al.* (1969) immunized monkeys against mouse cells and then transferred adult worms, from mouse donors, directly into the portal systems of these monkeys. The mouse worms were rapidly killed although they survived well in normal monkeys. Their death was due to the reaction of mouse antigens located at the surface of the cuticle with monkey anti-mouse antibodies; the role of these antibodies was unequivocally demonstrated by passive transfer experiments. Electron micrographs of mouse worms salvaged from the circulation of anti-mouse monkeys after various periods of time revealed that the worms were dying because of severe damage to the cuticle, which finally resulted in its complete destruction. We believe the electron micrographs (Fig. 1A–D) to be unique in showing the possibly destructive effect antibodies *can* have on schistosomes.

### E. HOST ANTIGENS IN SCHISTOSOMIASIS

Sprent (1959) suggested that during the evolution of host-parasite relationships a dual modification acting through natural selection resulted in reduced antigenic disparity between the parasite and its "natural" hosts. Dineen (1963a, b) expressed similar views, suggesting that because of this reduced antigenic disparity "natural" hosts will not produce antibodies harmful to the parasites as readily as will unnatural hosts.

The presence of antigens in extracts of homogenized schistosomes, indistinguishable from antigens of the vertebrate host, has been reported by Damian (1962, 1964, 1967) and by Capron *et al.* (1965b). Using immunodiffusion techniques, Damian demonstrated at least four common antigens between schistosome homogenate and the albino mouse serum in which the schistosomes were grown. Admitting the possibility that these antigens may be contaminants derived from the host, Damian favoured the view that they are parasite antigens which through the operation of mutation and selection have come to bear close resemblance to those of the host. These antigens, which will not evoke the production of antibodies by the host, have been termed "eclipse" antigens by Damian, who called the whole process "molecular mimicry".

Capron *et al.* (1965b) used a sensitive immunoelectrophoretic method and identified five antigenic fractions common to *S. mansoni* and the liver cells from the hamster in which these worms were grown; two of these antigens can also be identified in man. They were also able to demonstrate the persistence in the adult worm of antigens which were common to the intermediate host, *Australorbis glabratus*. Capron *et al.* (1968) summarized their work on immunological aspects of host-parasite relations and issued a thoughtful and provocative hypothesis which differs from Damian's views. They dismissed the idea that the antigens may be contaminants perhaps derived from host serum in the parasite's caeca, because of the apparent selection of host

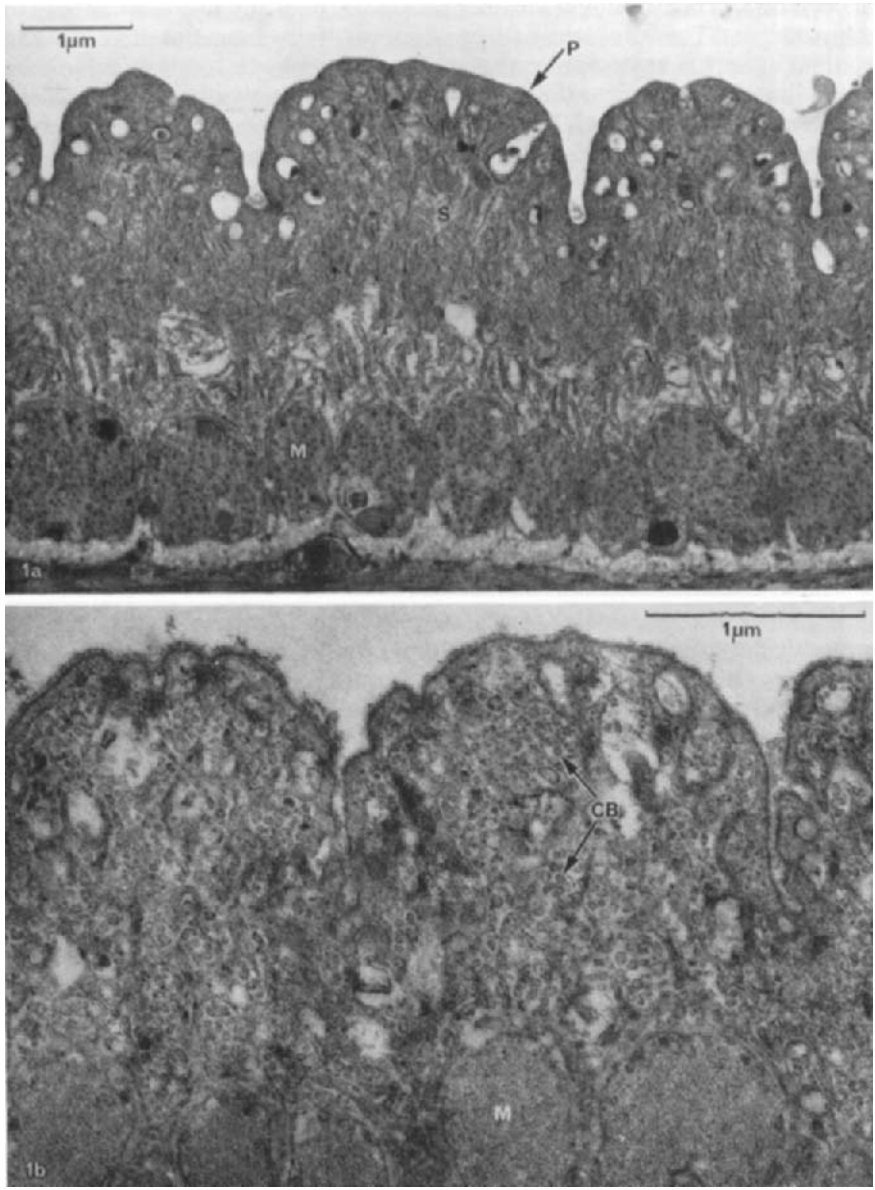


FIG. 1(*a*). Tegument of "mouse worm" recovered 45 h after transfer to a normal monkey. (p) plasma membrane, (s) outer syncytial layer of the tegument, (m) muscles.

FIG. 1(*b*). Tegument of "mouse worm" recovered 2 h after transfer to an "anti-mouse" monkey. The appearance is normal except for the round cytoplasmic bodies (cb) in the syncytium.


FIG. 1(*c*). Tegument of "mouse worm" recovered 6 h after transfer to an "anti-mouse" monkey. The syncytium is heavily vacuolated and is breaking down.

FIG. 1(*d*). Tegument of "mouse worm" recovered 25 h after transfer to an "anti-mouse" monkey. The syncytium has been destroyed exposing the underlying muscles.

Reproduced with permission from Smithers *et al.* (1969).

antigens that would have had to take place. They did not find antigens in their schistosomes which reflect the wide range of antigens present in hamster blood. Only five common antigens have been detected and two of these are common to man; this would be most unlikely with contaminating serum proteins. Their main disagreement with Damian's hypothesis, however, is that they do not accept that these communities of antigens of host and parasite could have arisen solely from pre-existing protein structures accidentally similar in the two partners. They agreed that some accidental "pre-adaptation" of antigens has occurred, but believed that most of the common antigens are synthesized by the parasite as a result of inductive processes. It is assumed that the parasites possess a range of "codes" for synthesizing proteins similar to those of their hosts. Induction occurs when the parasite enters a particular host, presumably as a result of the recognition of some "signal" from the host, and the parasite's cells then synthesize the relevant mimicking proteins. This is a bold hypothesis and the original article should be consulted for detailed supporting evidence. It is also an attractive hypothesis because, from this standpoint, we can explain parasitism as resulting from the possibilities of the inductive process, host specificity as the operation of the process within more or less narrow limits, and host insusceptibility or innate resistance as the parasite's lack of a code suitable for producing proteins which mimic those of the particular host.

The existence of these host antigens has been examined now by entirely different techniques. Smithers and Terry (1967) reported that, although adult worms transferred from both mouse and monkey donors into monkey recipients stimulated immunity to reinfection, the behaviour of the worms from these two hosts was different on transfer. Monkey worms survived the transfer well and continued egg production at about the same rate as they did in the donor host. In contrast, egg production from mouse worms ceased on transfer although the adult worm survived; mouse worms recovered a week or so after transfer were shrunken and the gut caecae were often empty. After 5–6 weeks, however, these mouse worms renewed egg production and this soon reached the same level as that of monkey worms; worms recovered at this time were normal in appearance. Clearly the worms had adapted themselves in some way to the donor host and when the worms were transferred from one species to another, this adaptation was upset, although re-adaptation to the new host could occur. It must be stressed that such adaptation could only be a short-term effect. Six weeks before transfer, all these parasites were in the same intermediate hosts, and before that again, all were in mice in which the strain is maintained by routine.

Believing that host antigens might be involved in this adaptation, Smithers *et al.* (1969) studied the fate of mouse worms transferred to monkeys which had been immunized previously against mouse liver or spleen cells or erythrocytes, combined with Freund's complete adjuvant. No mouse worm was recovered from ten monkeys immunized in this way although recovery of mouse worms from normal monkeys was $84 \pm 9\%$ of the transferred worms. In an experiment designed to determine the fate of the transferred worms, the majority were killed in 7–25 h and all were dead in 44 h. The death of the

worms was due primarily to the action of antibodies, for this immunity to mouse worms could be transferred passively in the serum from "anti-mouse" monkeys to normal monkeys.

These experiments confirmed the presence of mouse antigens associated with adult schistosomes grown in mice. Furthermore, these antigens appeared to be host species-specific, for worms transferred from another rodent, *Meriones libycus*, into anti-mouse monkeys survived normally. More surprisingly, it was found that mouse worms can quickly lose their mouse antigens. When mouse worms were transferred to a normal monkey and then one week later transferred for a second time into an anti-mouse monkey, they survived normally. Three days in a normal monkey was, however, insufficient for all worms to lose their mouse antigens.

These experiments also provided evidence on the location of these host antigens within the worms. Electron micrographs of mouse worms recovered from anti-mouse monkeys at various times after transfer showed that damage was confined to the tegument of the worm (Fig. 1). This suggests that the host antigens, which acted as targets for the antibodies, were also confined to the tegument, and this was confirmed by the ferritin-labelled antibody technique. There was some evidence that these host antigens are not mere contaminants loosely attached to the surface of the worms. The rapid loss of mouse antigens when the worms are transferred to normal monkeys could be looked upon as evidence for the hypothesis of induction of Capron *et al.* Certainly this finding does not support the hypothesis of Damian which would require a time scale of evolutionary magnitude for this to occur. Smithers *et al.*, however, put forward a third hypothesis to explain the presence of these host antigens in schistosomes. This might be termed a "selective contaminative hypothesis", for they suggest that these antigens or substantial fractions of them are in fact synthesized by the host and are then firmly bound to the surface or incorporated into the tegument. They favour this view "only on the grounds that it places less strain on our imagination than the alternative!"

Whatever the origins of these antigens, it is conceivable that their possession is advantageous to the parasite. Adult schistosomes liberate worm antigens into the blood of the host and it is likely that these provoke a state of anti-worm immunity in the host, which, although effective against invading immature forms from reinfections, would be ineffective against adult worms which had succeeded in "disguising" their surface as host tissue. The parasite would not only avoid destruction by the host but, through the agency of the host's immune response, it would create a barrier against continual reinfection that might otherwise lead either to gross overcrowding and stunting of the parasites or to the death of both host and parasites.

The hypothesis has at least the merit of explaining the long persistence of schistosomes in the blood, an immunologically hostile environment. It also affords an explanation for the phenomenon of concomitant immunity, discussed in an earlier section, how the adult worms escape the consequences of the immune response which they themselves are known to provoke. Experiments similar to those reported above should be carried out in other host-parasite relationships.

### F. HOW ACQUIRED IMMUNITY AFFECTS THE PARASITE

Stirewalt (1963) discussed the criteria used by various workers in determining the presence of protective immunity in experimental schistosomiasis. The most widely adopted and important of these criteria are as follows: a reduction in the expected worm burden from a given infective dose; the stunting of worms and their failure to mature; a reduced patent period and a reduced number of eggs deposited in the faeces or tissues; an enhancement of the host cellular reaction to the parasite; a reduced level of disease in the host, and particularly a reduced mortality and an increased survival time compared with control animals.

It is vital in our understanding of immunity to schistosomes that we should be able to explain the effects set out above in terms of the repertoire of immune mechanisms which the host is known to possess. We are far from achieving this aim, but attempts to do so are useful, even if they remind us of our ignorance in this field.

Acquired immunity is generally found to be dependent on specific allergic mechanisms, which have been discussed and summarized by Coombs and Smith (1968) and Coombs (1968), who stated that the repertoire of allergic reactivity is limited; they group allergic reactions into four modes which may be concerned in establishing immunity. Mode A involves the reaction of serum antibody, acting alone or with other soluble molecular co-factors such as complement; this mode is exemplified by the action of anti-toxins. Mode B involves serum antibodies, molecular co-factors and non-allergized cells; it is exemplified by the increased phagocytosis which often occurs in the presence of specific antibody. Mode C involves serum antibody which passively allergizes cells; it is exemplified by the cytophilic antibodies which passively allergize macrophages (Nelson and Boyden, 1967), and by the reagin-like antibodies which passively allergize mast cells. Finally, mode D involves actively all ergized cells; it is exemplified by the activities of sensitized lymphocytes in graft rejection.

The major difficulty in attempting to explain the mechanism of immunity to schistosomes is provided by the general failure to transfer resistance passively by means of serum from immune animals. At first sight, this finding rules out the possibility of any of the first three modes of allergic activity being involved; all are primarily antibody-mediated reactions and thus theoretically demonstrable by passive transfer experiments. This would leave mode D, involving actively allergized cells, as the only mechanism possible. Cell mediated immunity is technically more difficult to demonstrate than antibody-mediated immunity. Transfer of actively allergized cells should be carried out in isogenic hosts, in order to prevent the homograft reaction from confusing the results. This is possible in mice, where inbred strains have been developed, but immunity to schistosomes is an uncertain phenomenon in this species. Inbred strains of rhesus monkeys, a species which shows unequivocal immunity to schistosomes, are not available. It is worth noting that the only reported attempt to transfer immunity by means of cells was a failure (Hunter *et al.*, 1967), and it is probable that other negative results have gone unreported.

If these negative findings are accepted unreservedly, we must conclude that

immunity to schistosomes is dependent on mechanisms other than those known to exist in other infections. The alternative to this somewhat heretical standpoint is to accept that one or more of the established modes is effective against schistosomes, and that our failure to demonstrate its action by passive transfer is due to our lack of understanding and ineptitude in handling the reagents. In the absence of any positive evidence for a novel mode of allergic reactivity in schistosomiasis, we feel bound to accept this second alternative. Taking this position at least enables us to speculate on the possible particiption of the several modes in the immune reaction, although failure to demonstrate these unequivocally remains a constant irritation.

The contribution of Lichtenberg and Ritchie (1961) provides a convenient starting point for these speculations. Studying acquired immunity to *S. mansoni* in the rhesus monkey, they evinced the existence of three main immune phenomena. (1) The proportion of worms reaching the portal habitat is smaller in resistant hosts than in normal hosts, suggesting that the schistosomula are retained at their earlier points of migration. This was named the "trapping phenomenon". (2) Many of the worms which did arrive at the portal vessels remain immature up to the 42nd day, when ordinarily all should be fully developed. This was termed the "stunting phenomenon". (3) The worms reaching the portal circulation in some animals died before the 65th day. This was termed the "premature extinction phenomenon".

Histopathological examination revealed that the trapping of the migrating schistosomula occurred mainly in the lungs. Schistosomula seen in lung sections from control monkeys seemed to evoke little cellular reaction. In contrast, schistosomula from resistant monkeys were always associated with inflammatory reactions; about half of those seen were located within major inflammatory foci, called "tuft-like foci", which seemed to develop progressively, surrounding the larvae with a compound cellular reaction comprising polymorphs, eosinophils, lymphoid cells and large mononuclear cells. In the larger, presumably more developed foci, the schistosomula were degenerate and dying.

Lichtenberg and Ritchie (1961) favoured the idea that this reaction was mainly brought about by actively allergized cells, mode D, but Lichtenberg (1967) suggested that mode B is more probably involved. Antigen-antibody reactions, involving complement, have been shown to generate agents chemotactic for polymorphonuclear leucocytes (Boyden, 1962). Antibodies have been detected in schistosome-infected baboon sera which promote the adhesion of polymorphs to the surface of schistosomes (Newsome, 1962). Lichtenberg (1967) reasons that in the presence of such "immune adherence", the migration of schistosomula through the lung capillary beds would be slower. This would encourage the arrival of further host cells, homing on the target, and lead in time to the formation of the tuft-like foci seen in histological studies. According to Lichtenberg, occupation of the tegument by adhesive host cells might facilitate the action of circulating or cell bound antibodies (modes A and C) on the tegument, leading eventually to energy starvation and death of the worm.

There is little evidence yet either to support or refute these views, but we recall that schistosomes are vulnerable to attack by mode A, without cells

being involved except in the final disposal of the dead remains. This was demonstrated in a somewhat artificial situation, where the antibodies were directed against host antigens situated in the tegument (Smithers *et al.*, 1969), but this was not slow starvation of the worms; death was rapid and probably due to the antibodies and associated complement piercing the plasma membrane. The final dispatch of the worms in the inflammatory foci may be as rapid.

In terms of immunological mechanisms, reasoned explanations for the stunting phenomenon and the premature extinction phenomenon are even harder to find. Lichtenberg and Ritchie (1961) found that a considerable proportion of schistosomula did evade the "lung trap" of resistant animals and arrived in the portal vessels, where they were not subjected to any further cellular reaction until they died and were swept into the portal radicles and gave rise to large granulomas. Presumably, stunting and the death of the worms is brought about by mode A, antibodies acting alone or in concert with soluble co-factors such as complement. We imagine antibodies interfering with the biosynthetic mechanisms of the parasite, leading to failure of maturation and eventual death, but why is it that these antibodies, if they exist, cannot be demonstrated by passive transfer?

Lichtenberg and Ritchie (1961) proposed that the distinctive features of acquired immunity to schistosomes can best be explained on the basis of partial blockage of host antibodies by the parasite. This would be consistent with the capacity of schistosomes to survive in a humoral environment where anti-worm antibodies are known to exist. One possible mechanism for this blocking, they suggest, is that the permeability of the worm surfaces may be sufficiently selective to exclude antibody globulin. We now know this is not so; antibodies directed against host antigens in the cuticle are rapidly effective in killing the worm (Smithers *et al.*, 1969). It is more likely that the blocking mechanism is provided by the presence of these host antigens which may serve to disguise the worm, at least temporarily. Stunting of the worms could be explained by a partial failure of this stratagem, and premature extinction by the eventual unmasking of the worm in its true antigenic identity. Other mechanisms are possible. Lichtenberg and Ritchie (1961) suggested immunological paralysis as one, but this seems unlikely to us in view of the array of antibodies provoked by infection. Possibly, after an early stimulation of the host's immune mechanisms, the adult schistosome may shelter behind some antigenically inert outer layer of sialomucin such as may be associated with certain types of tumour cells (Sanford, 1967; Currie, 1967; Bagshawe and Currie, 1968) and perhaps with the trophoblast (Currie *et al.*, 1968). Also, if actively allergized cells, mode D, are involved actually or potentially in immunity to schistosomes, we may have to consider the phenomenon of enhancing antibodies. In some cases of tumour transplant immunity, if these antibodies are formed first, they can so coat the target tumour cells that they are unable to sensitize lymphocytes or even to interact with lymphocytes already sensitized which would rapidly damage them if they were uncoated (Uhr and Moller, 1968). Attempts to expose the stratagems by which schistosomes evade the immune mechanisms of the host provide a fascinating field of study.

Another aspect of the immune response which has gained a good deal of attention relates to the formation of the schistosome pseudotubercle. This is the granuloma which surrounds the individual egg trapped in the host tissue. The allergic reactivity in this case is specific and may be transferred with cells but not with serum (Warren *et al.*, 1967), and hence is almost certainly brought about through mode D, actively allergized cells. However, as there is little evidence that this reaction plays any part in acquired immunity to reinfection, the discussion of this phenomenon is deferred to Section IV.

Kloetzel (1967b) reported that *S. mansoni* eggs possess collagenase activity, which is inhibited in the presence of activated immune serum, and suggested that this inhibition would prevent eggs from passing through the intestinal wall and promote their retention in the tissues. This would explain the fall in faecal egg count that may occur even when the female worms are laying eggs at a steady rate. If this report is substantiated, mode A as well as mode D may be involved in the formation of pseudotubercles.

Finally, the state of immediate hypersensitivity is a constant feature of helminth infections. This allergic mechanism involves mode C; mast cells, passively allergized with reaginic antibody (IgE), react with antigen and disrupt, leading to the release of potent pharmacological agents. Although there has been no convincing demonstration of the role of reagins in schistosome immunity except in the abnormal rat host (Ogilvie *et al.*, 1966), it is difficult to dismiss them altogether. Their involvement in helminth immunity would provide some biological justification for the retention of what is otherwise a harmful response, and without this it is difficult to see why this response has been retained in spite of selection pressure.

In conclusion, we admit that none of the four modes of specific allergic reactivity has been unequivocally demonstrated in immunity to reinfection in schistosomiasis. Furthermore, none of these modes may be excluded as playing no part, but in this unhappy state we may take comfort in the remarks of Humphrey (1968), who has stressed the immediate difficulty of stating with conviction what role any particular kind of antibody or manifestation of cell mediated specific immunity is playing in a real-life complex biological situation. Few such situations may be more complex than the relationships between schistosomes and their vertebrate hosts. Yet we feel optimistic that soon solutions to some of the problems set out above will be found. The answers may come from other fields in which immune reactions occur; they may already be awaiting an astute mind.

## IV. IMMUNOPATHOLOGY

### A. THE SCHISTOSOME PSEUDOTUBERCLE

#### 1. *The nature of the pseudotubercle*

One major clinical manifestation of severe schistosomiasis is liver disease. Symmers (1904) described the hepatic lesion of schistosomiasis and suggested that its cause was schistosome eggs, many of which lodge in the liver and incite a granulomatous reaction in the portal areas. This opinion was supported by

many investigators, although some suggested that worm toxins, dead worms, or malnutrition may be other responsible factors.

The development by Warren and others of an experimental model of hepatosplenic schistosomiasis in mice which live for long periods after exposure to low numbers of cercariae, provided an opportunity to study the way in which the parasite caused the disease. Much evidence has now indicated that the eggs produced by the schistosomes and the subsequent granuloma reaction they invoke are prime factors which lead to lesions resembling Symmer's fibrosis and to overt hepatosplenic disease in mice (Warren, 1968).

The granuloma around an individual egg trapped in the host tissues is a circumscribed lesion, consisting of eosinophils, macrophages, lymphocytes, epitheloid cells and giant cells. In its acute form it is many times larger than the egg itself. Later on, when the inflammatory cells are gone, a residue of fibrous tissue remains. The miracidia within intact eggs surrounded by host cell granulomas can remain viable for 32 days (Maldonado, 1959).

The conditions which are necessary to induce schistosome pseudotubercle formation were investigated by Lichtenberg and his colleagues. The reaction around schistosome eggs differs markedly from that around insoluble plastic (polyvinyl) spheres, when both are injected intravenously into mice. Plastic spheres elicit only a transient inflammatory halo (Lichtenberg, 1962). Pure miracidia, when injected intravenously, cause a mild leucotactic response and disappear without trace within 48 h. Pure egg shells cause an inflammatory cell reaction lasting more than two weeks. Only living or dead (autoclaved) intact ova cause the formation of pseudotubercles, but the reaction to dead eggs is less in size and duration then that around viable eggs (Lichtenberg and Raslavicius, 1967). Thus, it seems likely that antigen in the intact egg is gradually and continuously released rather than quickly dissipated. Direct evidence of liberated antigen from the miracidia within the eggs and the consequential immunological reply by the host, is now available.

Andrade *et al.* (1961) were the first to demonstrate the presence of antigen and antibody in the schistosome pseudotubercle by the use of fluorescent antibody technique on cryostat sections from mice infected with *S. mansoni*. Some sections were treated with fluorescein conjugated rabbit anti-mouse $\gamma$-globulin in order to stain host antibody, and some with fluorescein conjugated $\gamma$-globulin from patients infected with *S. mansoni*, in order to locate parasite antigen. The anti-schistosome serum was found to stain structures within the schistosome ova, necrotic areas around the ova, and the cytoplasm of many macrophages in the granulomas. Host $\gamma$-globulin (host antibody) was also located in the cells of the granulomas. The fluorescent antibody of the anti-mouse $\gamma$-globulin (denoting host antibody) was removed by treatment of the sections at an acid pH. When these sections were further treated with anti-schistosome $\gamma$-globulin, fluorescent activity reappeared, denoting parasite antigen. This was taken to indicate that by lowering the pH, antibody had been split from its combination with antigen, leaving free antigen in the tissue. Using basically similar immunocytochemical techniques, the presence of antigen in the centre of the granulomas and in cells at the periphery, was confirmed by Magalhães Filho *et al.* (1965), and Moore (1967) showed that the

cephalic penetration glands of the miracidia within the egg in the tissues stain brightly for antigen.

Lichtenberg (1964) applied the indirect immunofluorescent technique to study the progression of the schistosome pseudotubercle. Tissues from mice infected with *S. mansoni* or injected intravenously with eggs of the parasite were treated with mouse anti-schistosome serum and then stained with fluorescein conjugated rabbit anti-mouse serum. A polysaccharide antigen was located in eggs in host tissue and antigen staining was most intense in well developed cellular granulomas, but older fibrotic nodules showed little or no staining.

The use of animals receiving an intravenous injection of eggs gave Lichtenberg a convenient method of studying the fate of the egg in sensitized and normal hosts. Soon after entry of eggs, antigen diffuses into lung vessels and alveoli. With the onset of the host cell response (after 24 h) antigen is sequestered in the granuloma centre, partly around the egg shell and partly inside host macrophages. After the formation of the schistosome pseudotubercle, antigen is progressively destroyed. Unsensitized mice show minute amounts of antigen in pulmonary granulomas up to 70 days after egg injection, whereas mice previously sensitized by intraperitoneal injections of eggs showed an accelerated and enhanced cell response with disappearance of antigen within 32 days of intravenous egg injection.

Peterson and Lichtenberg (1965) injected schistosome eggs into the lungs of donor mice via the tail vein and found that the sequestered egg remains actively antigenic for over 30 days. After successive intervals, the donor lungs were removed and their homogenates were used to sensitize recipient mice by intraperitoneal and subcutaneous injection. After a standard period, the recipients and appropriate controls were challenged intravenously with whole eggs. These mice were killed 8 days later and examined for the persistent antigen in the pulmonary granulomas by the indirect immunofluorescent technique. In this experimental system, the persistence of antigen in the donor lungs would manifest itself by a reduction in antigen in the granulomas of the recipient lungs, compared with those of unsensitized controls. Lung granuloma homogenates retained their antigenicity or sensitizing power through the 30th day of the evolution of the pulmonary pseudotubercle.

The sensitization of mice to granuloma formation was confirmed by Warren *et al.* (1967) and shown to be specific. Although prior intraperitoneal injection of *S. mansoni* eggs would sensitize for an intravenous challenge with similar eggs, a previous injection of *Ascaris* eggs would not sensitize for a later injection of schistosome eggs, although it did so for *Ascaris* eggs. These workers were able to transfer this sensitization with spleen and lymph node cells from mice infected with *S. mansoni*, but were unable to transfer the sensitivity with serum from the same mice. Cells from unisexually infected mice did not induce sensitivity, nor did cells from animals receiving egg injections. Granulomas around schistosome eggs in infected animals are larger than those in uninfected animals injected with eggs, and necrosis is more common; the failure to transfer sensitivity with cells from egg injected animals may have been due to a quantitative difference in sensitization compared with infected animals.

The fact that sensitization is specific and that it can be transferred with cells and not with serum, points to the possibility that the schistosome pseudotubercle is an immunological reaction of the delayed hypersensitivity type. Lichtenberg (1967) believed that both delayed hypersensitivity and circulating antibody have closely inter-related roles in mediating pseudotubercle formation. Antibody formation probably increases in importance proportionally with the degree of host sensitization. In acutely infected and highly sensitized hosts, clear cut antigen-antibody complexes appear around the egg in the tissues. The Hoeppli phenomenon is an example; this eosinophilic fringe which is sometimes seen surrounding schistosome eggs is correlated with substantial egg loads and with peak antibody titres. Immunofluorescent studies on *Mastomys coucha* infected with *S. mansoni* have shown the Hoeppli phenomenon to be an antigen-antibody complex (Lichtenberg *et al.*, 1966; Smith and Lichtenberg, 1967). It is probable that the formation of this insoluble complex around the egg would make antigen sequestration more efficient. Lichtenberg (1967) believed that the circumoval central necrosis of pseudotubercles which is frequently seen in heavily exposed animals, might be due to the formation of a soluble antigen-antibody complex similar to that responsible for other types of immunological damage.

2. *The significance of the schistosome pseudotubercle*

By inducing antigen sequestration *in situ* and potentiating antigen catabolism, it has been suggested that the schistosome pseudotubercle has a defensive role (Lichtenberg, 1967). It is also possible that antibody may be generated locally in cells that surround mature granulomas. This could account for the high immunoglobulin levels found in schistosomiasis. On the other hand, the granulomatous reaction of the host to the schistosome egg is a major factor in the development of hepatosplenic disease (Warren, 1968), and if this reaction were suppressed the development of overt hepatosplenic disease might be averted (Domingo *et al.*, 1967). In prolonged experimental infections, Andrade and Warren (1964) and Cheever (1965) showed granuloma formation to be lessened around new eggs deposited in the tissues. This diminished host response to eggs is considered to be just as much a manifestation of acquired immunity as is a decrease in the numbers of worms and eggs.

Recent studies on the suppression of granuloma by immunosuppressive drugs (Domingo *et al.*, 1967), neonatal thymectomy (Domingo and Warren, 1967) and heterologous anti-lymphocyte serum (Domingo and Warren, 1968), have all resulted in suppression of granuloma formation around schistosome eggs injected into the tissues of unsensitized mice. Partial suppression was found in sensitized animals, but significant degrees of suppression have not as yet been observed in infected animals. The results of suppressing granuloma formation and the subsequent systemic effect on the host of the antigen normally sequestered in the granuloma, are therefore still awaited. An encouraging report from Lees (1968), however, has shown that in two children with hepatosplenic schistomiasis, treatment with lucanthone hydrochloride followed by steroid therapy resulted in the rapid regression of hepatosplenomegaly.

### B. SPLENOMEGALY

Splenic changes in chronic schistosomiasis have been ascribed principally to congestive splenomegaly concurrent with an increase in portal pressure. However, reactions in the lympho-reticular tissue to parasite antigen are partly responsible for the splenomegaly of schistosomiasis.

Schistosome antigen has been detected in neutrophils infiltrating the spleen and also in the lungs, lymph nodes and endothelium of the blood vessels, as early as 15 days after exposure of mice to *S. mansoni* (Magalhães Filho *et al.*, 1965). A splenic hyperplasia was demonstrated in mice 3 days after exposure to *S. mansoni* by Magalhães Filho and Coutinho-Abath (1961); these splenic reactions reached a high degree around the 15th day. Using histochemical and immunocytochemical techniques, Andrade (1962) showed an intense proliferation of the reticuloendothelial cells of the red pulp of the spleen just after egg production had begun; they were followed by plasma cell differentiation and occurred in the absence of parasite elements in the spleen. The changes are similar to responses of the lymphoreticular tissue following antigenic stimulation and are believed to be the result of the reaction of the reticulo-endothelial system against antigens liberated by the parasite.

### C. AUTO-IMMUNITY

Auto-immunity is the production by the body of antibody against one of its own unmodified native components or against foreign antigen whose antibody cross-reacts with a native self antigen (Brent and Medawar, 1959). Auto-immunization has been proven or seriously suspected in a wide variety of diseases and certain clinical and laboratory findings in schistosomiasis seem to indicate that auto-immune disease plays a significant role in its pathology.

A chronic portal inflammation in schistosomiasis has been described by many authors, although no pathogenetic importance has been ascribed to it. Andrade (1964b) points out that the chronic portal inflammation is morphologically similar to a reaction of the delayed hypersensivity type, i.e. a diffuse infiltration of lymphocytes, plasma or plasmocytoid cells and a progression to fibrosis. Furthermore, there is frequent invasion of the parenchymal border by the inflammatory cells and focal ductular cell proliferation, indicating activity of the inflammatory process. There is also evidence of local formation of $\gamma$-globulin by the cells of the portal infiltration.

The presence of auto-antibodies in schistosomiasis was described, both in experimental animals and in man. Kurata and Noda (1965) demonstrated in rabbits infected with *S. japonicum* an auto-antibody to rabbit liver and colon extracts. The auto-antibody appears 3–4 weeks after infection and reaches maximum titre at 6–8 weeks. This appearance roughly parallels the antibodies which appear against parasite antigens (Kurata, 1966). Shamma *et al.* (1965) reported that 27 of 102 serum samples from patients with *S. haematobium* infections had a complement fixing antibody to human liver and lung homogenates. They then showed by an immunofluorescent technique that in treated lung sections the fluorescent staining was limited to the alveolar walls and

blood vessels, and in liver sections to connective tissue and vessel walls (Shamma *et al.*, 1966). Both groups believe that the auto-antibodies arise because of tissue damage due to egg deposition and possibly the presence of adult worms. Evidence for auto-immunization in schistosomiasis is at the moment, therefore, no more than suggestive or fragmentary. Further studies are necessary to determine the real significance of portal hepatitis and auto-antibodies in this infection and their relationship to auto-immune disease.

### D. SERUM PROTEIN CHANGES

Hypergammaglobulinaemia and hypoalbuminaemia have frequently been reported from both human and experimental infections of schistosomiasis (Evans and Stirewalt, 1958; Sadun and Walton, 1958; Ramirez *et al.*, 1961; Tomoda, 1963; Fiorillo, 1966; Sadun and Williams, 1966; Warren and Moore, 1966). Where experimental schistosome infections have been studied, the alterations in the concentrations of the serum proteins occur at the time of worm maturation and egg deposition. In infections where eggs are absent (e.g. in rhesus monkeys exposed to one sex of *S. mansoni*, or to the Formosan strain of *S. japonicum* or to irradiated cercariae of the Japanese strain of *S. japonicum*), alterations in the serum protein levels do not occur (Smithers, 1962b; Hsü and Hsü, 1964). It is likely that the serum protein changes are a result of the immunological processes after egg deposition and can therefore be included in immunopathology.

Decreased concentration of serum albumin is not always concomitant with hypergammaglobulinaemia (De Witt and Warren, 1959); the albumin concentration may fall only in heavy infections (Smithers and Walker, 1961; Hsü and Hsü, 1964). Studies using $I^{131}$-labelled albumin in rhesus monkeys exposed to *S. mansoni* indicate that at the 8th week of infection there is a fall in total body albumin followed by a recovery to normal values by the 12th week. Concurrent with the fall in albumin there was a marked increase in its catabolic rate. Calculations indicated that although the total body albumin values had fallen at week 8, there had been an increase in albumin synthesis, although insufficient to compensate for the increase in catabolism. At week 12 the albumin had recovered to near normal values, but its catabolic rate remained high, indicating that albumin synthesis was also greatly increased (Smithers and Walker, 1961). As the liver is the chief site of the albumin synthesis (Miller *et al.*, 1954) it is difficult to explain the fall in albumin concentration as a result of liver damage due to infection.

Large increases in the $\gamma$- and $\beta$-globulin levels as well as increases in the $\alpha_2$-globulins occur in schistosome infections. Most of the studies on raised immunoglobulin levels were made before the recognition of the five distinct immunoglobulin types, IgG, IgM, IgA, IgD and IgE (W.H.O., 1964, 1966, 1968), and the relative changes in each type are not known. Immunoglobulin increases occur at the time of egg deposition and may be due to the sudden and marked antigenic stimulation from the deposition of eggs in the host's tissues. It would appear from recent work, however, that the high immunoglobulin levels are not due to an increase in specific schistosome antibodies. This con-

clusion was reached from a study in which IgG from a monkey infected with schistosomes, i.e. IgG taken after the increase in concentration had occurred, was injected together with normal IgG into monkeys that had been infected 5 weeks previously so that their parasites had just begun to mature and lay eggs and therefore release large amounts of antigen. The "pathological" IgG and the normal IgG were labelled with different isotopes of iodine so that their rate of catabolism could be followed separately. If the "pathological" IgG had consisted mainly of antibodies specific to schistosome antigens, then it might have been expected to disappear more quickly from the circulation of the infected animal than the normal globulin did, because of its combination with antigen. In fact, the two IgG's were catabolized at the same rate. Within the limits of the experimental technique, this experiment shows that less than 5% of the "pathological" IgG was specific to schistosome antigens (Freeman *et al.*, in preparation).

The view that the hypergammaglobulinaemia response is non-specific and non-protective is strengthened by the experiments in which rhesus monkeys developed resistance to re-infection against schistosomes in the absence of raised immunoglobulin levels (Smithers, 1962b; Hsü and Hsü, 1964). It does appear, therefore, that the large increases in immunoglobulin values as a result of infection are probably not a reflection of specific schistosome antibodies, but are due to the synthesis of non-specific immunoglobulins. Askonas and Humphrey (1958) found that the stimulation of tissues of hyperimmunized rabbits by antigen produced at least as much non-specific immunoglobulin as antibody; and Humphrey (1958–1959) can see no reason why plasma cells should not be stimulated to secrete immunoglobulins that do not necessarily bear the imprint of any particular antigen.

High levels of macroglobulin have been reported in chronic schistosomiasis of man (Bour *et al.*, 1962; Fiorillo, 1966); these could be explained as a result of splenomegaly where IgM levels may become elevated (Lumsden, 1966), or perhaps in some protozoan diseases by the production of a 19S antibody against denatured IgG (Houba and Allison, 1966). The $\alpha_2$-globulin levels may be increased when tissue damage occurs (Belfrage, 1963).

Obviously the aetiology of hypergammaglobulinaemia in schistosome infections is complex and little understood. With the recent expansion of our knowledge of the immunoglobulins and development of techniques for their easy assay, we should be able to advance our understanding of the significance of raised immunoglobulins in the pathology and immunity of schistosomiasis.

Several workers have demonstrated an increase in the blood volume in patients with schistosomiasis and hepato-splenomegaly; showing that the increase in blood volume is due to an increase in plasma volume, the red cell mass showing no conspicuous change from the normal values (Fiorillo *et al.*, 1954; Khattab *et al.*, 1960; Saif, 1966). These changes are probably a reflection of hypergammaglobulinaemia. Anaemia has been reported in schistosome infections, and at least an associated if not the major cause must be attributed to haemodilution due to the high plasma volumes. Other types and mechanisms of anaemia in schistosomiasis occur, however, and they have been reviewed by Foy and Nelson (1963) and Jamra *et al.* (1964).

## V. General Conclusions

There are so many unanswered questions relating to immunity to schistosomes that any well-conducted experiment or observation on the subject is welcome. But there are certain aspects of the problem where research is needed most urgently and where it is likely to prove most profitable. The aspects have been discussed already, but they are set down again in summary.

1. The phenomenon of innate immunity requires further investigation; in particular it is important to determine for each host species how far this immunity is truly innate and how far it merely represents very rapidly acquired immunity.
2. Further evidence is required as to the acquisition of immunity by man. Long-term investigations of drug-treated patients exposed to possible reinfection would be valuable in this context.
3. It is important to determine whether the "self-cure" phenomenon seen in some host species is indeed due to immunological mechanisms. Immunosuppressive procedures and agents are likely to prove useful in such investigations.
4. Clarification is still required of the roles of the various stages in the life history of schistosomes in stimulating immunity. Investigations on the role of the adult worm, in host parasite systems other than *S. mansoni* in the rhesus monkey, would be particularly welcome.
5. There is great need for a more ordered approach to the study of schistosome antigens. There should be more vigorous attempts to isolate, purify, and characterize these antigens and there should be an agreed nomenclature. Success in this work will lead to improved immunodiagnostic agents, elucidation of the molecular architecture of the parasite, and perhaps agents which would prove useful in immunoprophylaxis.
6. The various antibodies characterizing schistosome infections should be redefined in terms of their immunoglobulin classes. This may prove easiest to carry out in primates, as specific reagents for the identification of primate immunoglobulins are readily available. The role of reaginic antibodies in schistosome infections requires further clarification.
7. The mechanism of immunity to schistosomes is very imperfectly understood and requires more enterprising study, particularly at the ultrastructural and molecular levels. Such studies should take account of the devices which schistosomes employ to evade the immune defences of the host.
8. It is necessary to determine how much the pathology of schistosomiasis owes to immunity, either as an exaggerated response to foreign antigen as in the pseudotubercle, or to auto-immunity. Should immunity to schistosomes in some situations be actively discouraged?

## Acknowledgements

We are grateful to Mrs. J. C. James and Mrs. P. M. Clark for their valuable help in preparing this review.

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# Experimental Fascioliasis in Australia

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## I. Introduction

The basic facts of the life cycle of *Fasciola hepatica* have been known for a long time, but most of the experimental research elucidating the details has been carried out within the last two decades. This is the result of a revolution in concept, which has transformed descriptive morphological parasitology to experimental and clinical parasitology. The most important force behind this revolution is the desire for increased agricultural production to provide the world with more animal products. As many bacterial and viral diseases are now successfully kept under relatively efficient preventive control, more attention has been paid recently to helminth diseases which, although usually subclinical, cause serious losses in production. *F. hepatica* is easily accessible, its distribution is cosmopolitan, and its fascinating life cycle is a rewarding challenge for zoologists, ecologists, pathologists and biochemists interested in trematode research.

The economic loss produced by fascioliasis due to *F. hepatica* or *F. gigantica* all over the world is enormous, and with the application of more intensive agricultural methods it is increasing. Using all the available resources of control measures, elimination of the disease is now theoretically possible. However, many biological, climatic, topographical and particularly human factors are involved in the epidemiology of the disease, and in spite of new encouraging improvements in control measures, eradication cannot be anticipated while the present methods of grazing are used.

Reference to many valuable contributions to fascioliasis research can be found in recent monographs (Dawes and Hughes, 1964; Taylor, 1964; Pantelouris, 1965). The present review is also a summary of the results of some experimental research in Australia aimed at various aspects of fascioliasis in order to control the disease economically and more efficiently.

In Australia, an estimated 40 000 000 sheep and about 5 000 000 cattle, about a quarter of their total population, are grazing on potentially endemic pastures, and fascioliasis causes severe economic losses due to mortality, condemned livers, reduction of meat and milk production, secondary bacterial infection and wastage through frequent anthelmintic treatment.

## II. Identity and Ecology of *Lymnaea tomentosa*, Snail Host of *Fasciola hepatica*

### A. Australasian lymnaeids and their response to environment

The taxonomic literature on the Lymnaeidae of Australia and New Zealand was reviewed by Boray and McMichael (1961). The Australasian lymnaeid snails can be divided into two major groups. One group comprises snails with large (25 mm or more) globose shells (Fig. 1G) which are not implicated in the transmission of liver fluke. There is no justification for the separation of these forms from *Lymnaea* s.s. (Hubendick, 1951), and, in general, there appears to be only a single, widely distributed species in Australia, New Zealand and New Guinea which varies greatly under environmental stress, and which should bear the name *Lymnaea lessoni* Deshayes.

The second group consists of snails with small to medium size shells (up to 16 mm long) of varying form and rather variable body characters, which have been implicated in the transmission of *F. hepatica.* The four generic and 24 specific names applied to living members of this group from Australia and New Zealand and type localities, were listed by Boray and McMichael (1961). Three generic names were regarded as synonyms of *Lymnaea* s.s., as was suggested by Hubendick (1951), and therefore all the Australasian Lymnaeidae are, in fact, referable to *Lymnaea* Lamarck 1799. The status of the various named forms as "species" is a more difficult problem. Cotton (1942, 1943) and Iredale (1943, 1944) admitted nearly all the 24 forms as full species with fairly limited geographic ranges, with the exception of a few obvious synonyms. However, Hubendick (1951) suggested that there were, in fact, only one or two widespread species which varied greatly. Dell (1956) reduced the number recognized in New Zealand to three endemic species. Authentic specimens from the Australian Museum collection of the several described "species" were compared.

A number of studies have been made on the variation in morphology of fluke-host snails, apart from the problem of taxonomy. Clunies Ross and McKay (1929) used the name *L. brazieri* Smith for what they considered to be the most important intermediate host in Australia and examined many snails from habitats in New South Wales, Victoria and Tasmania, finding no significant differences between the Tasmanian and mainland populations.

In order to clarify the taxonomy of these snails, a study was made of 61 populations from 42 localities in New South Wales, South Australia, Queensland, Victoria and Tasmania, and also some specimens forwarded by several collaborators in New Zealand. Two extreme morphological types ,"A" and "B", were recognized (Fig. 1), and both serve as intermediate hosts for *F. hepatica.* Snails collected in the field showed great variations in shell and body and there were many intermediates between the extreme variants. Many mixed populations of both types and many intermediate forms were found.

Samples of the various snail populations were cultured in isolation in the laboratory, and the structure of bodies and shells were studied. Egg masses from various populations were subjected to various environmental conditions in the laboratory, and samples of the two extreme variants were bred and their progeny observed during several generations. The morphology of the snails apparently depends on environmental conditions. Snails which originated from one population of uniform appearance produced large animals with thin shells when grown at a fast rate under good conditions with artificial feeding, but they produced small, thicker shells when grown under poor conditions with consequent slow growth. Snails of the type "B" collected from South Australia were reared under laboratory conditions for eight generations in isolated cultures. The subsequent generation offspring were less fragile, with the outer lip thicker and better developed (Fig. 1, E, F). The length of the body was reduced, and a striking change was the almost complete disappearance of the large mantle border present on the parent specimens (Boray and McMichael 1961).

Transverse sections of the prostate glands of types "A" and "B" from Aus-

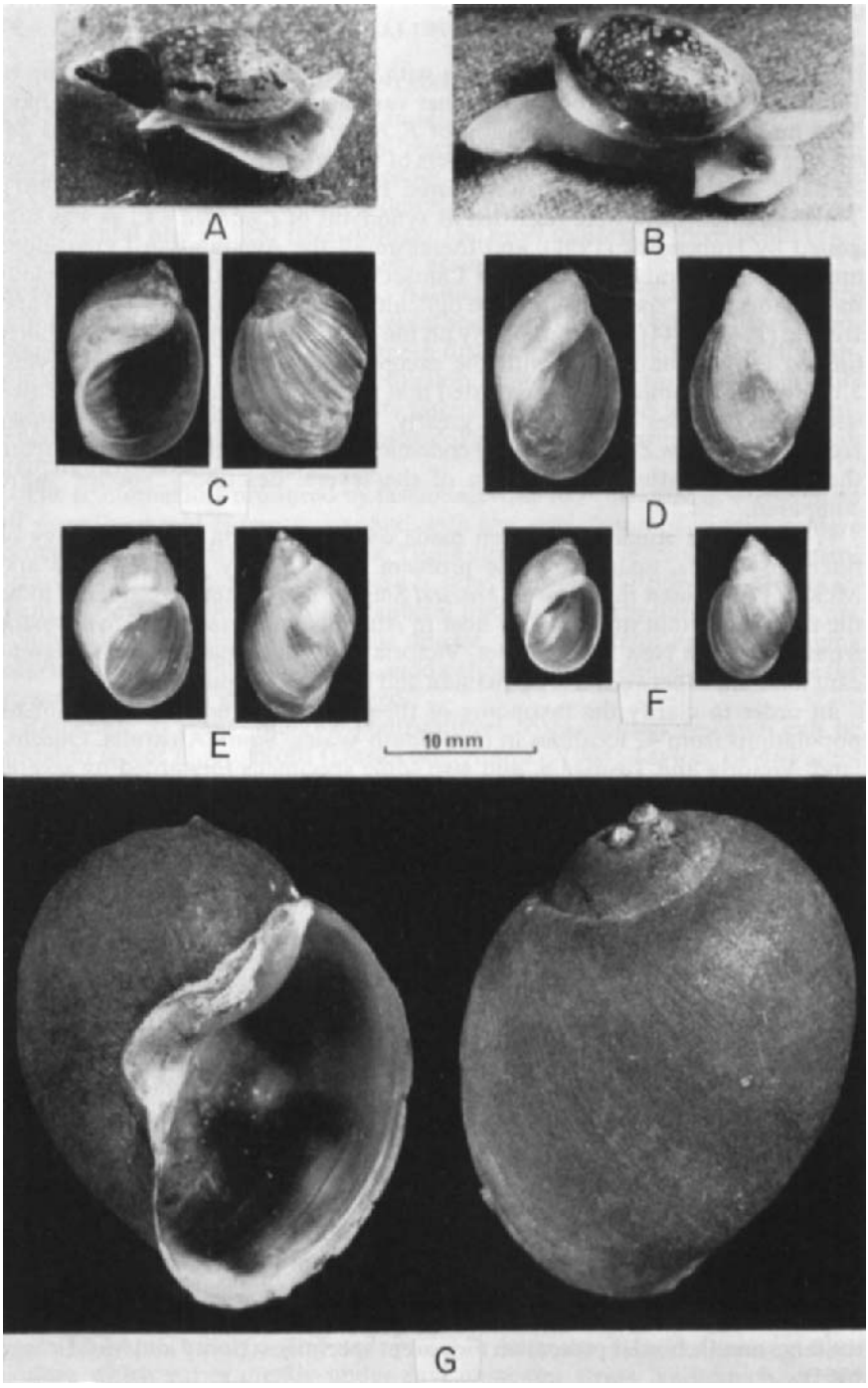


FIG. 1. **A,** *Lymnaea tomentosa* type "A". **B,** *L. tomentosa* type "B". **C,** Type "A" shell. **D,** Type "B" shell. **E,** Fourth generation offspring from type "B" parent. **F,** Fifth generation offspring from type "B" parent. **G,** *Lymnaea lessoni* (N.S.W.).

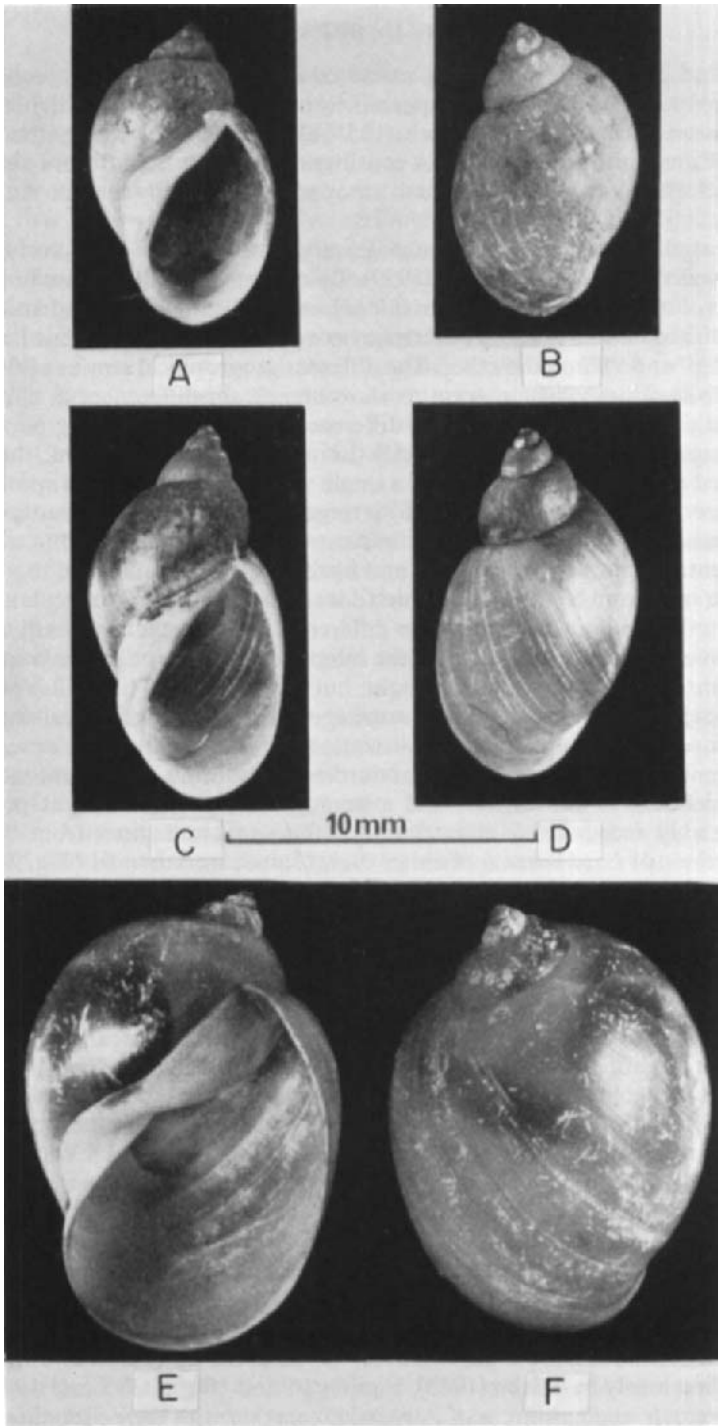


FIG. 2. **A, B,** *Lymnaea tomentosa*. Field specimen from the Eastern Highlands, New Guinea. **C, D,** Typical laboratory specimen of above snail (12th generation). **E, F,** *Lymnaea lessoni* (young specimen from Markham Valley, New Guinea).

tralia and New Zealand did not reveal consistent differences as recorded by Hubendick (1951), and there appeared to be wide variations in the length of the spermathecal duct. Hubendick (1951) showed that the spermathecal duct of the European *L. peregra* varies considerably in length, and Boray and McMichael (1961) revealed an equal amount of variability in the Australian species.

The application of paper chromatography to taxonomic studies on lymnaeid snails was described by Wright (1959). By this method, the chromatographic pattern of mucus from snails from our colonies indicated (Wagland and Boray, unpublished data) a marked difference between *L. lessoni* on the one hand and types "A" and "B" on the other. The different geographical strains of the intermediate snail host did not seem to show significant differences in chromatographic patterns. Apparently, the difference between the varying population is ecological rather than genetic. All the named forms, therefore, should be regarded as variant populations of a single very widely distributed species, and *Lymnaea tomentosa* (Pfeiffer, 1855) is regarded as the correct name for the intermediate host of *F. hepatica* L. in Australia and New Zealand.

Recent comparative anatomical and histological studies showed that a large globose snail from New Guinea, which does not transmit *F. hepatica*, is identical with the Australian *L. lessoni*. The difference between the smaller lymnaeids from New Guinea, which serve as the intermediate host for *Fasciola* spp., and *L. tomentosa* from Australia was slight, but recognizable. The snail is regarded as a geographical variation of the same species. Microgeographical variations of snail populations occurred in Australia as in New Guinea. However, some of these were maintained under standardized conditions in the laboratory for approximately 16 generations and a homogenous population was produced with readily recognizable characteristics. These were distinct from the field populations of *L. tomentosa* of either New Guinea or Australia (Fig. 2 A-F).

## B. DISTRIBUTION AND ENVIRONMENT

### 1. *Distribution*

The history, geographical distribution and physical, chemical and biological environment of *L. tomentosa* were discussed by Boray (1964a). The first experimental proof that this snail is the intermediate host of *F. hepatica* in Australia was by McKay (1926) and Bradley (1926). The early accounts of *L. tomentosa* showed it to be very widely distributed in the eastern part of N.S.W., the whole of Victoria and N.E. Tasmania, and it is also found in certain localities in South Australia and in the S.E. corner of Queensland.

Seddon (1950) recorded the distribution of *F. hepatica* in Australia, supplemented by Boray (1964a). Details of the endemic areas of South Australia were described by Ewers (1958) and Lynch (1965a). The distribution of snails in Queensland was recorded by Dixon (1963). A small endemic area was recently found in the mountain ranges 200 miles north of the limit of distribution described previously by Dixon (1963). Figures 15 and 16 (pp. 195 and 197) illustrate the main endemic areas of Australia, together with their climatic pattern.

Although the distribution of the intermediate host in Australia has not changed appreciably in the last decade, the snail has a great capacity to spread quickly whenever physical and meteorological factors are suitable. This feature is of particular importance in newly established irrigation systems, where a rapid expansion of fascioliasis can occur. A recent survey (Boray, unpublished) showed that *L. tomentosa* is widely distributed in the E. and W. Highlands of New Guinea at areas approximately 2500 ft above sea-level or higher. The most common snail habitats were found at about 5000 ft above sea-level, and snail colonies were found as high as 7000 ft.

Brunsdon (1967) summarized many reports on the distribution of *L. tomentosa* in New Zealand and found that the snail is often confined to restricted areas. It is present on both the main islands, particularly at Hawke's Bay, Poverty Bay, Bay of Plenty, Northland, King Country, Nelson, South Canterbury and Central and E. Otago; and the incidence of *F. hepatica* infection is increasing in New Zealand.

The occurrence of wide microgeographical variations of *L. tomentosa* in Australia and New Guinea was discussed above. The long, but only temporary isolation of populations is due to the long distances with dry barriers and the variable environment of the continent. The presence of small isolated populations on the mountain ranges north of the endemic areas of Queensland shows the link between the snails of Australia and New Guinea, once possibly connected by land.

It seems that *L. tomentosa* is an indigenous species of Australia and New Guinea, but snails from New Guinea show a more distinctive morphological deviation due to the wide sea barrier and the unsuitable tropical climate of northern Queensland. A similar but more pronounced link is the presence of *L. lessoni* in Australia and New Guinea, but this snail is present on the northern coast of Australia and the separating barrier is narrower. There is little evidence of geographical connection between New Zealand and the Australian continent, and it would seem that *L. tomentosa*, as with some other lymnaeids, could have been introduced to New Zealand by man.

## 2. *Habitats*

The main habitats of the snails are temporary or permanent springs, which exist as natural resources, undergoing great expansion in wet years and providing good refuge during long dry spells. Big creeks and rivers are not very suitable, but adjacent backwaters, swamps and billabongs provide better conditions. However, the large permanent waters harbouring only a few snails are important in the recolonization of temporary streams and carry the snails for long distances. Natural and artificial dams provide drinking water for animals and good living areas for the snails, if connected with springs or small streams, where they are protected against high or low temperatures, flood or drought. Large lakes are usually not suitable for the snails. However, at the mouth of the Murray River in South Australia there are good habitats in the swamps around the edges of Lake Alexandrina and Lake Albert and in the wide channels linking various sections of the lakes, where the water is usually

in motion depending on the direction of the wind. The snails are usually found in irrigation channels in N.S.W., Victoria and South Australia. The main irrigation areas where the snails are present are the Murray River, the Murrumbidgee River and the Goulburn River Valley.

In New Guinea the snails were found in fast-running streams and in the beds of rocky creeks in deep water. In Australia these snails do not favour such aquatic habitats, prefering shallow, marshy conditions. It seems that the higher temperatures in New Guinea are unfavourable for the snails if they are in shallow water, but by adapting to more aquatic conditions they are able to survive.

In Australia, permanent habitats provide indefinite security for the maintenance of snail populations, but the number of snails found in them is much lower than in some temporary habitats. Large populations built up in small temporary streams carrying water into permanent creeks during favourable seasons, when migration and reproduction were stimulated by physical changes. The common snail habitats had an equivalent calculated salinity of 25–160 p.p.m. The highest salinity where permanent snail populations were found in the field was 940 p.p.m. In isolated stagnant dams, existing snail populations are often drastically reduced when the salinity of the water increases due to evaporation.

Clunies Ross and McKay (1929) recorded observations on *L. tomentosa* in 21 habitats in Australia, and found it present from pH 5·4 to 7·3. Boray (1964a) found *L. tomentosa* in 48 natural habitats within a range of pH 5·0–8·0, but about 90% of the suitable habitats were in the range pH 6–7, and the larger proportion of these was slightly acid. There was no significant difference in the pH range for *L. tomentosa* and that for *L. lessoni* and *Physastra* spp., but some planorbids were only found at pH 5–6·5. *Melania* and *Vivipara* spp. were found at a narrower range of pH 6·5–7. No freshwater snails were found when the pH was under 5 or over 8. It seems that *L. tomentosa* can live and breed at a fairly wide range of pH, but appears to be adapted to the slightly acid condition which is predominant in Australia and Tasmania. If the pH is lower than 4·5, which is usual in some parts of the coastal regions of Queensland and N.S.W., it indicates that *L. tomentosa* is absent. A high pH, which occurs mainly in bore waters and some areas rich in carbonates, also indicates the absence of *L. tomentosa*.

## C. REPRODUCTION

### 1. *Sexual activity*

A number of studies on the method of fertilization of European and American lymnaeids were referred to by Kendall (1953), Barraud (1957), DeWitt and Sloan (1958) and Boray (1964b). Both self-fertilization and copulation occur in several species. Self-copulation in isolated *L. stagnalis* never occurs but the snails produce eggs. Egg production was also observed in snails which had their male copulatory organs removed. The sperm derived from the partner at copulation is stored in the receptaculum seminis and transported to the sper-

moviduct where fertilization occurs. Kendall (1953) reported that, after hatching, isolated individual *L. truncatula* produced large numbers of eggs. He did not observe copulation in *L. truncatula* kept in colonies, but Boray (1966) showed it to be common in the laboratory. Isolation is generally unfavourable for reproduction.

Boray (1964b) reported studies on the sexual activity of the Australian *L. tomentosa*, which were observed to copulate in the laboratory and in the field. Copulation by two snails and by more than two partners was observed on many occasions, and chain copulation occurred, involving four to five partners. Field observations suggested that cross-fertilization may be the usual method of reproduction. Experiments with large numbers of snails isolated from the time of hatching showed that neither self-copulation nor reciprocal copulation took place and single isolated snails may reproduce by internal self-fertilization.

*L. tomentosa* usually does not reproduce by self-fertilization when it has the opportunity to copulate. In both laboratory cultures and in the field copulation did not occur below 16°C or above 30°C, or in very dense populations, but was resumed when population density was reduced. Snails kept in pairs produced the first egg masses earlier than single snails kept in isolation. In the field copulation mostly occurred in running streams where conditions seemed to be favourable, giving more rapid growth of the snails and greater egg production. Moderate activity was noted in a dam with some water movement, but little activity in stagnant dams. Copulation was usually induced after sudden physical changes in the habitat, e.g. heavy rainfall or a rise of temperature. Copulation was frequent in newly established channels or in habitats after a dense population had been drastically reduced by flood or molluscicides. The biotic potential of *L. tomentosa* is very great because of the reproductive capacity of an isolated individual. This ensures repopulation in cases where populations are depleted by control measures, by the natural enemies of the snail, or by physical factors.

The preference by *L. tomentosa* for cross-fertilization may be the reason for the wide variety of strains appearing throughout the Australian continent, Tasmania, New Zealand and New Guinea, and for the atypical forms found in single populations (Boray and McMichael, 1961). The effect of ecological conditions, which produce widely different morphological characteristics in the snail, may be complicated by genetical factors due to the opportunity for cross-fertilization by the linkage of different habitats in irrigation systems or during floods.

### 2. *Egg production*

Some data on the egg production capacity of *L. tomentosa* were reported by Boray (1959, 1963a). The observations were carried out in the laboratory on mud slopes with an area of 500 $cm^2$ each at 25°C, with an equal amount of food per snail. Snail populations were observed for a period of 12 weeks, and the maximum egg production was recorded during the most productive 30-day period. The egg masses were removed when counted, so the populations remained constant. The observations were repeated four times and the average

number of eggs was recorded in each population group (Fig. 3). The maximum number of eggs of a single snail was 1265 during its most productive 30 days. Under these experimental conditions, the optimum egg production per snail was dependent on the density of population resulting in similar total egg production per population. An increase of the surface area apparently did not increase egg production.

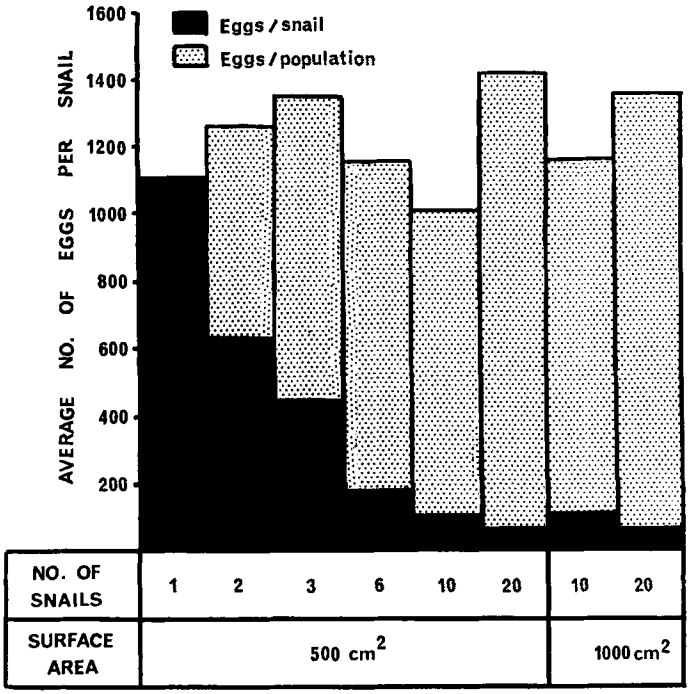


FIG. 3. Egg production of *L. tomentosa*.

Lynch (1963) found generally lower egg production in laboratory cultures. There was a significant reduction of egg production when the surface area was reduced to one quarter of the control boxes, and larger populations produced fewer eggs. In his experiments most eggs were laid around the edge of the experimental area and relative overcrowding occurred irrespective of the surface area. Our field observations seem to confirm this result; *L. tomentosa* has amphibious characteristics and is most abundant around the edges of natural habitats. Some factors such as close contact of snails to one another, excretions and relative food shortage acting simultaneously may be responsible for the crowding effect. Boray (1964b) showed that sexual activity is greatly stimulated by physical changes and reduction of population. These changes were also usually associated with increased production of eggs.

3. *Hatching time*

Some of the results of experiments on the hatching of *L. tomentosa* under controlled temperatures in the laboratory, using more than 5000 eggs, were reported by Boray (1963a). Snail populations were observed daily, the newly produced egg masses were marked, and the time of hatching was recorded when the young snails left their egg-shell. The number of eggs which failed to develop was also recorded (Table I). No development occurred at −2°C

TABLE I

*Hatching of* L. tomentosa

| Temperature °C | No. of eggs | Hatching time in days | | % of normal hatching | Note |
|---|---|---|---|---|---|
| | | Mean | Range | | |
| 2 (±0·5) | 450 | Nil | — | — | No development occurred for 70 days |
| 5 (±0·5) | 480 | 57·5 | 56–60 | 84 | — |
| 7·5 (±0·5) | 510 | 49·0 | 47–50 | 92 | — |
| 16 (±1·0) | 608 | 18·0 | 17–19 | 86 | — |
| 18 (±1·0) | 515 | 15·0 | 14–15 | 88 | — |
| 20 (±1·0) | 621 | 11·7 | 9–14 | 79 | — |
| 23 (±1·0) | 492 | 8·7 | 7–10 | 74 | — |
| 25 (±1·0) | 522 | 6·5 | 5–8 | 67 | — |
| 27 (±1·0) | 492 | 7·8 | 7–9 | 52 | Irregular development |
| 30 (±1·0) | 511 | 9·3 | 9–10 | 20 | „ |

to 2°C, but the embryos survived for more than two months and hatched if brought to higher temperatures. Slow development occurred down to 5°C. The hatching time, and also the hatching rate, decreased with the increase of temperature, but at temperatures higher than 25°C development was irregular, the hatching rate decreased appreciably and the hatching time slightly increased again. Lynch (1963) reported similar results with the South Australian strain of *L. tomentosa*. On the Central Tableland egg production was continuous during the whole year, but numerous eggs were produced in spring and early summer. The hatching of eggs is delayed by low temperatures during winter, but massive spring hatching may contribute to an early increase of snail populations. During the warmer seasons egg masses may hatch 1–3 weeks after being laid. Laboratory and field observations showed that the growth rate of snails also depends on the temperature and other environmental conditons. Snails under favourable conditions reached a mean length of 3mm at 6 days, 4 mm at 14 days and 5·8 mm at 17 days after hatching, and snails aged 20–27 days may produce eggs. One generation may take only about 1 month in the field from late spring until early autumn.

## D. SEASONAL DISTRIBUTION

Field observations were carried out on six different habitats, typical of most Australian endemic areas, and on 26 marked observation areas for a period of 2 years. The total number of adult and juvenile snails were counted and recorded (Fig. 4). The presence of *L. tomentosa* depends largely on climatic factors. Snails were present during all seasons, but in large numbers during the

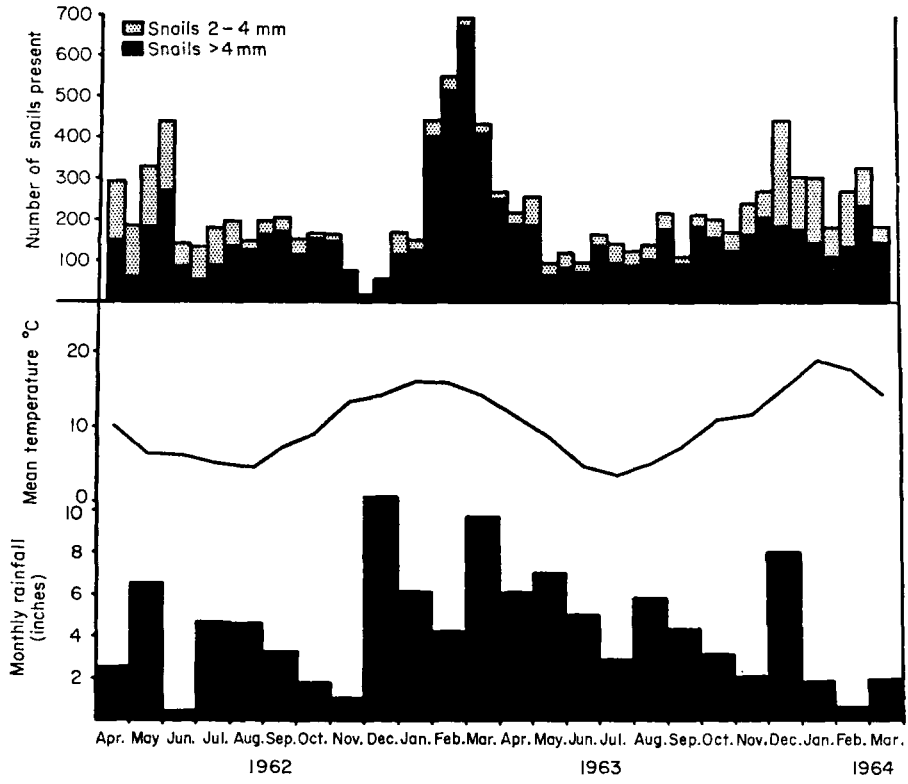


FIG. 4. Seasonal variation of snail population in the field (Central Tableland, N.S.W.).

warmer months, particularly in spring and summer, depending substantially on the prevailing rainfall. Figure 4 also shows that during favourable seasons smaller numbers of adult snails were associated with proportionally larger numbers of juvenile snails, due to increased egg production in populations of lower density. In high density populations reduced reproduction may limit further increase of the number of snails, but drastic reduction of the population results in a sudden response and repopulation would quickly occur.

### E. SURVIVAL OF SNAILS UNDER ADVERSE CONDITIONS

Most areas where *L. tomentosa* occurs in S.E. Australia are exposed to extreme climatic changes depending on the seasons, and particularly on the seasonal distribution of rainfall. The climatic conditions also vary from year to year, and lasting drought and floods are common. Many areas have subzero temperatures during winter, and diurnal variations of $\pm 20°C$ may occur in autumn and spring. Temperatures above 30°C are often encountered in summer. These extreme climatic conditions do not seem to have a long-range influence on snail populations in the endemic areas, and the absence of snails at certain periods is only temporary.

Lymnaeids are apparently excluded from large areas in Australasia, such as N. Queensland and areas of New Guinea less than 2000 ft above sea level, due to persistent high temperatures. However, *L. tomentosa* was found to be resistant to temporary increase of temperature, and in the laboratory snail populations survived for at least 6 weeks when kept at 36°C, although reproduction was impaired.

Lymnaeids are more resistant to low temperatures and various species, including the European *L. truncatula*, do not suffer from the long, cold winter (Mehl, 1932). The Australian *L. tomentosa*, which is adapted to the subtropical climate, was also found to be resistant to low temperatures. In the field, snails were not affected by frost, and an experimental population remained alive and active for 3 years when kept at 2–5°C in the laboratory. The snails may for some time remain dormant under the frozen mud, but they are active again when the temperature has risen in the field (Boray, 1959). Temperature influences the vertical distribution of snails. During the winter months at temperatures near freezing point, snails are usually found in deeper water where the temperature is slightly higher, sometimes at a depth of 12–24 in. They can live for long periods without emerging from the water, due to their low metabolic rate and low oxygen consumption. During the winter, snails migrate in the direction of the springs where the temperatures are higher. At hot summer temperatures most snails are found in shallow water or around the edges of dams or out of water on damp mud; they have to leave the water more frequently at high temperatures to satisfy their oxygen requirements.

Dry conditions would be expected to influence snail populations, particularly in Australia where dry conditions are usually associated with high temperatures.

The ability of lymnaeids to spend a dormant period in drought was reported by Dupuy as early as 1847–1852. Mehl (1932) referred to the early literature, and stated that *L. truncatula* can survive for up to $4\frac{1}{2}$ months in dry mud. Kendall (1949a) referred to some later literature, and found that *L. truncatula* may aestivate for more than a year in the laboratory, but he stated that the laboratory results could be far behind the maximum possible in the field. He found that many snails survive with the aperture attached to the mud surface; burrowing was occasionally observed but was not essential for aestivation. Olsen (1944) observed in Texas that *L. bulimoides* often burrows into soft mud in the field and aestivates for about 5 months.

In Australia, Seddon (1928) reported that the snail may survive for 3 weeks

on damp mud. Clunies Ross and McKay (1929) suggested that the wide-mouthed snail with a low shell may not be able to aestivate, and resistance of different species of lymnaeids to desiccation may vary. In field observations large populations of *L. tomentosa* survived for at least 77 days in summer. Before the observation area dried up the average length of snails was 6·0 mm, but was 3·5 mm when the area was covered with water again, suggesting that young snails may be the more resistant to desiccation. Immediately after they regained activity in the laboratory, previously aestivating snails were gradually desiccated in a dish containing 10 cm deep mud and 80% of them survived for an additional 75 days. Laboratory experiments were carried out in earthenware dishes at 22–24°C and 55–75% relative humidity. Some of these results were reported (Boray, 1959, 1963a). Snails survived for only 7 days when mud was absent, for 121 days when they were allowed to attach to the surface of the mud, and for 332 days under the mud (Table II). Snails were observed actively

TABLE II

*Aestivation of* L. tomentosa (*average length 6 mm*)
*Temperature:* 22–24°C. *Relative humidity:* 55–75%

| Experimental conditions | Time of aestivation in days | % of snails survived |
|---|---|---|
| Earthenware dish, dry surface without mud | 7 | 10 |
| | 10 | 0 |
| Dry surface of mud | 4 | 80 |
| | 20 | 71 |
| | 32 | 70 |
| | 60 | 60 |
| | 100 | 30 |
| | 121 | 6·6 |
| Burrowed under 8–10 cm deep mud | 40 | 72 |
| | 62 | 50 |
| | 62 | 3·3* |
| | 130 | 70 |
| | 332 | 14 |

* Length of snails > 10 mm.

burrowing into the mud if its consistency was not too hard but, as in the results of Kendall (1949) with *L. truncatula*, burrowing was not essential for the aestivation of *L. tomentosa*. In the field *L. tomentosa* aestivated for long dry periods in winter at low temperatures. A dry winter is usual on the N. Tablelands of N.S.W. The aestivation of another wide-mouthed lymnaeid, *L. auricularia natalensis*, was reported by Bitakaramire (1967 and personal communication) in Kenya.

In the assessment of various factors influencing the epidemiology and control of fascioliasis due to *F. hepatica* or *F. gigantica*, it should be assumed that all lymnaeid snails may aestivate for a considerable time during dry periods at

both low and high temperatures. Lynch (1965b) studied the physiological process of aestivation, concluding that the external stimulus to aestivation is desiccation. In our experiments the metabolic rate of snails was reduced during aestivation, and also during the dormant period (hibernation) at low temperatures. This was indicated by the reduction of the normal rate of heart beat of active adult snails from 66/min at 26°C to 5–21/min at 4°C, or during aestivation at 18–24°C. The possible physiological response of the snails for temporary increased retention of water, which was assumed to depend on internal factors (Fisher, 1931), remains to be investigated.

### F. DISPERSAL OF SNAILS

The sudden reappearance of snails at dried-up habitats often led to speculations on vigorous migration of snails from one area to another. Waterfowl were suspected to be a means of transport (Kew, 1893). However, our present knowledge on the ability of lymnaeids to aestivate and hibernate seems to support observations by Mehl (1932) that the relatively small *L. truncatula* did not migrate considerable distances on moist soil when the habitat was drying. Similar restricted movements of *L. tomentosa* were observed in the field in Australia during dry periods. Active migration of European *L. truncatula* in streams was not noted, but Mehl (1932) suggested that lowered oxygen tension could provoke the snail to leave stagnant pools, and also that snails may be carried many kilometres by streams.

In Australia, the stream gradient of habitats has an important influence on distribution by providing a faster or slower flow of water (Boray, 1964a). If the water lies between steeply sloping banks, it is less likely to harbour a big snail population. A medium stream velocity of about 15–20 cm/sec is associated with algal production, and with active migration and rapid reproduction of the snails. A faster flow removes the suitable alluvial mud, and the snails, from their environment. Flushing of streams by frequent local floods increases the amount of passive migration, reduces populations from certain areas, and increases snail colonies at other locations. Floods affecting larger districts introduce snails into new areas hundreds of miles away from their origin.

The active and passive migration of *L. tomentosa* was observed in the field with marked snails, but because of large and rapidly changing populations the marked snails were diluted and their recovery was not reliable enough to evaluate the results. For an accurate assessment of their ability to migrate actively, experimental channels of 25–30 cm wide, about 10 cm deep and 150 m long were dug in dry land on slopes which had never been inhabited by snails. The deepest end of the channels was connected with a small dam with a slow water movement, which contained populations of approximately 150 snails/$m^2$ at the beginning of the experiment. The upper end of the channel was supplied with water from a dam through plastic pipes. The water was forced through an elaborate filtering device before entering the channel, to prevent possible contamination by snails or snail eggs from the upper dam. One experiment was carried out during winter when the channel was occasionally covered with snow and the water surface was often frozen, and a second experiment was

TABLE III

*Number of* L. tomentosa *counted in experimental channel with a water current of* 15 *cm/sec in winter*

| Date 1960 | Time in weeks | Distance in metres 1–10 | 11–20 | 21–30 | 31–40 | 41–50 | 51–60 | 61–70 | 71–80 | 81–90 | 91–100 | 101–110 | 111–120 | 121–130 | 131–140 | 141–150 | Average Temperature °C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8/6 | 0 | 0 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 5·8 |
| 22/6 | 2 | 22 | 0 | — | — | — | — | — | — | — | — | — | — | — | — | — | 5·9 |
| 6/7 | 4 | 26 | 0 | — | — | — | — | — | — | — | — | — | — | — | — | — | 4·8 |
| 20/7 | 6 | 32 | 0 | — | — | — | — | — | — | — | — | — | — | — | — | — | 4·7 |
| 3/8 | 8 | 24 | 2 | 0 | — | — | — | — | — | — | — | — | — | — | — | — | 4·5 |
| 17/8 | 10 | 26 | 28 | 24 | 15 | 17 | 2 | 0 | — | — | — | — | — | — | — | — | 4·6 |
| 31/8 | 12 | 24 | 19 | 34 | 19 | 11 | 14 | 1 | 2 | 1 | 4 | 0 | — | — | — | — | 5·1 |
| 14/9 | 14 | 127 | 102 | 46 | 47 | 17 | 16 | 10 | 21 | 3 | 1 | 6 | 0 | 2 | 2 | 1 | 6·2 |

TABLE IV

*Number of* L. tomentosa *counted in experimental channel with a water current of* 18 *cm/sec in late spring*

| Date 1961 | Time in weeks | Distance in metres 1–10 | 11–20 | 21–30 | 31–40 | 41–50 | 51–60 | 61–70 | 71–80 | 81–90 | 91–100 | 101–110 | 111–120 | 121–130 | 131–140 | 141–150 | Average temperature °C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11/10 | 0 | 0 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 8·9 |
| 25/10 | 2 | 26 | 0 | — | — | — | — | — | — | — | — | — | — | — | — | — | 9·5 |
| 8/11 | 4 | 21 | 41 | 6 | 0 | — | — | — | — | — | — | — | — | — | — | — | 12·2 |
| 22/11 | 6 | 29 | 31 | 12 | 9 | 0 | — | — | — | — | — | — | — | — | — | — | 14·0 |
| 6/12 | 8 | 21 | 44 | 31 | 45 | 21 | 6 | 6 | 12 | 24 | 0 | 18 | 11 | 14 | 2 | 3 | 15·0 |

carried out in a new channel during late spring. The average speed of the water current was 15 and 18 cm/sec respectively. Tables III and IV show that the speed of migration depended on the temperature, but snails migrated up to 150 m at temperatures as low as 4·5–6·2°C in approximately 3 months. Most of the migration occurred during the last 4 weeks of the experimental period. At higher temperatures the snails migrated 150 m in 8 weeks, but the last 100 m was achieved by the snails in 2 weeks.

The passive migration of snails was measured by applying wire screen as a snail trap on to the outflow of a small dam at the bottom of a 5-acre paddock containing several small water dams connected with channels. Snails can attach their foot to the water surface, floating and drifting with the current for long distances, and a total of 2803 snails drifted out of the experimental area in about a year (Table V). The number of snails which left the area monthly was depen-

TABLE V

*Passive migration of* L. tomentosa

| | Months | No. of snails recovered | Monthly rainfall in points | Highest rainfall during one week in points | Snail population |
|---|---|---|---|---|---|
| 1960 | December | 764 | 491 | 328 | + + + + |
| 1961 | January | 251 | 255 | 125 | + + + |
| | February | 0 | 267 | 176 | + |
| | March | 0 | 403 | 240 | + |
| | April | 359 | 302 | 165 | + + + |
| | May | 0 | 45 | 18 | + |
| | June | 99 | 253 | 102 | + |
| | July | 80 | 293 | 155 | + |
| | August | 178 | 487 | 245 | + + |
| | September | 212 | 126 | 53 | + + |
| | October | 0 | 338 | 154 | + + |
| | November | 446 | 678 | 570 | + + + + |
| | December | 414 | 460 | 170 | + + + + |
| | Total | 2803 | | | |

dent on population size in the area and on rainfall. Evidently *L. tomentosa* is capable of active and passive migration, which must be considered in molluscicide treatment of an area. Small springs and streams are usually connected permanently or temporarily with more permanent snail habitats in larger creeks or irrigation channels. Molluscicide treatment has to be carried out from the origin of the water to prevent rapid recolonization by passive migration, but if snail habitats are connected with permanent populations usually inaccessible to molluscicide treatment, recolonization of the habitats may also occur by active migration.

### G. BREEDING AND MAINTENANCE IN THE LABORATORY

It is relatively easy to breed truly aquatic snails in the laboratory, but difficult to provide suitable laboratory conditions for amphibious or semi-aquatic snails such as *L. truncatula* or *L. tomentosa*. Schumacher (1938) described a successful system in earthenware dishes with a continuous flow of water. The use of spring water was recommended, but accumulation of minerals through evaporation renders the dishes unsuitable for snails and the mud must be changed every 8 days (Lämmler, 1955). The use of mud slopes inoculated with algae, described by Taylor and Mozley (1948), was successful for breeding *L. truncatula*, and has been improved and standardized by Ollerenshaw (personal communication), with relatively pure algal cultures as food. The method is widely used for the propagation of European intermediate snail hosts.

The basic principle of breeding lymnaeids in the laboratory, particularly suitable for *L. tomentosa*, was described by Boray (1963b). Boxes 1 m by 60 cm and 10 cm high are lined with plastic sheeting and filled with sterilized mud taken from areas where the snails abound. The mud is soaked with de-ionized water and zig-zag channels 3–4 cm deep are formed in it. They are filled with water and one end of the box is raised to provide a moderate slope. De-ionized water is dripped into a high point at a speed of 100–200 ml/h, i.e. sufficient to replace water lost by evaporation and to remove metabolic products. The lower end of the box has a specially designed outflow syphon which serves as drainage and also controls the water level.

Originally, the boxes were exposed to natural or artificial light to facilitate algal growth, but smaller perspex boxes are now used, the amount of mud is reduced into one corner of the box, more intensive aeration is applied and algae are totally excluded as a source of food in order to achieve further standardization. As a standard laboratory snail food, 3% calcium sulphate is added to a mixture of 25% dried ground lettuce, 25% dried ground lucerne leaves and 50% ground wheat germ. All experimental snails are now fed on this diet in this laboratory. The snail boxes are kept at 22–24°C in thermostatically controlled rooms, with no direct natural light. The technique which was developed for *L. tomentosa* is equally suitable for *L. truncatula*, and breeding boxes used for *L. tomentosa* serve also for rearing *L. auricularia* (*natalensis*). The survival rate of snails infected with *F. gigantica* was appreciably higher in the boxes than in the aquaria (Boray, 1963b).

All other lymnaeids used in our experiments are bred in aquaria 40 cm by 25 cm and 26 cm deep containing sterilized mud. The aquaria are filled with de-ionized water, constantly aerated by bubbling, and circulated through a perlon-wool or fibre glass–wool filter. Periodically some water from the bottom of the tanks is removed, together with excreta of the snails, and replaced with de-ionized water. The cultures are kept at a constant temperature of 22–24°C. This simple technique has also been used successfully for the breeding of large numbers of *L. auricularia* (*natalensis*), *L. peregra*, *L. palustris*, *L. stagnalis*, *L. lessoni* and many planorbid species, including *Bulinus* and *Physastra* spp. (Boray, 1966 and unpublished data).

## III. Susceptibility of Snails and Adaptation of the Parasite

### A. Relative Susceptibility

*F. hepatica* and/or *F. gigantica* occur throughout the world wherever conditions are suitable for the completion of their life cycles. In most of these areas, *F. hepatica* is transmitted by *L. truncatula*, and *F. gigantica* by *L. auricularia* s.l.,* the intermediate host, or by varieties insufficiently distinctive to be regarded as separate species. In some countries where *Fasciola* spp. were introduced with infected stock, e.g. Australia, Canada, North America and some Pacific Islands, the parasites seem to have established a new relationship with indigenous snail species by adaptation. The speed of such adaptation would depend on the relative ecological and biological suitability of the particular snail for the larval development of the fluke, and on the longevity of the fluke in the mammalian host.

Species other than *L. truncatula* may act as an alternative host for *F. hepatica* in Europe (Kendall, 1950, 1965; Berghen, 1964; Furmaga and Gundlach, 1967a, b). Full development of the parasite may occur in a few very young specimens of *L. stagnalis*, *L. palustris*, *L. glabra* and *L. auricularia* s.l., but development terminates at the redia in *L. peregra* (*peregra*), and no development at all occurs in *L. auricularia* s.s. and *L. peregra* (*ovata*). However, *L. peregra* (*ovata*) is susceptible to artificial infection (Goderdzishvili, 1955; Bogomolova, 1961). Natural infections with cercariae were claimed to occur in *L. peregra* (*peregra*) in Scotland (Taylor, 1922) and in Russia (Goderdzishvili, 1955), but no evidence was given that the cercariae observed were, in fact, the cercariae of *F. hepatica*. *L. peregra* (*peregra*) was considered unsuitable for full larval development in experimental infections with *F. hepatica*. Boray (1966) studied the relative susceptibility of some unusual lymnaeid hosts to infections with *F. hepatica* and *F. gigantica* in laboratory experiments, and the results are summarized in Table VI. Viable metacercariae were produced from young *L. peregra* (*peregra*) after artificial infection with *F. hepatica*, and also from other lymnaeids which previous authors found to be susceptible.

*L. truncatula* is widely distributed and is usually found in semi-aquatic habitats. The successful infection and production of metacercariae by *L. peregra* (*peregra*) might be of more importance than that of the aquatic *L. palustris* and *L. stagnalis*. *L. peregra* (*peregra*) may live under aquatic conditions, but it is found more often than other lymnaeids in habitats similar to those of *L. truncatula* where natural infection may occur. Boray (1966) showed that *L. peregra* (*peregra*) shed cercariae for at least 179 days in the laboratory. The snail might serve as an alternative reservoir host for *F. hepatica* in certain areas.

Studies on infections of the snails with *F. gigantica* of different origin (Boray, 1966) showed that this parasite is not as host-specific as *F. hepatica*. Kendall (1954) suggested that *F. hepatica* and *F. gigantica* are clearly separated by the ecological demarcation of their respective snail hosts, and that there is

* The taxonomic names of the species of lymnaeids used in this review accord with the concept of Hubendick (1951).

TABLE VI

*Species and origin of snails and their susceptibility to flukes in first passage*

| Snail | | Origin of fluke | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Fasciola hepatica* L. | | | *Fasciola gigantica* Cobb. | | |
| Species (type or strain) | Origin of snail | Weybridge, England | Hanover, Germany | N.S.W., Australia | Kenya, East Africa | Java, Indonesia | Ipoh, Malaysia |
| *Lymnaea stagnalis* | Lower Saxony, Germany | — | YC | — | YC | — | YC |
| *L. palustris* | Lower Saxony, Germany | — | YC | — | YS | — | — |
| *L. peregra* (*peregra*) | Burgenland, Austria | — | YC | YC | YC | — | YC |
| *L. peregra* (*ovata*) | Lower Saxony, Germany | — | YC | — | YC | — | YC |
| *L. truncatula* | Lower Saxony, Germany | AFC† | AFC† | AFC* | ADC* | — | ADC* |
| *L. auricularia* (natalensis) | Kenya, East Africa | YC | YC | — | AFC† | AFC* | AFC* |
| *L. tomentosa* (Strain "A") | N.S.W., Australia | ADC† | ADC* | AFC† | AFC* | AFC* | AFC* |
| *L. tomentosa* (Strain "B") | South Australia, Australia | — | ADC* | AFC† | AFC* | — | AFC* |
| *L. tomentosa* (Strain "X") | N.S.W., Australia | — | ADC* | AFC† | AFC* | — | — |
| *L. tomentosa* (McMaster strain) | N.S.W., Australia | ADC* | ADC* | AFC† | — | — | — |
| *L. tomentosa* (Goroka strain) | Eastern Highlands, New Guinea | — | — | ADC† | ADC* | — | — |
| *L. lessoni* | N.S.W., Australia | — | YS | YS | N | — | — |
| *L. lessoni* | Markham Valley, New Guinea | — | — | YS | — | — | — |

AFC = Adult snails fully susceptible, viable cercariae produced
ADC = Adult snails susceptible, host-parasite disparity, viable cercariae produced
YC = Only young snails susceptible, viable cercariae produced
YS = Only young snails susceptible, development terminates at sporocyst stage
N = Susceptibility nil
* = The relationship is an important potential source of transmission
† = The relationship is the common source of transmission

no evidence of the existence of a common snail host for both *F. hepatica* and *F. gigantica*. Alicata (1946) showed that *L. ollula* in Hawaii was potentially a suitable host for both *F. hepatica* and *F. gigantica*. Alicata (1953) found a second species, the introduced *L. columella*, to be a good host for *F. gigantica*, and the same snail was said by Krull (1933a) to be an intermediate host of *F. hepatica*. Dinnik and Dinnik (1957) found *L. truncatula* susceptible to infections with *F. gigantica*, and found it to be the common snail host of *F. gigantica* in the irrigation area near the slopes of Mount Kenya. Boray (1966, 1967c) demonstrated that *L. tomentosa* from both Australia and New Guinea is fully susceptible to *F. hepatica* and to *F. gigantica*, and can be infected at any age; also that adult *L. truncatula* can be infected with *F. gigantica* and produce metacercariae. Full development of *F. gigantica* occurred in a very high proportion of *L. peregra* (*peregra*) and *L. peregra* (*ovata*) more than one month old. All three types of *L. peregra* (*peregra, ovata and lagotis*) and *L. truncatula* were claimed to be naturally infected with *F. gigantica* in the Turkmen S.S.R. (Kibakin, 1960). In certain areas, mixed infections of stock with *F. hepatica* and *F. gigantica* have been reported (Kendall, 1954; Güralp *et al.*, 1964).

The less specific requirements shown by *F. gigantica* for its intermediate host as compared with *F. hepatica*, might be a manifestation of appreciably greater adaptability. This permits the speculation that *F. gigantica* evolved from the more stable *F. hepatica*, which originally inhabited the temperate climate of the Northern Hemisphere (Boray, 1966). However, the apparent introduction of *L. truncatula* and *F. hepatica* into most endemic areas of *F. gigantica* might have caused a break in the isolation of *F. gigantica*. In some areas, such as the Gulf Coast of North America and Japan, *F. gigantica* was introduced into the endemic areas of *F. hepatica*. Mixed infections with *F. hepatica* and *F. gigantica* resulted in the emergence of intermediate types (Price, 1953; Watanabe, 1962). The microgeographical strains of *L. tomentosa*, the common intermediate host of *F. hepatica* in Australia, New Zealand and New Guinea, were susceptible to *F. gigantica* (Boray, 1966, 1967c; Boray and Dinnik, unpublished data), which could spread rapidly if the parasite was introduced from neighbouring Indonesia and Malaysia.

### B. ADAPTATION OF *Fasciola* SPP. TO LYMNAEIDS

The speciation of digenetic trematodes and the potential evolution of new host-parasite relationships between larval stages of flukes and freshwater snails were discussed by Wright (1960, 1962, 1966). Hubendick (1954) suggested that temporary isolation may lead to the formation of microgeographic strains, but not to new species of freshwater snails. Some differences in appearance were found in the Australian intermediate host of *F. hepatica* by Boray and McMichael (1961), but these strains were variant populations of the widely distributed species, *L. tomentosa*. Boray (1966, 1967c) discussed the question of evolution in geographical races between lymnaeids and *Fasciola* spp. Although many lymnaeids were to some degree susceptible to infection with both *F. hepatica* and *F. gigantica*, there was great variation in their susceptibility; experimental evidence of the adaptation of *F. hepatica* to lymnaeids

was obtained by laboratory passages. He suggested that similar adaptation to less susceptible snails may occur in the field when infected mammalian hosts are present and the common intermediate host is absent.

The results of the studies on subsequent laboratory passages of the European and Australian strains of *F. hepatica* in *L. tomentosa* from Australia and New Guinea, and the European *L. peregra* (*peregra*) in homogenous populations of the two lymnaeids, were reported by Boray (1967c), and further experimental results are reported in the present review (Tables VII, VIII and IX). The

TABLE VII

*Infections of adult* L. tomentosa (*McMaster strain*) *with* 20 F. hepatica *miracidia per snail*

| Passage | Origin of *F. hepatica* | No. of snails | Infection rate with rediae % | Mortality of infected snails % | Mean no. of viable metacercariae per snail |
|---|---|---|---|---|---|
| 1 | Germany | 200 | 65·0 | 93·0 | 678·7 |
| | Australia | 200 | 90·0 | 48·9 | 726·1 |
| 3 | Germany | 47 | 90·0 | 21·6 | 316·0 |
| | Australia | 39 | 100·0 | 25·0 | 392·0 |
| 5 | Germany | 51 | 100·0 | 41·0 | 352·0 |
| | Australia | 50 | 100·0 | 36·0 | 374·0 |
| 6 | Germany | 200 | 98·0 | 55·5 | 473·0 |
| | Australia | 200 | 96·0 | 51·5 | 345·0 |

Australian *L. tomentosa* was less susceptible in the first passage to the European strain of *F. hepatica* originating from Germany; irregular larval development occurred in the snails with consequent high mortality, but a degree of adaptation was observed as early as in the 3rd passage (Boray, 1966). Table VII shows the results of subsequent passages of the German strain of *F. hepatica*, which apparently adapted fully to the Australian *L. tomentosa*. The *F. hepatica* strain from Weybridge, England, showed similar incompatibility in the first passage, but a rapid adaptation (Boray, 1967c). This strain showed full adaptation in further passages in this laboratory. Although a certain degree of disparity was demonstrated between the European *F. hepatica* and the indigenous Australian *L. tomentosa*, it was rapidly overcome by the selection of larvae which found the snail suitable for the completion of their development to the infective stage.

Boray (1967c) showed that the susceptibility of the New Guinea strain of *L. tomentosa* to the Australian *F. hepatica* was lower in the first passage, mortality was not higher but larval development was slower, with fewer rediae, and consequently fewer cercariae developed. In the 2nd and 3rd passages the susceptibility increased, but fewer cercariae developed and the development was still slower. The development of cercariae was quicker in the 4th and 5th passages but still fewer cercariae were produced (Table VIII).

TABLE VIII

*Infections in adult* L. tomentosa *with* 20 F. hepatica (*Australia*) *miracidia per snail*

| Origin of snail | Passage | No. of snails | Infection rate with rediae % | Mortality of infected snails % | Minimum development time of cercariae at 22–24°C in days | Mean no. of metacercariae per snail |
|---|---|---|---|---|---|---|
| New Guinea (Goroka strain) | 1 | 100 | 25·3 | 25·0 | 42 | 56 |
| | 2 | 75 | 54·0 | 35·0 | 38 | 102 |
| | 3 | 50 | 68·0 | 35·0 | 45 | 65 |
| | 4 | 30 | 60·0 | 42·0 | 30 | 70 |
| | 5 | 50 | 71·0 | 38·0 | 30 | 90 |
| Australia (McMaster strain) | X | 200 | 96·0 | 51·5 | 28 | 345 |

The introduction of *F. hepatica* to the Territory of Papua and New Guinea occurred during the last 20 years. The New Guinea strain of *L. tomentosa* is a less suitable snail for *F. hepatica.* A different aspect of the host-parasite disparity was manifested by slower and lesser production of infective larvae. Under natural conditions full adaptation is likely to occur but might take many generations. The economic losses due to *F. hepatica* infection in domestic animals in New Guinea might not be as serious as they are in Australia for many years to come.

*L. peregra* (*peregra*) was only susceptible to *F. hepatica* and produced cercariae if it was infected during the first 7 days after hatching in the first passage. Some evidence of adaptation was demonstrated in subsequent passages, in which snails aged 2–3 weeks were successfully infected. As evidence of further adaptation, some snails about 6 weeks old were infected and produced metacercariae in the 3rd and 5th passages, but snails with a minimum age of 7 weeks which became infected in the 5th passage lost their infection, possibly due to a tissue reaction of the host (Table IX). The destructive effect of this tissue reaction was demonstrated in *L. stagnalis* when rediae were transplanted into the haemocoel of adult snails; the rejection of *F. hepatica* rediae by adult *L. peregra* was also reported (Boray, 1966).

The adaptation of *F. hepatica* to *L. peregra* (*peregra*) may be an extremely slow evolutionary process, and could only take place in isolated areas where the presence of more suitable hosts would be excluded. In such areas, *F. hepatica* may become slightly better adapated, through passages, to *L. peregra* (*peregra*), which may serve as a reservoir intermediate host for the parasite, although because of strong host-parasite disparity perhaps unimportant in the transmission of the disease. Disparity between lymnaeid snails and *Fasciola* spp.

TABLE IX

*Infections of the Laboratory strain of* L. peregra (peregra) *with* 25 F. hepatica *miracidia per snail*

| Passage | No. of snails | Age of snails in days | Infection rate with sporocysts or young rediae % | Number of snails shedding cercariae | Minimum development time of cercariae at 22–24°C in days |
|---|---|---|---|---|---|
| | 700 | 1–7 | 70·0 | 63 | 49 |
| 1 | 350 | 14–21 | 0 | 0 | — |
| | 100 | 28–30 | 0 | 0 | — |
| 2 | 150 | 1–7 | 77·0 | 74 | 28 |
| | 150 | 14–21 | 24·7 | 30 | 28 |
| | 400 | 1–5 | 68·0 | 36 | 40 |
| 3 | 67 | 21–30 | 28·5 | 0 | — |
| | 150 | 42–50 | 6·6 | 1 | 42 |
| | 100 | 1–3 | 72·0 | 0 | — |
| 4 | 100 | 14–21 | 21·0 | 7 | 56 |
| | 100 | 28–32 | 0 | 0 | — |
| | 200 | 1–7 | 75·0 | 21 | 28 |
| | 100 | 28–40 | 18·5 | 3 | 38 |
| 5 | 500 | 50–60 | 9·0 | 0* | — |
| | 250 | 60–90 | 0 | 0 | — |

* Rediae died before cercariae developed

is manifested by absolute resistance, age-resistance, low susceptibility and high mortality of the adult snails (Boray, 1966), and in the low susceptibility and slower development of fewer rediae, which was demonstrated in adult *L. tomentosa* from New Guinea (Boray, 1967c).

It may be concluded that in newly formed relationships between *Fasciola* spp. and an unusual snail host, the adaptation of the trematode might occur rapidly as a result of passage if the snails have a degree of susceptibility in their adult stage, e.g. various strains of *F. hepatica* in the various geographical races of *L. truncatula* of Eurasia and Africa; *L. bulimoides* and *L. columella* of North America; the *L. viator* and *L. diaphana* complex and *L. columella* of South America; *L. tomentosa* of Australasia; and *L. viridis* (*ollula*) of the Pacific region.

Similarly, rapid adaptation may occur by the geographical races of *F. gigantica* in various races of *L. truncatula* and *L. auricularia* s.l. in Eurasia, in *L. columella* of North and South America, in *L. tomentosa* of Australasia and in the *L. viridis* (*ollula*) complex of the Pacific areas. It seems, however, that rapid adaptation cannot be expected in relationships where strong age-resistance is present, such as between *F. hepatica* and *L. stagnalis*, *L. peregra* s.l., *L. palustris* and *L. auricularia* s.l. Slightly more rapid adaptation may be expected between *F. gigantica* and *L. stagnalis*, *L. palustris* and *L. peregra* s.l.

Some susceptible species of microgeographical races of planorbid snails are not equally competent intermediate hosts for different strains of *Schistosoma haematobium* and *S. mansoni* (Files and Cram, 1949; Wright, 1962; Paperna, 1968), and similar host-parasite disparity was found between various species and races of *Oncomelania* and various strains of *S. japonicum* (DeWitt, 1954; Hsü and Hsü, 1967). A strong age-resistance was not evident in these relationships with some host-parasite disparity. The present evidence of the adaptability of *F. hepatica* to different relatively non-susceptible *Lymnaea* spp. suggests the possibility that in some of the less competent race combinations within established specific relationships, the observed disparity may be only temporary. Many schistosome strains introduced by movements of human populations may adapt rapidly to a relatively less susceptible snail within a few passages.

## IV. Biology of *Fasciola hepatica*

### A. Eggs and miracidia

The literature concerning the development of *F. hepatica* eggs and the hatching of miracidia was summarized by Pantelouris (1965). It has been stated by many authors that development does not take place below 10°C. Rowcliffe and Ollerenshaw (1960) showed that the hatching time depends on the temperature, but development is inhibited above 37°C and below 9·5°C.

The development of eggs was observed in the field for a period of 18 months, at a typical endemic area of Australia on the Central Tablelands of N.S.W. (Boray, unpublished data). Large numbers of eggs in open jars were exposed to different environmental conditions in snail habitats. Samples were collected weekly, refrigerated immediately and taken to the laboratory for examination. A standard method, based on physical and biological criteria, was developed for testing the viability of miracidia. Their movements, speed and penetrability into susceptible snails were observed. The infectivity of miracidia was tested in young susceptible snails, and the presence of sporocysts was easily detected 24 h after exposure. Miracidia swimming in circles were usually not infective. The speed of the swimming miracidia after hatching was a good indicator of their infectivity. The speed of infective miracidia was 4–12 sec/cm. If miracidia were slower than about 30 sec/cm they were not infective to snails.

During winter the hatching time was 90–100 days at an average temperature of 5°C with a range of 1°C to 11°C. Eggs exposed in winter for 80 days hatched normally after incubation in the laboratory and were infective to *L. tomentosa.* About 60–90% of the exposed eggs survived the winter period and hatched in early spring. The shortest incubation period in summer at an average temperature of 15°C (range: 10°C–25°C) was 21 days. During summer the development of eggs was delayed and the hatching rate was lower in stagnant pools with a lot of organic matter compared with habitats with moderate water movement. In laboratory experiments eggs survived at any stage of their development for at least 12 months at 4–5°C, and were infective to *L. tomentosa* after incubation. *F. hepatica* eggs kept in the refrigerator at 4–5°C, after removal from the gall bladder of infected animals, survived for at least 2 years

and after incubation the miracidia were infective to the snails. However, when eggs were kept below freezing point they lost their viability. Eggs kept at −15°C for 24 h did not develop after incubation.

### B. EFFECT OF ENVIRONMENT ON DEVELOPMENT

The effect of the physical environment of the host snails on the parthenogenetic development of *F. hepatica* was discussed by Kendall (1949a, b) and Kendall and Ollerenshaw (1963). Davtyan (1956) considered the problem in relation to both *F. hepatica* and *F. gigantica*, and Dinnik and Dinnik (1956, 1963, 1964) in respect of *F. gigantica*. Kendall (1965) gave a summary and references to the literature.

In Australia, Boray (1963a) studied the effect of temperature on the development of cercariae in *L. tomentosa*. Below 10°C the larval stages of *F. hepatica* survived for at least 100 days without completing their development, and without causing apparent damage to the snails. Larval development was completed and large numbers of metacercariae were produced when the temperature was raised to 20°C. One snail population survived for 3 years at 2°C–5°C (see Section IIE). These larvae may survive much longer at low temperatures than is necessary for overwintering, which explains the possibility of survival by the parasites in northern Europe, Scandinavia, the Baltic area and some parts of Siberia, where one generation of metacercariae may take 2–3 years to develop. Boray (1963a) showed that the increase of temperature decreased the development time, which agrees with other reports summarized by Kendall (1965). Metacercariae production in *L. truncatula* could not be carried out at temperatures higher than 28°C (Kendall, 1965). It seems that *L. tomentosa* is more adaptable to higher temperatures, and that *F. hepatica* has an extremely wide range of temperature tolerance. However, Boray (1963a) showed that higher temperatures during development may affect the viability of metacercariae. He used two expressions: "minimum development time" was recorded when the first "free cercariae" (cercariae which have left the rediae) were visible through the transparent shell of the snail, and which, if emerged, were able to encyst and infect a definitive host; and "maximum development time" was regarded as the time when the majority of cercariae are free in the snail's body ready for emission and encystment. In an experiment carried out with more metacercariae and more sheep (Table X), the developmental period was shorter when the temperature was increased, and the viability of metacercariae was not affected up to 23°C. The infectivity of metacercariae decreased slightly at 26°C and substantially at above that temperature, but some metacercariae produced at 35°C were infective to sheep.

Snails were usually short-lived in temperatures above 35°C. In Australia and other subtropical or tropical countries, three generations of metacercariae may be produced during the warmer half of the year and an additional generation may complete its development during the remainder. The number of generations may be increased in certain districts, such as the warmer irrigation areas in northern N.S.W. and Victoria, but the infectivity of the metacercariae may decrease.

TABLE X

*Recovery of* F. hepatica *from sheep infected with* 200 *metacercariae each*

| Temperature °C | Development time in days | | No. of flukes recovered from 5 sheep | | Recovery % |
|---|---|---|---|---|---|
| | Minimum | Maximum | Average | Range | |
| 15±1 | 57±8 | 84±10 | 96·0 | 52–144 | 48·0 |
| 23±1 | 30±2 | 41±5 | 98·0 | 56–151 | 49·0 |
| 26±1 | 28±1 | 38±7 | 82·5 | 28–114 | 41·2 |
| 30±1 | 24±3 | 27±4 | 31·4 | 11–71 | 15·7 |
| 35±1 | 23±4 | 26±3 | 12·2 | 0–26 | 6·1 |

If snails are kept on a higher plane of nutrition, more metacercariae are produced (Kendall, 1949b; Kendall and Ollerenshaw, 1963; Boray, 1963a). Relative shortage or abundance of food may occur in the field, often depending on the density of snail populations. Boray (1963a) showed that dense populations or small isolated populations may produce similar numbers of metacercariae, and the presence of numerous snails is usually associated with a high infection rate in sheep, but a survey revealing only a few snails does not necessarily indicate less contamination of the pasture with metacercariae.

Kendall (1965) referred to studies on the larval development of *F. hepatica* in aestivating snails, using *L. truncatula.* Field and laboratory experience in Australia supports these data, and *F. hepatica* was found to survive for long periods and develop in aestivating *L. tomentosa.* Stimulation of natural emission of cercariae was observed, due to sudden physical changes after aestivation or hibernation. This stimulation, particularly in aestivating snails, plays an important part in intermittent massive infections of sheep and cattle in the irrigation areas of Australia (see Section VIIIA).

### C. STANDARD PRODUCTION OF METACERCARIAE

Many laboratories are engaged in experimental work on various aspects of fascioliasis, and information on the disease may be emerging from series of experiments carried out over several years. The results of such experiments should be comparable and reproducible. We have seen that external factors, such as temperature during development, may modify the infectivity of metacercariae. Variable results after experimental infection may also accrue from different methods of storage and administration of metacercariae. The basic requirements of a standard method to achieve reasonable uniformity of different batches of metacercariae were described by Boray (1963b). Steps were taken to eliminate variables in the maintenance of snails during infection by keeping the cultures at a constant temperature and using a standard diet (Section IIG). Later, generally higher and uniform numbers of flukes were recovered from experimental animals after artificial infections with metacer-

TABLE XI

*The production of metacercariae by* L. tomentosa *experimentally infected with* F. hepatica *miracidia at* 23 ± 1°C. *Average length of Shells:* 6 *mm* (4–9)

| Experimental conditions | No. of infected snails | No. of metacercariae produced | Average no. of metacercariae |
|---|---|---|---|
| Various strains, algae | 360 | 54000 | 150 |
| Selected strain, algae + food mixture | 1348 | 337000 | 250 |
| Homogenous strain, food mixture only | 895<br>2003 | 412000<br>1022000 | 460<br>510 |
| Homogenous strain, modified snail boxes, intensive feeding, high oxygen tension | 495 | 308000 | 622 |
| Total | 5101 | 2133000 | 418 |

cariae produced in this laboratory (Boray, 1967a). A similar standard procedure was used for the successful production of *F. gigantica* metacercariae (Boray, 1966; Hildebrandt, 1967, 1968a, b). Table XI shows the increased efficiency in the production of metacercariae and the production capacity of *L. tomentosa*, as the conditions for maintenance and adaptation improved during the evolution of the standardized technique in this laboratory. Several modifications in the Weybridge technique were also carried out by Ollerenshaw (personal communication) to produce standard metacercariae of *F.hepatica* from *L. truncatula.*

1. *The selection of strains*

Considerable morphological changes may occur in populations of *L. tomentosa* subjected to different environmental conditions (Boray and McMichael, 1961). Variants have been described. The laboratory strain found to be most susceptible to *F. hepatica* was selected and maintained for many generations. These snails were more susceptible to infection and more successful in producing large numbers of metacercariae than snails collected freshly from the field, or even their first progeny.

Taylor and Mozley (1948) found that *L. truncatula* reared in the laboratory usually grew bigger than snails found in the field. It was observed (Boray, 1963b) that first generations of *L. truncatula* collected in the field and reared in the laboratory with the shallow water system suffered high mortality. After several generations, an adapted strain emerged, the average size of the snails increased and lower mortality occurred after infection. Similar observations have been made by Dinnik (personal communication) with *L. auricularia natalensis*. For metacercaria production a highly susceptible strain adapted to laboratory conditions should be used.

2. *Miracidia*

Bile from the gall bladder of sheep artificially infected with metacercariae in the laboratory (homogenous sheep strain) is collected, filtered through an 80 mesh/in bronze sieve in a conical urine glass and decanted several times. The eggs in water are placed in sterile disposable petri dishes within black boxes, or petri dishes may be painted outside with black plastic paint, so that eggs can be incubated in complete darkness. Dishes are filled with aerated tapwater and contain only one layer of eggs, because too many eggs can delay development. The eggs are incubated at 26°C for 12 days and used immediately for infection.

3. *Infection phase*

Snails 4–6 weeks old (4–6 mm) are placed in plastic petri dishes in groups of ten. The dishes are filled up to the top with de-ionized water, and approximately 20 eggs containing miracidia are added for each snail. Aliquots are used to determine the number of miracidia in the water before infection. Infection may also be carried out by adding the required number of miracidia in the snail-maintenance boxes. The success of infection may be determined within 24 h. Young sporocysts are readily recognizable in the tissues, in the heart or in the haemolymph system of live snails.

4. *Developmental phase*

If infection occurs in petri dishes, the snails are moved into the maintenance boxes, fed adequately and kept at a constant temperature of $23 \pm 1$°C. The boxes are checked every day, or every 2nd day, to feed the snails if necessary and to remove dead snails. Two weeks after infection the rediae are recognized in live snails by using a dissecting microscope with direct light. Sufficient standard food is necessary to produce more and larger rediae and more cercariae (Kendall, 1949b; Boray, 1963a). During the minimum development time of approximately 30 days, about 30–40% mortality in the snails may be expected. After this period cleaning, spraying or flushing is avoided, because any sudden physical change can induce shedding of cercariae already developed. From the 40th day the snails are examined microscopically, and if a large proportion of rediae contain developing and already pigmented cercariae, or if some of the cercariae are already free, the snails are removed from the boxes.

5. *Accumulation phase*

During aestivation of infected snails, an accumulation of free cercariae occurs in the body cavity. This phenomenon can be used for completing the final stage of development of the cercariae. Snails with many rediae which contain numerous cercariae are placed in plastic petri dishes lined with filter paper soaked in de-ionized water. The snails are kept on the wet filter paper at room temperature, fed with the standard food mixture, and moved into new dishes every second or third day. During this period, large numbers of cercariae are completing their development and leaving the rediae. Because they

are unable to emerge from the snail without submersion, the cercariae apparently ready for encystment accumulate in large numbers in the body cavity of the snail and are easily visible microscopically. The snails are examined daily until the majority of cercariae are free in the body.

6. *Shedding phase*

Schumacher (1938) observed with *L. truncatula* that sudden physical changes, mainly of temperature, induce the shedding of cercariae. He used the "cold shock" method to obtain cercariae from snails. With *L. tomentosa* and *L. L. truncatula* many other changes, such as flushing of stagnant habitats, and particularly the rewatering of aestivating populations, produced massive emission of cercariae. Boray (1963b) found that if snails were removed from the filter paper when most of the cercariae appeared to be free and were put into water, massive shedding occurred. However, a few days later most of the snails died, together with cercariae which had been unable to emerge. If the cercariae were liberated mechanically by destroying the snails when they were still active, many more cercariae encysted. Their viability and infectivity was comparable with that obtained by natural emission. In the routine technique, therefore, the cercariae accumulated in the snail's body are liberated into sterile plastic petri dishes by means of two dissecting needles. The foot of the snail is removed because the mucus produced hinders proper encystment of some cercariae. Encystment on cellophane (Urquhart, 1954) was satisfactory, but the cysts were not suitable for long storage. After liberation of cercariae, rediae and encysting cercariae are mixed by a fresh water jet and the petri dish is allowed to stand overnight. After about 16 h the whole contents of the dish are washed out with water; dead rediae and non-encysting cercariae are then removed and the dish is placed in the refrigerator.

7. *Counting and storage of metacercariae (cysts)*

The metacercariae encysted in the petri dish are counted under a dissecting microscope on a glass slide ruled by a diamond pencil. The bottom of the plastic dishes can also be ruled for easy counting. Incomplete cysts are not counted. After counting, all the metacercariae are brushed off the surface with a soft hair brush and washed into a funnel fitted with a filter paper of 9 cm diameter. The washing is repeated with aerated tap water. The filter papers with collected metacercariae are removed from the funnel, placed in 2 oz jars with a loosely closed lid, and these stored at temperatures of 4–6°C.

8. *Testing the viability of metacercariae*

Before use in an experiment, every batch of metacercariae produced by the standard technique is tested for viability by physical and biological means, as described by Boray (1963b). If the metacercariae are alive, the typical formation of excretory granules is visible under the microscope. For easier examination, the outer layer of the metacercariae may be removed. Active movement of the young fluke may be observed if the cysts are placed on a warm stage at

38°C. Dead cysts appear as diffuse masses without any typical structure, and occasionally only empty cysts are seen. Metacercariae may also be excysted in artificial digestive fluids (Susuki, 1931; Wikerhauser, 1960; Dixon, 1966b).

Guinea-pigs and mice were found to be the most susceptible animals for the viability test. Each batch of metacercariae is tested in 20 mice using a single metacercaria for each mouse, and in two guinea-pigs given ten metacercariae each. The mice are killed 2 or 3 weeks later, examined for migratory damage and the flukes recovered from the liver. In routine viability tests, the appearance of the liver damage alone is satisfactory proof of viability. The guinea-pigs are killed 4 weeks later, examined and the flukes recovered. The mouse test is the more suitable and only mice are now used in regular tests. The metacercariae are regarded as fully viable if at least 18/20 mice are positive.

9. *Administration of metacercariae to experimental animals*

The metacercariae are injected into the stomach of mice, rats and guinea-pigs under general ether anaesthesia with specially designed needles (Boray, 1963b). Metacercariae are placed in a small glass vessel. A tuberculin glass syringe is attached to the needle and water is drawn into the syringe from another vessel. The cysts are then sucked into the needle so as not to penetrate more than a few mm into it. The contents of the syringe are then emptied, the water washing the cysts into the stomach. After administration the needle is washed out and checked microscopically for cysts left in the needle. Metacercariae may be given to guinea-pigs in small gelatine capsules, or on a piece of lettuce, but should be given to rabbits in gelatine capsules with a specially designed tube, and to sheep and cattle with a commercial instrument employed for giving drugs in bolus or in capsules.

## D. VIABILITY AND RESISTANCE OF METACERCARIAE

It has been generally believed that several days are necessary after encystment for metacercariae to reach their full infectivity for definitive hosts (Dawes, 1962a). Hughes (1963) found that 2-day-old metacercariae were infective. Boray (1963a) found that cercariae did not infect mice before or immediately after their encystment, but were fully infective 24 h after encystment. No difference was found in the viability of groups of metacercariae tested 2, 5, 8, 16 and 30 days after encystment. Metacercariae which passed through the snail's digestive system several times were fully viable. About 10% of cercariae encysted on the water surface by enclosing small air bubbles, which enabled them to float for long periods, and thus to infect hosts through drinking.

The survival of metacercariae encysted on the herbage serves to maintain the life cycle of the parasite during periods which are unsuitable for the production of cercariae. Boray and Enigk (1964) surveyed the literature which showed that metacercariae may survive long periods, but there was no evidence on the effect of temperatures lower than 0°C, and very little information on their resistance to temperatures higher than 25°C. Boray and Enigk (1964) studied the survival and infectivity of *F. hepatica* and *F. gigantica* metacercariae produced under standard conditions and exposed to different temperatures

and relative humidities in the laboratory. Survival was tested in thermostatically controlled incubators at temperatures from −20 to +35°C, with a deviation of not more than ±1°C. Some of the tests were carried out at a controlled relative humidity in climate chambers. The metacercariae were exposed to experimental conditions on wet filter paper and controls were stored similarly. Viability was tested by physical and biological means (see Section IVC).

Experiments with *F. hepatica* showed that after sudden, gradual or intermittent exposure to −20°C for 12 h, the metacercariae were unable to infect susceptible hosts. Most metacercariae retained their viability for 7 days and some were still infective after 28 days at −10°C, but if they were frozen in water at −10°C they were destroyed at 7–28 days. Some metacercariae survived and were infective at −5°C for 28 days, but non-infective after 56 days. If they were kept at −5°C for 12 h and at +10°C for 12 h daily, a high proportion were infective after 70 days. At −2°C they were infective for at least 92 days. The results indicate that *F. hepatica* metacercariae can survive during the usual winter conditions in Australia and even in the coldest countries in Europe, particularly if the infected pastures are covered by snow. Taylor (1949) suggested that repeated freezing and thawing may have a more detrimental effect than a constant low temperature, but metacercariae were fully infective for at least 70 days after exposure to repeated freezing and thawing at −5°C and +10°C. The metacercariae were more susceptible to desiccation than to freezing (Boray and Enigk, 1964). Freezing at low temperatures did not kill the metacercariae, but apparently caused irreversible changes which made them unable to infect the hosts. Thus, the results of microscopical examination are not sufficient as a test of viability, but may serve in the case of cysts exposed to high temperatures, when they agree with the result obtained by biological test.

A high proportion of *F. hepatica* metacercariae are viable and infective for at least 130 days at +10°C, 36 days at +25°C and 14 days at +30°C. About 50% of the metacercariae survived for 60 days at +25°C and at least 20% for 36 days at +30°C. The metacercariae died when kept at +35°C for 14 days. The short survival period at high temperatures has particular application to Australian climatic conditions, and confirms the observations made in Britain by Ollerenshaw (1959), who found that metacercariae on grass lived twice as long in winter as in summer.

Boray and Enigk (1964) showed that metacercariae lost their infectivity in most tests, when exposed in a climate chamber to an atmosphere of 75–80% relative humidity at 20°C for 3 days; at the same temperature and a relative humidity of 90% some metacercariae were infective after an exposure of 14 days, but none was infective after 27 days. Some metacercariae survived for 31 days at 10°C and a relative humidity of 75–80%, but they were fully infective for at least 122 days at 10°C and 90% R.H. It was found by Marek (1927) that metacercariae survived for 8 months in moist hay collected during a rainy period. Nöller and Schmid (1929) stated that heavily contaminated hay, incompletely dried and stored under the roof of a stable for 1–3 weeks, resulted in only light infection when fed to animals, and was non-infective after 5–6 months. Clunies Ross and McKay (1929) in Australia found that metacercariae are very susceptible to desiccation. In summer, the cysts lived for only 2 days when exposed

to sunshine and for 17 days when in the shade. Ono *et al.* (1954) found that all metacercariae died in 10 days on grass dried at 25–32°C. Boray and Enigk (1964) suggested that as high as 90% relative humidity is required for metacercariae to survive in hay, which would have to be stored at a low temperature. Under European winter conditions, inadequately dried hay from fluke-infested areas stored in cold and wet conditions may cause infection when fed to animals, but properly dried and stored hay is unlikely to produce substantial infection. Enigk and Hildebrandt (1964) found, however, that metacercariae placed in hay at low temperatures may survive for 2–3 months at apparently lower relative humidity. It seems that in Europe the feeding of hay from contaminated areas in winter may produce infection in sheep and cattle, but the risk of infection from hay may be negligible in Australia or other tropical and subtropical countries.

Metacercariae of *F. gigantica* and *F. hepatica* did not survive in silage (Alicata, 1938; Wikerhauser and Brglez, 1961), and may be fed to animals in winter without risk of infection. Metacercariae seem to be less resistant to high temperatures and desiccation, which suggests that under Australian conditions they may have a limited life. The infection of stock is more likely to be due to recently produced metacercariae. Boray and Enigk (1964) found that the metacercariae of *F. gigantica* survived longer at higher temperatures (114 days and 21 days at 30°C and 35°C respectively) than those of *F. hepatica*. *F. gigantica* metacercariae were less resistant to exposure to 90% relative humidity than those of *F. hepatica*, and therefore infection with *F. gigantica* by feeding dried hay may not be possible. *F. gigantica* is confined to subtropical and tropical countries and its intermediate host is an aquatic snail. The experimental results are in accord with the adaptation of *F. gigantica* to aquatic conditions and to higher temperatures.

### E. STRUCTURE OF METACERCARIAE, ENCYSTMENT AND EXCYSTMENT PROCESSES

The cyst wall of *F. hepatica* and *F. gigantica* has been described as a structure with two layers (Wright, 1927; Alicata, 1938). A survey of the literature on the structure of the metacercariae of digenetic trematodes was provided by Dixon (1965), and he deduced that metacercariae which encyst externally may have a more complex structure than those encysting in a second intermediate host. He studied the structure of *F. hepatica* metacercariae produced from *L. tomentosa* in Australia by histological and histochemical techniques, regarding the cyst wall as an outer and an inner cyst complex. The outer cyst wall consists of two layers; the external thick layer is a tanned protein and covers the metacercariae dorsally and laterally; another thin layer adheres to the inner surface of the outer layer, and is composed of mucoprotein and acid mucopolysaccharide. The inner cyst wall consists of two layers, the first one made up of three sub-layers. The first complex layer of the inner cyst consists of (a) mucoprotein, (b) acid mucopolysaccharide, (c) neutral mucopolysaccharide. In the dorsal and lateral regions, the second major layer in the inner cyst appears to be formed of lamellae held in a protein-lipid matrix, and is composed

of protein stabilized by disulphide linkages. In the ventral region of this last layer, a thickened area consisting of mucopolysaccharide forms a ventral plug. Dixon (1965) concluded that the outer cyst wall acts as a barrier against bacterial and fungal infections, but it also fastens the cysts to grass or any solid object during the encystment process. Strong adhesion to grass for long periods is important for the survival of metacercariae and infection of the hosts. Because the cysts may survive for long periods and remain infective if the outer cyst wall is removed (Boray, 1963a), the inner cyst walls must play a more important part in the survival of the metacercariae.

Dixon (1965) suggested that the young flukes within the cyst may secrete a mucopolysaccharidase capable of digesting the ventral plug of the cyst wall, as suggested by Schumacher (1938) and Hughes (1959). The structure of the layers described above was confirmed by Dixon and Mercer (1964) by electron microscopy. They found the inner layer of the inner cyst very dense and compact, composed of protein sheets formed from tightly wound scrolls. The formation of these scrolls seems to explain the cyst formation process, from intracellular vacuoles unrolled at the surface of the cercariae after secretion. The cyst wall is produced by the cystogenic cells of the mature cercariae. The presence of such cells was observed by Thomas (1883), and the related literature has been reviewed by Stirewalt (1963). Further references to the literature were made by Dixon (1966a) in his study of the morphology and histochemistry of the secretory cells of the cercariae of *F. hepatica.* He found four types of cystogenic cells, which could be characterized by their position and by their histochemical properties, and were related to the four layers in the cyst wall of metacercariae (Dixon, 1965). The fine structure of these cystogenic cells was subsequently described by Mercer and Dixon 1967), using an electron microscope, and revealed further details of the encystment process. In recent work, Dixon and Mercer (1967) produced a film of the entire process, and successive stages were studied by fixing metacercariae at intervals during encystment. The layers of the cyst walls of *F. hepatica* are formed from precursors synthesized in the cystogenic cells of cercariae, separate components being released in sequence during encystment. Separate groups of cells are producing the outer cyst and the rapid process is completed in a few minutes. After the production of a further polysaccharide layer, rod-like scrolls of sheets of the laminated component of the inner wall emerge at the surface of the cercariae, and movements of the cercaria within the enveloping outer cyst wall compact the material into the lamellar inner wall. The rodlets are enclosed in vacuoles, and their secretion is effected by the fusion of the vacuolar membrane with the plasma membrane; the cells will constitute the epithelium of the juvenile fluke.

The excystment process of *F. hepatica,* carried out under *in vitro* and *in vivo* conditions, was studied by many authors, and Dawes and Hughes (1964) referred to most of the relevant literature. The excystment has been basically attributed to the action of digestive enzymes. However, Hughes (1959) and Dawes (1961a) showed that young flukes emerge from cysts injected into the abdominal cavity of experimental hosts. Two sheep were given 1000 metacercariae each by intraperitoneal injection in this laboratory, and 420 and 352

mature flukes respectively were recovered from their livers 14 weeks after the injection. This recovery was similar to reports regarding oral infection. Evidently excystment is an active process which does not require digestive enzymes of the host.

Dixon (1966b) carried out intensive studies on the excystment process in Australia and referred to many previous papers. Excystment of the metacercariae of *F. hepatica* is an active process occurring in two stages, activation and emergence. Activation is initiated by carbon dioxide, reducing conditions and a temperature about 39°C. The emergence phase is triggered by bile. He suggested that the presence of bile may activate an enzyme secreted by the parasites, inducing muscular movements of the young fluke. He also suggested that an enzyme produced by the fluke may digest the ventral plug region of the cyst facilitating the emergence of the fluke. He assumed that the activation may take place in the rumen or stomach of the hosts, and emergence would take place in the small intestine below the opening of the *ductus coledochus*. However, the information on the apparently normal excystment of metacercariae in the peritoneal cavity of hosts suggests that the process of active excystation, apart from a temperature of about 39°C and possibly the presence of a low concentration of carbon dioxide, may require very little additional stimulus, and further studies on the problem may be justified.

### F. INTERMEDIARY METABOLISM

References to metabolic studies on *F. hepatica* were given by Pantelouris (1965). The concept of biochemical uniformity between host and parasites has been suggested in the past, based on the identification of some intermediates of the metabolic pathways. Many enzyme systems of trematodes are similar to the vertebrate systems, but some recent *in vitro* experiments showed that delicate differences may exist between the metabolism of parasites and their hosts.

Extensive studies were made of the intermediary metabolism of adult *F. hepatica*, and further references regarding the problem were given by Prichard (1968) and Prichard and Schofield (1968a, b, c, d, 1969) in Australia. The volatile fatty acid production, glycolysis, carbon dioxide fixation, tricarboxylic acid cycle, electron transport, glyoxylate cycle and glyconeogenesis were investigated. In the case of glycolysis and the tricarboxylic acid cycle a direct comparison was made with a vertebrate tissue, namely rat liver. Whole *F. hepatica* were found to excrete volatile fatty acids. Quantitatively, the major acids were acetic and propionic, accounting for 33 and 61% respectively of the volatile fatty acid production. In addition, formic, *iso*butyric, *iso*valeric, 2-methyl-butyric and traces of *n*-butyric, *n*-valeric, 2-methyl-valeric and 2-methylcrotonic acid were produced. By the use of labelled glucose, it was demonstrated that the volatile fatty acids were produced as end products of carbohydrate metabolism.

Overall glycolysis and the enzymes of the Embden-Meyerhof pathway were investigated in cell-free extracts from *F. hepatica* and rat liver. Glycolysis from

glucose was demonstrated in the presence of fructose-1, 6-diphosphate and pyruvate. The rate of lactate formation in the trematode was lower than that with rat liver. The following enzymes were found to have similar specific activities and cofactor requirements in both tissues: hexokinase, glucose-6-phosphate dehydrogenase, phosphoglucomutase, glucosephosphate isomerase, mannosephosphate isomerase, phosphofructokinase, aldolase, triosephosphate isomerase, glycerolphosphate dehydrogenase, glyceralde hydephosphate dehydrogenase, phosphoglycerate kinase, glycerate phosphomutase and phosphopyruvate hydratase.

Very little pyruvate kinase activity could be detected in *F. hepatica*, although this enzyme was readily demonstrated in rat liver. Pyruvate phosphate ligase (AMP), an enzyme with the same glycolytic function as pyruvate kinase, could not be demonstrated in the fluke. Lactate dehydrogenase activity was also much lower in the parasite, being only one-fortieth that of rat liver. It appears that only in the last two steps of glycolysis, those catalysed by pyruvate kinase and lactate dehydrogenase, does the parasite differ from the vertebrate tissue; glucose is degraded to phosphoenolpyruvate, but the major fate of this substance in *F. hepatica* appears not to be metabolized to pyruvate. The fate of phosphoenolpyruvate was found to be carboxylation to form dicarboxylic acids. Carbon dioxide is fixed by whole flukes, resulting in the formation of dicarboxylic acid of which the major constituent is succinate. The carboxylating enzymes, phosphoenolpyruvate carboxykinase and malate dehydrogenase (decarboxylating), were detected in *F. hepatica*. Phosphoenolpyruvate carboxylase, phosphoenolpyruvate carboxytransphosphorylase and pyruvate carboxylase could not be demonstrated in the fluke.

Oxaloacetate formed by the carboxylation of phosphoenolpyruvate was found to be largely reduced to succinate through the action of malate dehydrogenase (non-decarboxylating), fumarate hydratase and the reversal of succinate dehydrogenase, and the reduction of fumarate by NADH. In this series of reactions, NADH can be reoxidized anaerobically in the reductions of oxaloacetate and fumarate.

As well as reduction to succinate, oxaloacetate formed by phosphoenolpyruvate carboxykinase action is metabolized to pyruvate by malate dehydrogenase (non-decarboxylating) and malate dehydrogenase (decarboxylating). This pathway of pyruvate formation was found to be sensitive to the presence of pyridine nucleotides, and may be an important point of metabolic control over the level of reduction of the pyridine nucleotides, an important requirement for an organism living in an almost anaerobic environment.

The succinate and pyruvate formed by these pathways may then be converted to the excretion products, propionic and acetic acids respectively. The pathway to succinate and propionate appears to be quantitatively more important than that to pyruvate and acetate.

The cofactor requirements of the phosphoenolpyruvate carboxykinase in *F. hepatica* were for IDP, GDP or ADP, the level of effectiveness descreasing respectively. Those of malate dehydrogenase (decarboxylating) were for $NAD^+$, activity with $NADP^+$ being considerably lower. These carboxylating reactions were readily demonstrated in both directions. Phosphoenolpyruvate carbo-

xykinase was found to be dependent on the presence of a divalent cation, the following metal ions being effective in decreasing order: $Zn^{2+}$, $Co^{2+}$, $Mn^{2+}$ and $Mg^{2+}$. Maximum activity for this enzyme was detected at pH 5·9. These two carboxylating enzymes were found to be distributed in both the supernatant and particulate fractions.

The enzymes of the tricarboxylic acid cycle have been studied in cell-free preparations from *F. hepatica*, and their cofactor requirements, specific activities and subcellular localization compared to the corresponding enzyme from rat liver. Succinate dehydrogenase, oxoglutarate dehydrogenase, pyruvate dehydrogenase and glutamate dehydrogenase were primarily located in the particulate fraction in both tissues. Succinate dehydrogenase was assayed in both directions in each tissue; the reverse reaction, the reduction of fumarate, could be accomplished in *F. hepatica* with NADH as cofactor. Glutamate dehydrogenase was much more active in the parasite than in the vertebrate tissue, and it is possible that this enzyme is involved in the production of ammonia by *F. hepatica*. This enzyme was considerably more active with $NAD^+$ than with $NADP^+$, especially in the trematode.

Citrate synthase and malate dehydrogenase (non-decarboxylating) were demonstrated in both tissues, and appeared to be evenly distributed between the particulate and supernatant fractions in each case. The specific activities were similar for each tissue.

Aconitate hydratase, *iso*citrate dehydrogenase and fumarate hydratase were largely confined to the supernatant fraction in *F. hepatica*, no aconitate hydratase or fumarate hydratase activity being detected in the particulate fraction. The levels of aconitate hydratase and *iso*citrate dehydrogenase were considerably lower in the fluke than in rat liver, while the latter enzyme was only active with $NADP^+$ and not $NAD^+$ as cofactor in *F. hepatica*. Both $NAD^+$-and $NADP^+$-specific *iso*citrate dehydrogenases were demonstrated in the vertebrate tissue.

The particulate fraction from *F. hepatica* did not metabolize $C^{14}$-2-pyruvate to $C^{14}O_2$, and this, together with the absence or low activity of some of the tricarboxylic acid cycle enzymes in this fraction, suggests that this cycle does not operate in isolated mitochondria from the fluke. However, some tricarboxylic acid cycle activity could be detected when the whole homogenate was used; this preparation contains all the enzymes necessary for the activity of the cycle. While the reactions of this pathway may function to some extent in a cyclical manner, many of them appear to be more important in contributing to the production of succinate and volatile fatty acids by fermentative pathways.

Respiration was demonstrated in cell-free fractions from *F. hepatica*, and was found to be stimulated by succinate and NADH, but not by malate. Azide and Antimycin A reduced respiration only at very high concentrations, while cyanide did not inhibit oxygen uptake, and at high concentrations markedly increased it. These results suggest that a mammalian type of cytochrome chain is not primarily responsible for respiration in *F. hepatica*, while the effect of cyanide suggests it is removing an inhibition on a flavoprotein enzyme possibly caused by the accumulation of hydrogen peroxide. The oxidation

of NADH was found to be inhibited by amytal and rotenone, suggesting the involvement of a flavoprotein enzyme. Succinoxidase activity was reduced by malonate and 2-thienyltrifluoroacetone, which indicates the presence of the dehydrogenase and non-haem iron in the pathway of succinate oxidation.

The oxidation of NADH and succinate in the particulate fraction from *F. hepatica* resulted in the production of hydrogen peroxide. A low level of peroxidase activity was measured in the supernatant fraction, this activity possibly being associated with a haemoglobin pigment which was detected.

Difference spectra of the particulate fraction from *F. hepatica* showed the presence of a *b*-type cytochrome with absorption maxima at 557, 526 and 426 mμ. Evidence was obtained, based on carbon monoxide spectra, of a possible *o*-type cytochrome with peaks at 570, 536 and 418 mμ and troughs at 555 and 441 mμ. This *o*-type cytochrome may contribute to the absorption about 557 mμ. Spectral evidence was also obtained for the presence of flavoproteins, cytochromes *b* and $c_1$, while possible traces of cytochromes *o* and *a* were detected. The component with absorption peak at 557 mμ was found to be readily reduced by NADH and rapidly oxidized by fumarate. Fumarate and oxygen may act as terminal electron acceptors in *F. hepatica*, the fumarate being the preferred acceptor.

The pathway of electron transport from NADH appears to involve the dehydrogenase, flavoprotein components, the *b*-type cytochrome, *o*-type cytochrome and either fumarate or oxygen. That from succinate may include the dehydrogenase, flavoprotein components, non-haem iron, the *b*-type cytochrome, the *o*-type cytochrome and oxygen. Although vestiges of a mammalian type of cytochrome chain are apparent, they do not appear to be contributing significantly to electron transport in *F. hepatica.*

Evidence was obtained for the presence of a glyoxylate cycle in *F. hepatica*, the two key enzymes of the cycle, *iso*citrate lyase and malate synthase, being detected. This cycle may be significant in the formation of succinate as the precursor of the propionate which is excreted, or in glyconeogenesis under suitable conditions.

Fructose diphosphatase was found to be active in *F. hepatica* and to have an absolute requirement for $Mg^{2+}$, while EDTA also increased its activity. AMP was found to inhibit this enzyme. Maximum activity was found at pH 9. Little glucose-6-phosphatase activity could be shown in *F. hepatica*, suggesting that gluconeogenesis may not be important in this parasite.

All of the reactions necessary for hexosephosphate formation from succinate were demonstrated, and it is possible that succinate and its precursors may be important as substrates for glyconeogenesis; $C^{14}$-succinate and other metabolites were found to be incorporated into glycogen by whole flukes. It is possible that one part of the fluke, such as muscle, is fermenting carbohydrate, while another part is synthesizing carbohydrate in a manner analogous to vertebrate muscle and liver. In this respect there is some suggestion that the anterior cone is implicated in glyconeogenesis. In view of the extremely high carbohydrate content of the eggs of *F. hepatica* (Horstmann, 1962; Wilson, 1967), glyconeogenesis may be of some importance to the adult liver fluke.

In more recent studies, the method of Flatt and Ball (1964), which uses

glucoses labelled with radioactive carbon together with the resultant determination of isotope incorporation in the end products, was used to test the effect of some hormones on the metabolism of *F. hepatica* (Buist and Schofield, personal communication). It was shown that hydrocortisone, ACTH, epinephrine and insulin had no effect. Serotonin, however, increased glucose uptake and the rate of breakdown of glycogen, which confirm the results of Mansour (1962).

In the studies summarized above, some enzyme activities in the metabolic pathways of *F. hepatica* were determined and some of them were compared with those of a vertebrate system. It was concluded that there was a certain conformity in both systems, but sufficient evidence was presented of biochemical diversity between the host and the parasite.

Thorsell (1967) showed that adenosine triphosphatase was activated, but succinate oxidase and cholinesterase were inhibited, when homogenized flukes were incubated in some concentrations of hexachlorophene, a highly efficient anthelmintic against *F. hepatica*. Empirical screening for new and more efficient anthelmintics against *F. hepatica* has been fairly successful during the last 10 years, but has still failed to produce the ideal drug for safe, economic and preventive medication of fascioliasis. More knowledge on the intermediary metabolism of *F. hepatica*, particularly of enzyme activity in the pathways, may lead to a new revolutionary approach for selecting a substance for the elimination of the parasites.

## V. *Fasciola hepatica* IN DEFINITIVE HOSTS

### A. GROWTH

Marek (1927) reported that the length of *F. hepatica* was 1–4 mm at 4 weeks, 1–17·0 mm at 6 weeks, 7–23 mm at 8 weeks, and 7·5–24·0 mm at 11 weeks after infection in ruminants, thus showing considerable variation in size. The growth of the fluke in mice was studied by Dawes (1962a, b), who also found great variation in the size of fluke of similar age. A summary of his findings, together with a discussion on the possible reasons for variation of growth rate, was given by Dawes and Hughes (1964).

The growth rate of *F. hepatica* in most small laboratory hosts appeared to be faster during the first few weeks than in ruminants. Table XII shows the results of observations by Boray *et al.* (1967a) and Tewari (1969). It appeared that the growth rate of fluke in the albino rat and in the guinea-pig is comparable with that in lightly infected ruminants, with great variation in length of fluke of the same age and within one animal. It was found in other experiments in this laboratory that, similar to observations in ruminants, the growth of fluke was also retarded in guinea-pigs with heavy infection (See Table XII).

Table XIII shows data on the length of flukes at different ages and different levels of infection in sheep. The information is the result of measurements of many thousands of flukes with a standard technique, and reported earlier by Boray and Happich (1966a, b), Boray *et al.* (1967b) and Boray (1967a). There was a 3-fold increase in the average length of flukes between the 6th and 8th

TABLE XII

*The length of* F. hepatica *in* 166 *albino rats and* 20 *guinea-pigs infected with* 25 *and* 10 *metacercariae each respectively*

| | | Albino rat | | Guinea-pig | |
|---|---|---|---|---|---|
| Age of flukes weeks | No. of flukes measured | Length of flukes in mm Mean | Range | Length of flukes in mm Mean | Range |
| 4 | 30 | — | — | 3·6 | 3·0– 6·0 |
| 5 | 173 | 3·7 | 2·0– 5·5 | — | — |
| 7 | 49 | 4·9* | 3·0– 8·0 | — | — |
| 8 | 17 | — | — | 7·0* | 6·0—11·0 |
| 9 | 118 | 6·8* | 3·0–13·5 | — | — |
| 11 | 69 | 9·6* | 5·0–16·0 | — | — |
| 12 | 17 | — | — | 12·5* | 9·0–15·0 |
| 13 | 48 | 10·6* | 5·0–16·0 | — | — |
| 16 | 6 | — | — | 17·0* | 14·0–20·0 |
| 20 | 132 | 13·0* | 5·0–18·5 | — | — |

* *F. hepatica* eggs were present in bile

TABLE XIII

*The length of* F. hepatica *in* 49 *experimentally infected sheep aged* 4–5 *years*

| | | Heavy infection | | Light infection with a mean fluke burden of 102 | |
|---|---|---|---|---|---|
| Age of flukes weeks | Mean no. of flukes | Length of flukes in mm Average | Range | Length of flukes in mm Average | Range |
| 4 | 986 | 2·4 | 1·0– 3·5 | 2·8 | 1·0– 4·5 |
| 6 | 1630 | 3·9 | 1·5– 7·5 | 3·0 | 1·5– 6·0 |
| 8 | 2193 | 5·2 | 1·5–11·5 | 8·9 | 4·0–14·0 |
| 9 | 1536 | 5·8 | 1·5–13·0 | — | — |
| 10 | 1314 | 7·3 | 1·5–16·0 | 10·2 | 3·0–17·0 |
| 12 | 934 | 6·9 | 2·0–15·0 | 13·7 | 6·5–19·5 |
| 13 | 930 | 8·0 | 2·5–17·0 | 14·3 | 3·0–20·0 |
| 14 | 780 | 15·4 | 2·5–25·0 | 16·4 | 9·0–23·0 |
| 15 | 464 | 10·1 | 2·5–24·0 | 14·5 | 9·5–23·0 |
| 16 | 646 | 9·9 | 3·5–18·5 | — | — |
| 30 | — | — | — | 20·5 | 10·5–29·0 |
| 46 | — | — | — | 20·3 | 11·0–31·0 |

weeks, and then the length increased more gradually until the 14th week, in light infections. There was a very wide range in the length of flukes, particularly in heavy infections. One reason for individual variation in the length of fluke in the early migration period may possibly be the long interval between the entry of the first and the last flukes from the peritoneal cavity into the liver. The flukes which enter earlier may have faster growth before the resultant pathological changes make the liver less suitable for penetration and nutrition (Boray, 1967a). After an initial period of feeding on liver cells (Dawes, 1961b), the nutritional requirements of flukes may be better satisfied in the bile ducts, and the flukes which reach the bile ducts early develop faster than others which are still migrating in the tissues. It was shown that in light infections, after an initial period of development in the liver tissues, a proportion of the flukes were already in the bile ducts at 6 weeks and most of them were there at 8 weeks. From the present information it is evident that retardation of growth in *heavy* infections is appreciable and does not commence before the 6th week after infection, which is the critical period in sheep when tissue fibrosis may prevent normal entry into the bile ducts and prolong tissue migration. Apart from a minority of flukes which succeeded in entering the bile ducts and contributed to the increase of the average length, the length of most flukes did not increase appreciably for up to 13 weeks, and about one-third were smaller than the average (Boray, 1967a). Large numbers of 4-month-old flukes were only 3·5–5 mm long. The practical significance of this observation in the occurrence of a delayed type of subacute fascioliasis is discussed in Section VIII B.

Table XIV shows the comparative growth rate of flukes in heavily and lightly infected cattle, taken from reports by Dixon (1964) and Boray (1967b; unpublished data). The retardation of growth 10 weeks after infection, in heavy infections compared with light infections, was more pronounced in cattle than in sheep. Flukes in light infections may reach their maximum length in 19 to 21 weeks, but in heavier infections there was very little growth for as long as 26 weeks. The presence of stunted flukes in the liver in heavy infections may be observed for a very long period, but in sheep it is terminated by the death of the host due to destruction of the liver tissues and in cattle by the death of the parasite and spontaneous recovery from infection.

It was stated by Montgomerie (1928) that immature flukes were not as susceptible to anthelmintics as mature flukes. Kendall and Parfitt (1962) suggested that the relative resistance of young flukes to anthelmintics is due to their different state of physiological development and not to their location in the host. Boray (1963b) stated that after artificial infections the development of flukes showed considerable variation within the one animal, and development was slower in heavy infections. Efficacy of anthelmintics, therefore, may depend on the state of development and not necessarily on the age of the fluke. Boray and Happich (1966b) showed that in infected sheep treated with an anthelmintic, most of the larger flukes were killed and those that survived did so only if their development was retarded. However, Boray *et al.* (1967b) suggested that at 6 weeks after infection the physiological development of flukes may be more important than their size, and efficiency may also depend on whether the flukes are located in the tissues or are already in the bile ducts.

TABLE XIV

*Length of* F. hepatica *in cattle aged* 12–24 *months*

| | Length of flukes in mm in heavy infections (Boray, 1967b and unpublished data) | | | | Length of flukes in mm in light infection. Mean fluke burden: 45·4 (Dixon, 1964) | |
|---|---|---|---|---|---|---|
| Age of flukes Weeks | No. of flukes recovered | Mean length | Range | Average | Mean length | Range |
| 10 | 85 | 7·2 | 3·5–14·0 | | | |
| | 5190 | 6·0 | 1·5–10·5 | 6·0 | 12·2 | 7·5–19·0 |
| | 6165 | 4·9 | 1·0–15·5 | | | |
| 14 | 2660 | 7·4 | 2·0–16·5 | | | |
| 16 | 654 | 11·8 | 4·5–22·5 | | | |
| | 681 | 10·2 | 5·5–20·0 | | | |
| | 845 | 11·7 | 3·0–22·0 | 10·3 | | |
| | 1602 | 9·9 | 5·0–18·0 | | | |
| | 2138 | 7·6 | 4·0–13·5 | | | |
| | 2367 | 10·5 | 5·0–20·0 | | | |
| 19 | | | | | 19·7 | 12·0–28·0 |
| 21 | 64 | 13·8 | 4·5–19·5 | | | |
| | 69 | 16·0 | 9·0–26·0 | | | |
| | 119 | 14·9 | 6·0–21·0 | 14·4 | 20·9 | 18·0–24·0 |
| | 130 | 14·1 | 7·0–20·0 | | | |
| | 230 | 15·0 | 5·0–22·0 | | | |
| | 407 | 12·7 | 6·0–19·0 | | | |
| 26 | 2862 | 9·0 | 2·5–19·5 | 9·0 | | |

The metabolism of young flukes may differ from the older ones (Williams and Bryant, 1963). From the practical point of view, the size of flukes in an acute outbreak may not be fully indicative of the exact age of the infection. Therefore, the dose rate of an anthelmintic which was highly efficient against 6-week-old flukes in the standardized efficiency tests (Boray and Happich, 1968) should be used against an acute or subacute infection.

However, in many experiments the different growth rate of flukes appeared to be the only indication of the presence of innate or acquired resistance (Kerr and Petkovich, 1935; Urquhart *et al.*, 1954). It is obvious that in this complex system the recording of the length of *F. hepatica* has great importance in the comparative pathogenesis and resistance in different hosts, and in the diagnosis, prognosis and treatment of the disease.

The prepatent period of *F. hepatica* varies considerably from host to host, and also depends on the number of flukes in the liver. Dawes (1962a) found that the first eggs appeared 35–37 days after infection in mice. Boray (1963b) found the first eggs to appear in rats 42 days after infection. Tewari (1969)

stated that the prepatent period in guinea-pigs averaged 55 days irrespective of the size of the infective dose. The earliest appearance of eggs in the faeces in sheep was observed by Sinclair (1962) and Boray (1967a) 56 days after light artificial infections. Boray (1967a) found that the faecal samples contained eggs 9 weeks after infection in all sheep infected with 200 metacercariae. Sheep infected with 2000 metacercariae first contained eggs 13–15 weeks after infection. The egg output in faeces reached its peak at 17–18 weeks after infection in some surviving sheep. These animals then showed more severe clinical symptoms, and the number of eggs/g became appreciably higher because of the smaller volume of faeces. Sheep infected with 4000 to 10 000 metacercariae each either died earlier than the usual prepatent period or the infection did not reach patency at all. Dixon (1964) found the first eggs to appear 61 days after infection in 1-year-old cattle, but Boray (1967b) found the prepatent period to be 56 days in both 3-month-old and 14-month-old cattle. The peak of egg production was reached in both sheep and cattle about 18–19 weeks after infection, which persisted in sheep but soon declined in cattle (Boray, 1967a, b; Happich and Boray, 1969b). The eggs disappeared and a spontaneous recovery from fluke infection occurred after single infections; a negligibly small peak and a much faster decline and recovery occurred after reinfection in cattle (Boray, 1967b).

## B. CLINICAL SYMPTOMS AND PATHOLOGY IN SHEEP AND CATTLE

### 1. *Sheep*

There is an extensive literature on the pathology of fascioliasis due to the wide distribution of the parasite and the substantial pathological changes produced by the disease. These are summarized by reviews (Dawes and Hughes, 1964; Taylor, 1964; Pantelouris, 1965). More recently, Sinclair (1967) provided an excellent description of the disease process, and referred to most of the important contributions in this field up to 1966. The present chapter will mainly deal with the experimental work carried out in Australia, and will refer to some more recent work relevant to the problem with particular attention to acute fascioliasis in sheep and cattle. Sinclair (1967) stated that in many studies the number of metacercariae administered was kept low to avoid the occurrence of severe traumatic hepatitis. However, evidence supports the present author's view that the acute disease is due mainly to a mechanical process, and it is subclinical unless a large proportion of the functional liver tissues is destroyed during the migration of a large number of immature flukes.

Outbreaks of acute and subacute fascioliasis may occur with considerable losses when seasonal and climatic conditions result in the massive intake of metacercariae during a relatively short period. There is little information available on the successful reproduction of acute and subacute fascioliasis experimentally. Certain parasitological and pathological aspects of experimental infections in 148 Merino sheep, aged 4–5 years and artificially infected with *F. hepatica* metacercariae, were reported by Boray (1967a) in Australia. The animals were kept free from nematode infections and were vaccinated

TABLE XV

*Experimental infections with* F. hepatica *in* 259 *sheep aged* 4–5 *years*

| No. of metacercariae given to each sheep | No. of sheep | No. of flukes recovered at death, *in extremis* or at slaughter | % recovery of flukes | Mean life expectancy of sheep in weeks | | Clinical manifestation of the disease | |
|---|---|---|---|---|---|---|---|
| | | | | Lower recovery range | Higher recovery range | Lower recovery range | Higher recovery range |
| 200 | 119 | 103 (39–156) | 51·5 | > 35 | 35 | Subclinical, chronic, slight compensated anaemia | Clinical, chronic, progressive anaemia, some deaths |
| 500 | 15 | 204 (125–275) | 40·8 | 36 | 26 | Clinical, chronic, progressive anaemia, death | |
| 1000 | 27 | 400 (212–699) | 40·0 | 25 | 20 | Clinical, chronic, profound anaemia, death | Clinical, subacute and chronic, profound anaemia, death |
| 2000 | 26 | 708 (352–1353) | 35·4 | 22 | 15 | Clinical, chronic, profound anaemia, death | Subacute with some chronic changes, haemorrhages, anaemia, death |
| 4000 | 58 | 1535 (700–2730) | 38·4 | 10 | 7 | Subacute, haemorrhages, anaemia, death | Peracute and subacute, severe haemorrhages, anaemia, death |
| 6000–10 000 | 8 | 2542 (1083–3778) | 30·9 | 8 | 7 | Peracute, subacute, severe haemorrhages, anaemia, death | |
| Field infection | 6 | 1384 (1030–1782) | — | 11 | 7 | Subacute, severe haemorrhages, anaemia, death | |

against Black Disease. The sheep were kept in pens and fed on 50% lucerne chaff and 50% wheaten chaff *ad lib.* The metacercariae were produced from the laboratory strain of *Lymnaea tomentosa* by a standard technique (Boray, 1963b; Section IV C). The metacercariae were carefully counted after viability tests and administered in gelatin capsules. The experimental results of Boray (1967a) are combined with the results of new experimental infections (Boray, unpublished data) carried out in an additional 105 sheep (see Table XV).

The numbers of flukes recovered after artificial infections, reported by various workers, were summarized by Dawes and Hughes (1964). The recovery rate of adult flukes varied considerably, but averaged 26·6–39·1% after artificial infections with low numbers of metacercariae. Kendall and Parfitt (1962) gave 3000 metacercariae each to seven sheep and the average recovery was 26·4%. It was shown by Boray (1967a) that the heavier infections resulted in significantly lower percentage yields than the lighter infections, and there is some indication that the yield was higher when the metacercariae were given in single rather than in divided doses. Table XV shows that the average recovery rate from 119 sheep, which received 200 metacercariae each, was 51·5%. The average recovery rate from 42 sheep which received 500 to 1000 metacercariae each was 40·4% and from 92 sheep which received 2000 to 10 000 metacercariae each 34·9%.This additional information confirms the previous results, in which a negative regression of percentage recovery on the dose of metacercariae was found to be statistically highly significant (Boray, 1967a).

Roberts (1968) found a comparable fluke recovery of 39% of 5000 metacercariae given to each of seven sheep, but with a very wide range of 13–81%. Boray (1967a) suggested that the lower yield in heavy infections was possibly due to the damage caused by the entry of the first flukes into the liver, which made the tissues less suitable for the establishment of those entering subsequently. Dawes and Hughes (1964) stated that "the principal sources of loss are to be sought in the early stages of infection, when technical difficulties of recovery are greatest". There is no doubt that even with the most careful technique which was used in this laboratory, a small proportion of flukes may not be counted in heavily infected animals. There is sufficient evidence, however (Thorpe, 1965b, c; Ross, 1965; Boray *et al.*, 1967b; Boray, 1967a), that the "wastage" of flukes observed, which varies from host to host and also depends on the number of metacercariae given, may be due largely to an early or delayed tissue reaction eliminating some of the migrating flukes (see Section V F). Table XV shows that some sheep given 2000 metacercariae, and which survived for 16 weeks or more, had similar or higher fluke burdens and died earlier than some sheep infected with 4000 metacercariae each with a resultant fluke recovery in the lower range. It seems that a higher invasion by young flukes may initially cause more serious tissue damage and clinical symptoms, but more of the flukes will be eliminated in the fibrous tissues.

There was a considerable variation from sheep to sheep in the effect of the dose of metacercariae and the resultant fluke burdens. Fluke burdens of more than about 100 were lethal. Sheep which had 100–1000 flukes in their livers died of chronic fascioliasis, and most of those with more than 1000 flukes died of the peracute, acute or subacute form of the disease. It was shown within

each infection group that sheep with the higher range of fluke burden died earlier (Table XV), and it was apparent that the more flukes there were in the liver the earlier death occurred.

The sheep infected with an average of 103 flukes were observed for up to 25 weeks after infection, and some similarly infected sheep (Happich and Boray, 1969b) were observed for up to 30 and some for up to 46 weeks (Boray and Roseby, unpublished data). They showed no obvious clinical symptoms. The sheep with a fluke burden in the higher range showed slowly progressing anaemia and some died 32-39 weeks after infection. The fate of the sheep with a fluke burden in the lower range was shown by Boray and Roseby (unpublished data) to depend on their nutrition (Table XVII), but usually they survived for a long period.

Sheep infected with an average of 204 flukes gained an average of 4·68 kg during the first 12 weeks after infection and showed no clinical symptoms of fascioliasis, apart from slight inappetance for a few days 2 weeks after infection. Ten of these sheep were observed for a longer period and showed clinical signs of progressing anaemia from the 15th week after infection. The sheep with the higher range of fluke burden died or were killed when moribund 24–29 weeks, and the others 31–39 weeks after infection.

Infections averaging 400–708 flukes in the liver caused temporary inappetance and loss of weight 2–4 weeks after infection, but the sheep gained weight afterwards up to 7–8 weeks. Inappetance was shown from this stage, the animals lost weight, the conjunctivae were yellowish-red and some sheep became constipated. Ascites was evident in some cases. The condition of the sheep improved slightly from the 10th to the 12th week, but deteriorated again 14 weeks after infection. At this stage the conjuctivae became pale, the sheep lost weight, and the wool was dry and easy to pull out. Serious ascites developed in some sheep, and the animals eventually became emaciated and died 16–26 weeks after infection.

Infections averaging 1384–2542 flukes caused inappetance from the 2nd week after infection, and the sheep gradually lost weight from the third week. The symptoms became very severe at the 8th week with evidence of abdominal pain and ascites, the conjunctivae became yellowish and pale, the animals passed very little dry faeces, became emaciated and died. The sheep lost an average of 9 kg before they died, but some lost up to 18 kg. Two sheep showed few clinical symptoms and suddenly died 4 and $5\frac{1}{2}$ weeks after infection with 4000 and 6000 metacercariae respectively. All sheep artificially infected with 4000 or more metacercariae showed clinical signs of very severe anaemia at 5–6 weeks after infection.

The summary of most pathological changes given by Boray (1967a) was modified in the present Table XVI including the necropsy results. Further experiments confirmed the previous report (Boray, 1967a) that in lighter infections the typical haemorrhagic tracts resulting from the migration of immature flukes were concentrated mainly in the left lobe of the liver. In heavier infections these changes and the resultant fibrosis, though more severe in the left lobe, were also found throughout the other lobes of the liver.

At necropsy the flukes were found in the bile-ducts of the sheep infected with

TABLE XVI

*Necropsy results in sheep infected with* 200–10 000 F. hepatica *metacercariae*

| Necropsy results | Average number of flukes in liver: 103–204 | | 400–708 | | > 1000 | |
|---|---|---|---|---|---|---|
| | Weeks after infection: 4–5 | 14–25 | 8 | 16–26 | 4–8 | 9–13 |
| Liver haemorrhage | + | . | ++ | . | ++++ | +++ |
| Liver rupture and haematoma | . | . | . | . | +++ | . |
| Liver fibrosis | + | ++ | +++ | +++ | ++++ | ++++ |
| Icterus | . | . | . | ++ | ++++ | ++++ |
| Chronic cholangitis | . | ++ | . | ++++ | . | ++ |
| Biliary occlusion | . | + | ++ | +++ | ++++ | ++++ |
| Venous congestion | . | . | + | ++ | ++++ | ++++ |
| Fibrinous peritonitis and perihepatitis | + | . | +++ | ++ | ++++ | ++++ |
| Blood-stained fluid in peritoneum | . | . | + | . | ++++ | + |
| Clear fluid in peritoneum | . | + | . | +++ | . | +++ |
| Flukes in *ductus pancreaticus* | . | . | + | ++ | +++ | +++ |
| Flukes in peritoneum and in organs other than liver | . | . | ++ | + | +++ | ++ |
| Flukes in lungs and pleura | . | + | ++ | + | ++++ | +++ |
| Pleural fluid | . | . | + | . | +++ | ++ |
| Enlarged hepatic lymph nodes | . | + | + | + | ++++ | ++++ |
| Oedematous lymph nodes | . | . | . | + | +++ | ++++ |

less than 1000 metacercariae, but in heavier infections most or all of the flukes were still in the parenchyma for up to 13 weeks, or occasionally longer.

The clinical symptoms and the pathology in the six sheep exposed for six weeks to natural infection in the field (Table XV) resembled, or were identical to the changes found in sheep experimentally infected with 4000–10 000 metacercariae. In lighter infections the typical haemorrhagic tracts were found mainly in the left lobe of the liver 4–5 weeks after infection. The liver and the surrounding peritoneum showed evidence of well localized fibrinous perihepatitis and peritonitis. Twelve to 14 weeks after infection there was little evidence of this earlier acute damage and the livers were of normal size and showed typical chronic cholangitis with enlarged whitish bile ducts containing the mature flukes. In some livers partial or total occlusion of the smaller ducts was present. In many cases the walls of the gall bladder and *ductus coledochus* were thickened, occasionally containing mature flukes. The hepatic lymph nodes were also enlarged.

In sheep which were killed 8 weeks after infection with 1000–2000 metacercariae the haemorrhages were more severe, and the tracts wider than at 4–5

weeks after infection. Some of the larger flukes were already in the bile ducts, but there were still numerous flukes in the tissues. There was intensive fibrinous perihepatitis and peritonitis in many well defined areas through the parietal and visceral peritoneum. Some haemorrhagic tracts containing a few small flukes were seen in the parietal peritoneum. In some cases the liver was connected by fibrinous adhesions with the surrounding abomasum, duodenum, diaphragm and other organs, and the mesenteric veins were greatly extended. One to five l. of clear or slightly yellowish peritoneal fluid was present in many cases. 16–26 weeks after infection the livers showed chronic cholangitis and total biliary occlusion was seen in large areas, particularly in the left lobe. Most of the livers were enlarged, deformed, lighter in colour, fibrous and hard. The wall of the bile ducts was enlarged, protruding to the liver surface, filled with flukes and dark yellow or brown granular detritus. In many cases the *ductus coledochus* contained large numbers of flukes, but flukes were never found in the duodenum of freshly killed animals. The migration of flukes into the duodenum was observed, however, if the sheep had been dead for some time before examination. Flukes were often found in the pancreas and *ductus pancreaticus*, the wall of which was enlarged similarly to the bile ducts. Flukes found in the lung parenchyma were surrounded by a well localized tissue reaction, and some contained fully developed eggs.

It was observed that many adult flukes in the bile ducts were intermittently attached with their suckers to the epithelium, and hyperaemic areas were observed after the removal of flukes if the examinations were carried out immediately after death. The presence of hyperaemia in the epithelium was more evident in sheep which were killed with a mechanical stunning apparatus, were not bled, and in which the main blood vessels of the liver were tied before examination.

In infections with more than 1000 flukes, migratory tracts and intensive haemorrhages were seen throughout the liver parenchyma, the parietal and visceral peritoneum, spleen, pancreas, pleura and lungs. Rupture of the liver parenchyma and haemorrhage into the peritoneal cavity was found in the two sheep which died suddenly 4 and 5½ weeks after infection respectively. In many other sheep which rapidly became anaemic, haematoma 1–3 cm in diameter were seen in several confined areas of the liver parenchyma. The peritoneal cavity contained 1–8 l. of mostly dark red, but occasionally yellow fluid. The fluid was usually darker and blood stained in the sheep which died up to 9 weeks after infection. The fluid became clearer and yellowish in most sheep which died later. Yellow discolouration of the omentum, mesentery and in some cases the subcutis was observed. The hepatic lymph nodes were enlarged, sometimes haemorrhagic and oedematous. The mesenteric veins were dilated. The fibrinous perihepatitis and peritonitis with adhesions to other organs were more severe than in the lighter infections. In all sheep, but particularly in those which died later than 10 weeks after infection, cholangitis and biliary occlusions were also seen. Flukes were often recovered on haemorrhagic tracts surrounded by inflammatory changes in the lungs. Lung damage with chronic pneumonia and fibrinous pleuritis with pleural fluid were found in most animals.

Haemorrhagic tracts were often observed in the diaphragm, suggesting that

most flukes found in the pleura and lungs migrated there through the diaphragm. The flukes which were recovered from organs other than the liver were all stunted and their length was only 1–2·5 mm. These flukes were less motile than the ones from the liver tissues. All flukes in the liver were within the parenchyma for up to 6–7 weeks after infection. Some flukes entered the bile ducts 7–8 weeks after infection. More flukes were located in the bile ducts as the infection became older, but about one-third of the flukes were still migrating in the parenchyma 13 weeks after infection causing fewer but wider haemorrhagic tracts. The flukes already in the bile ducts were all immature.

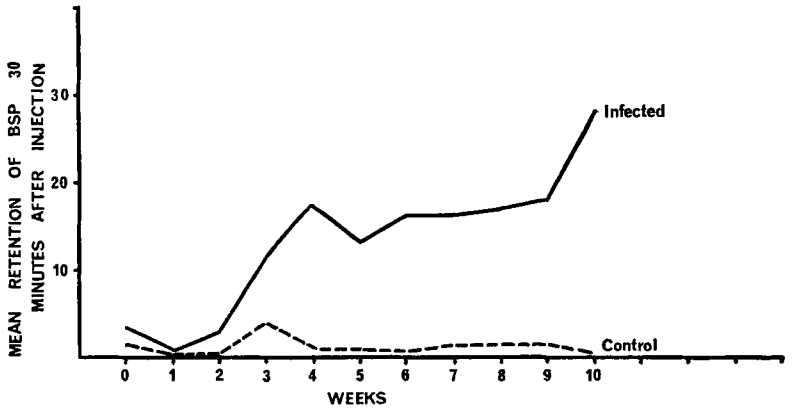


FIG. 5. B.S.P. retention in sheep infected with 6000 *F. hepatica* metacercariae each and in fluke-free sheep.

Boray (1967a) stated that there was always sufficient liver damage by flukes to explain death. However, in most cases secondary pathological lesions, such as peritonitis, pleuritis and traumatic damage in the lungs and pancreas, contributed to the condition. These lesions were found to be always localized around the trauma caused by the migration of flukes, but some sheep infected with 4000 metacercariae showed acute peritonitis and bacteraemia and died 4½–5 weeks after infection. Boray showed that liver damage was detected in sheep with heavy infections by a single bromosulfalein clearance test as early as 3 weeks after infection, thus demonstrating the failure of liver function (Fig. 5), which is in accord with the results of other workers (Sinclair, 1967). The method may be useful in the field for the early diagnosis of acute fascioliasis in sheep.

In many cases of acute fascioliasis investigated in the field, and in six experimental sheep which were exposed to heavy, natural infection in the field, the clinical and pathological changes were similar to those produced experimentally (Boray, 1967a). In the field, however, acute lesions were often superimposed on typical chronic changes.

If examination was carried out immediately after the death of the sheep, there was no evidence that flukes already present in the liver tissues during

early migration would emerge again into the peritoneal cavity and be responsible for the perihepatitis, as was suggested by Dawes and Hughes (1964). It is possible that in heavy infections the fibrous perihepatitis may be due to irritation caused by the flukes entering the liver tissues. In heavy infections the period of entry is usually extended and the tissues are exposed to the prolonged effect of the sustained migration of flukes (Dawes, 1963; Boray, 1967a).

All the above experiments were carried out in adult sheep of similar condition and fed on a standard diet, but the clinical effect of the infections varied considerably from sheep to sheep. Recent studies (Boray, unpublished data) showed that young sheep fed on a low protein diet died earlier and showed typical pathological changes of acute fascioliasis. One group of five lambs was fed on 50% crushed wheat, 25% lucerne chaff and 25% wheaten chaff *ad lib.* The other group of five lambs was fed on 75% wheaten chaff and 25% lucerne chaff *ad lib.* The mean survival time of the first group was 13 weeks (9–16) and of the second group 6·7 weeks (4·5–8) after they were each dosed with 3000 metacercariae.

Similar results were shown by Boray and Roseby (unpublished data, Table XVII) in adult chronically infected sheep, and in adult sheep infected with 2000 metacercariae each (Boray, unpublished data, Table XVIII).

TABLE XVII
*The effect of nutrition on* F. hepatica *infection in adult sheep*

| Diet | No. of sheep | Mean no. of flukes in liver | Age of infection at slaughter in weeks | Mean P.C.V. at slaughter % | Average daily egg production per fluke, weeks after infection | |
|---|---|---|---|---|---|---|
| | | | | | 17–22 | 25–29 |
| 50% crushed | 4 | 41 | 46 | 35·0 | | |
| wheat+ | 6 | 72 | 30 | 34·8 | 11000 | 16000 |
| 50% lucerne | 5 | 0 | — | 41·0 | | |
| chaff | | | | | | |
| 100% wheaten | 4 | 50 | 46* | 19·3 | | |
| chaff | 6 | 79 | 30 | 23·3 | 19600 | 19000 |
| | 5 | 0 | — | 31·8 | | |

* One sheep died 31 weeks after infection due to profound anaemia

Table XVIII shows that heavily infected sheep fed a higher plane of nutrition lived longer, and the growth of flukes was more retarded compared with sheep fed a low plane of nutrition. In chronic infection the peak of the egg producing capacity of flukes was delayed in sheep fed the higher plane of nutrition.

Boray (1967a, b) could not produce evidence of acquired resistance to *F. hepatica* in sheep fed a standard diet. However, from the above data it is evident that the nutrition of the host may greatly influence the manifestation of infection. Animals fed a high plane of nutrition may be in an increased state of resistance, possibly producing a more vigorous cellular reaction and thus retarding the growth and hindering the tissue migration of flukes.

TABLE XVIII

*The effect of the nutrition on* F. hepatica *infection in adult sheep each dosed with* 2000 *metacercariae*

| Diet | No. of sheep | Mean no. of flukes | % recovery | Mean life expectancy of sheep after infection in weeks | Age of fluke in weeks | Length of flukes in mm | |
|---|---|---|---|---|---|---|---|
| | | | | | | Average | Range |
| 50% crushed wheat<br>25% lucerne chaff<br>25% wheaten chaff | 5 | 689 | 34·5 | 13·0(9–16) | 9 | 4·3 | 1·5– 9·0 |
| | | | | | 13 | 5·9 | 2·5–10·5 |
| | | | | | 15 | 7·5 | 2·5–14·0 |
| 75% wheaten chaff<br>25% lucerne chaff | 5 | 725 | 36·2 | 10·6(6–15) | 9 | 3·6 | 1·5– 7·0 |
| | | | | | 13 | 10·0 | 3·0–17·0 |
| | | | | | 15 | 12·8 | 4·0–24·0 |

Boray (1967a) and Roberts (1968) showed that the most characteristic symptom of the disease was a severe anaemia. All sheep infected with approximately 200–700 flukes showed chronic, progressive anaemia from about 12 weeks after infection. Severe anaemia developed from about the 5th week after infection in all sheep which harboured approximately 1000 or more flukes. Anisocytosis, polychromatophilic erythrocytes, punctate basophilia and macrocytes were present in heavy infections, suggesting intensive erythropoiesis. The pronounced poikilocytosis may also indicate active erythropoiesis, but may suggest that the released erythrocytes are continually disappearing from the circulation.

Obara *et al.* (1964) demonstrated absorption of Vitamin $B_{12}$ by *F. gigantica*, and suggested that Vitamin $B_{12}$ deficiency might be the cause of anaemia in infected animals. Boray (1967a) and Sinclair (1967) showed that infection of Vitamin $B_{12}$ did not improve the anaemia caused by subacute or chronic infection. In further experiments, sheep were injected 3 times with 1000 μg Vitamin $B_{12}$ or weekly with 500 mg Iron Dextran commencing soon after they were dosed with metacercariae. The treatments did not improve the anaemia in sheep compared with untreated controls.

Dawes and Hughes (1964), Pantelouris (1965) and Sinclair (1965, 1967) gave summaries from recent literature concerning anaemia due to fascioliasis in sheep. Most of the experimental work on the disease was carried out with small numbers of animals not sufficiently heavily infected to produce acute fascioliasis. The evidence on the nature of the anaemia in the chronic form of the disease is conflicting.

Dawes and Hughes (1964) showed evidence of the feeding by adult flukes on hyperplastic epithelium in the bile ducts of the mouse. They concluded that it was unlikely that the anaemia of fascioliasis was due to ingestion of blood by *F. hepatica*. Later, Sinclair (1965) supported this conclusion and stated that the sheep he bled daily had a higher rate of iron utilization by erythrocytes than infected sheep. He believed the anaemia was secondary to a disorder of the reticulo-endothelial system.

There seems, however, to be sufficient evidence (Stephenson, 1947; Jennings *et al.*, 1956; Pearson, 1963; Todd and Ross, 1966; Dargie *et al.*, 1968; Sewell *et al.*, 1968) that removal of blood by the flukes occurs, which may play a important role in the chronic anaemia in fascioliasis. This is supported to some extent by observations in this laboratory on the apparent blood sucking of adult flukes in the bile ducts of sheep, with evidence of hyperaemic areas of the epithelium. This has been also observed in guinea-pigs and rabbits. It seems that removal of blood by flukes is possible both by direct sucking action and by the removal of the hyperaemic epithelium in chronic infections. It is concluded that peracute, acute and subacute fascioliasis result from simple mechanical lesions causing extensive haemorrhage, and from the subsequent reparative tissue reaction which prolongs continuous migration of flukes, further increasing the initial mechanical damage. Deaths are due to profound anaemia and failure of liver function.

Although some earlier workers used isotopes to follow certain aspects of iron metabolism or erythrocytic loss, a comprehensive study of iron metabolism in both the acute and chronic forms of the disease was first carried out by Symons and Boray (1967, 1968). Sheep were infected with 4000 metacercariae each and injected with plasma labelled with $^{59}Fe$ in the 6th and 8th weeks of infection. Others which were infected with 2000 metacercariae each were injected in the 13th week when the flukes were in the bile ducts. The change of radioactivity was measured in the plasma, over the sacrum (for bone marrow), the liver and spleen and in the erythrocytes. In the 13-week sheep the activity was also measured in the faeces. There were five sheep in each of these groups and eight control sheep. Other chronically infected sheep were given $^{59}Fe$-plasma, and the radioactivity in the fluke before and after discharge of their caecal contents was also recorded. A spectrophotometric examination was made of the characteristics of the brown-black material of the caeca and of the contents of the gall bladder.

The infected sheep were anaemic, and liver function, as shown by bromosulphalein clearance and icterus of the plasma, was disturbed when the fluke were in the parenchyma of the liver. In none of the sheep was there any evidence of plasma iron deficiency; in fact, the mean concentration was higher in the acutely diseased sheep than in the controls. The plasma iron concentration tended to be normal when the fluke were in the bile ducts. In some chronically diseased sheep which had been infected for some months the plasma iron did fall below the level of the controls, but it was clear that this deficiency was the result of a long-standing anaemia and not the cause of it.

Assessments of erythropoiesis were made by estimating the rate of clearance of radioactivity from the plasma, its appearance and disappearance from the

bone marrow and its incorporation into erythrocytes. The plasma activity was reduced to 50% of the initial value in about 2 h in the acutely diseased sheep and their controls. The rate of clearance from the plasma of the 8-week sheep was significantly faster than from the 6-week sheep ($P < 0{\cdot}05$), but neither differed significantly from that of the controls. On the other hand, there was a significantly faster rate of plasma clearance than from their controls in the 13-week sheep.

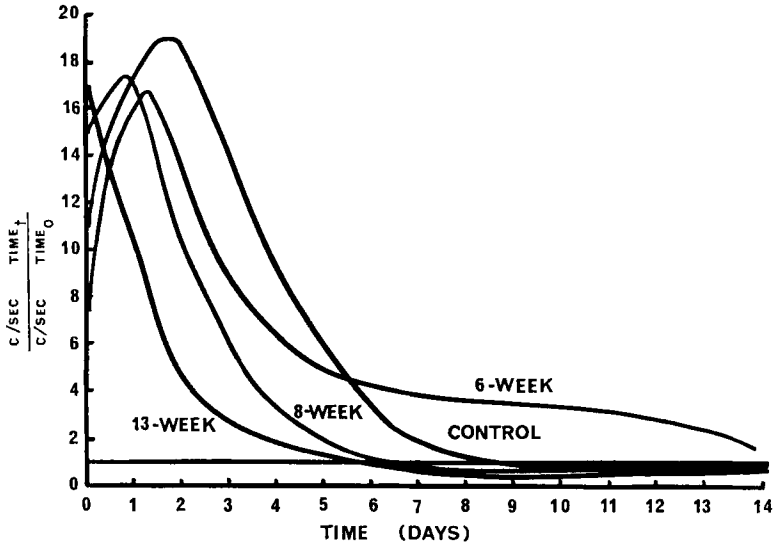


FIG. 6. Mean radioactivity in sacral bone marrow compared with radioactivity at time zero. (Reproduced with permission from Symons and Boray, 1968.)

The transfer of radioactivity from plasma to bone marrow was rapid in all sheep. This activity fell again almost as rapidly as it was incorporated in the erythrocytes. These results are shown in Fig. 6, in which activity is plotted as the ratio of counts/min at time "t" to the estimated counts/min at time zero. Here it can be seen that the ratio returned to one by about day 9 after infection in the 8-week and control sheep, but remained elevated above one in the 6-week sheep until about day 12–14. This suggested a return of iron to the marrow, possibly by haemolysis. In the 13-week sheep, however, the ratio approached close to zero by day 14 and indicated a rapid and complete incorporation in erythrocytes.

This incorporation into erythrocytes is illustrated in Fig. 7. Firstly, it can be seen that incorporation in the control sheep did not reach a maximum until about day 50, and then had fallen only slightly by about day 100. In the infected sheep the rate of incorporation was appreciably more rapid and reached a higher percentage of the total $^{59}Fe$ injected as the infection progressed from 6–13 weeks, in which sheep it was close to maximal. The activity measurements

which were only continued in the 6- and 8-week sheep for 14 days had already begun to fall by that day. Although no absolute measurement was possible of the half-life of erythrocytes in the control sheep, that in the 13-week sheep was less than 6 days and almost all activity had disappeared between days 35 and 40. All the evidence, therefore, indicated that in all infected sheep there was an increased rate of erythropoiesis which reached the maximum in the 13-week animals. There was no evidence of a relative failure of erythropoiesis, nor was there any evidence of haemolysis and re-incorporation of $^{59}Fe$ in the 13-week group.

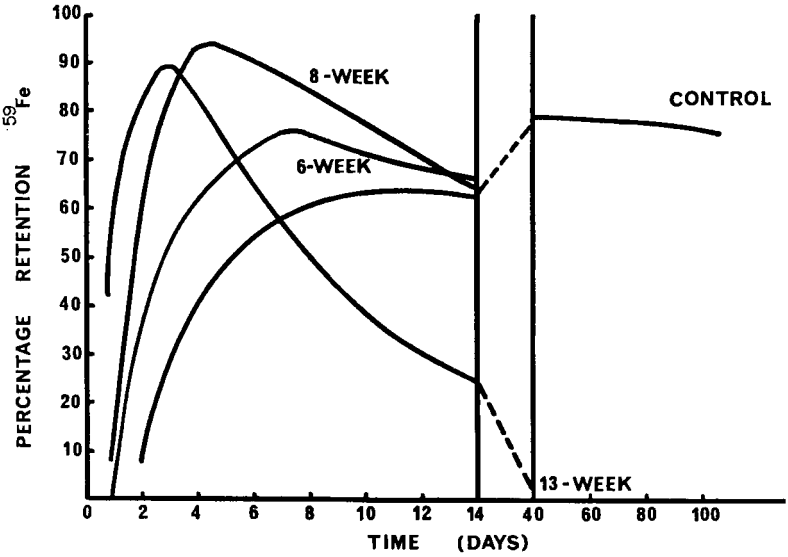


FIG. 7. Mean percentage of $^{59}Fe$ incorporated in erythrocytes. (Reproduced with permission from Symons and Boray, 1968.)

One interesting result was the finding that the pattern of activity over the spleen of normal sheep differed markedly from that for man (Figueroa and Weinstein, 1962). In these sheep, the activity over the spleen rose rapidly and parallel with that in the erythrocytes until it was considerably higher than that over the liver. In man the activity over the spleen and liver are very similar, as was found in the 6- and 8-week sheep. In 6- and 8-week sheep, activities over liver and spleen fell slowly after reaching a maximum, as they do over the liver of normal man. In the 13-week sheep the mean activity rose higher in the spleen than it did over the liver, due to an abnormally high level in one sheep. The important fact is, however, that the activities over both organs of the 13-week sheep fell to near zero together with that in the erythrocytes. Again, there was no evidence of haemolysis in any of the infected sheep.

The question of the whereabouts of the radioactivity that was lost from the erythrocytes in the 13-week sheep, and which did not reappear in the bone marrow, spleen or liver, was readily answered when it was found in the faeces.

The problem of its passage from the erythrocytes to the faeces was also just as readily answered by an examination of the fluke and the bile in a separate group of chronically infected sheep given $^{59}$Fe-plasma. When these fluke were made to discharge the brown-black material from their caeca over 90% of their radioactivity was lost. A spectrophotometric examination with a pyridine haemochrome reagent (Lemberg and Legge, 1949) showed that the discharge material must be haematin or haemoprotein (Fig. 8). As this material was soluble in water or phosphate buffer and was precipitable by protein precipitants, it was

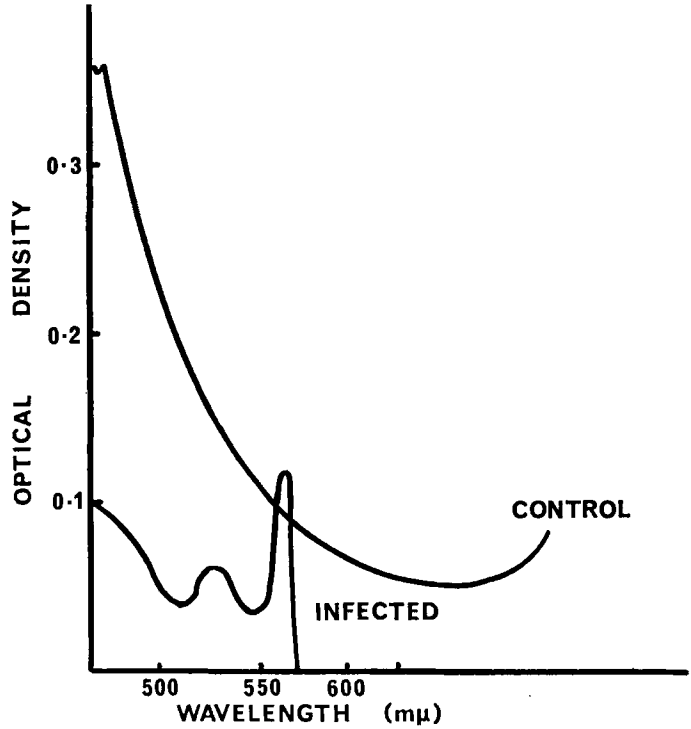


FIG. 8. Typical absorption spectra of caecal material discharged from fluke or in bile of infected sheep compared with bile of non-infected sheep (control). (Reproduced with permission from Symons and Boray, 1968.)

concluded to be haemoprotein. Spectrophotometrically and by electrophoresis a small amount of the haemoprotein was shown to be oxyhaemoglobin. No free organic iron was detected. Haemoprotein, but no recognizable oxyhaemoglobin, was also recovered from the bile of these sheep, but none from the bile of the controls.

Dawes and Hughes (1964) stated that they could find no evidence of blood sucking in the bile ducts. The evidence of the above experiments by Symons and Boray (1967, 1968) does not support this finding. In addition, it was found that when flukes were removed from their attachment to the bile ducts the

mucosa was missing and the region was occupied by a clot containing whole erythrocytes. Symons and Boray (1967, 1968) concluded that the anaemia of acutely infected sheep, when the fluke are in the substance of the liver, was due to loss of blood into this organ and into the abdominal cavity. There was no evidence of generalized haemolysis. The return of the isotope to the bone marrow that probably occurred in the 6-week sheep was apparently due to haemolysis within the deranged liver. When the fluke are in the bile ducts they ingest blood, and the very short half-life of the erythrocytes, and hence the cause of the anaemia, is due to ingestion of blood by the fluke and the consequent loss via the bile into the faeces. It is suggested that the conclusion of Sinclair (1965), which depended upon a comparison between bled and infected sheep, that there was insufficient stimulus to erythropoiesis, now requires amendment. He was able to use only two sheep in each group, the relevant pair of which was so lightly infected that the blood ingested by a round figure of 100 flukes and based on 0·2 ml ingested blood/fluke/day (Jennings *et al.*, 1956) would only be about one sixth of the volume he took from another pair by bleeding. The stimulation of erythropoiesis in his sheep would, therefore, be less in the diseased than in the bled sheep. In the sheep in the experiments described by Symons and Boray (1968) there was no failure but a marked increase in the rate of erythropoiesis.

Studies in this laboratory (Tewari, 1968) showed that guinea-pigs became anaemic after artificial infections with *F. hepatica*, and the reticulocytes increased from 2·7 to 14·7%. There was relation between the appearance of anaemia and the number of reticulocytes. The increase of reticulocytes may be interpreted as an indication of a progressive haemorrhagic type of anaemia resulting in increased bone marrow activity.

2. *Cattle*

There is relatively little information available on experimental infections in cattle, and in reports from the field the age, breed, nutrition and other intercurrent diseases of the animals make the interpretation of results very difficult. It has been generally recognized that cattle are more resistant to fascioliasis than sheep. The problem has been discussed by Ross (1966b, 1967c), Boray (1967b), Sinclair (1967), and in this review (Section V G). Because of increased resistance of the host, variation of host reaction from animal to animal is greater in cattle than in sheep. Difficulties, therefore, are expected in drawing conclusions from artificial infections of small numbers of cattle at various dose levels. This was clearly shown in the results of Ross (1965, 1966a, 1967a, b), Ross *et al.* (1966) and Boray (1967b). Ross (1965) found that high level infections with metacercariae failed to produce clinical fascioliasis in calves. Boray (1967b), however, found in Australia that typical acute, subacute or chronic disease could be produced in calves with increased doses of *F. hepatica* metacercariae. Ross and Dow (1966) attributed most deaths of calves infected with immature flukes to pneumonia in the laboratory and in the field. In sheep (Boray, 1967a) severe pleuropneumonia contributed to the pathological lesions in acute fascioliasis. In experiments in cattle, however, pneumonia was rarely observed, but small isolated nodules were found in the lungs of some of the

infected calves from which calcified or live specimens of *F. hepatica* were removed. Live fluke recovered from the lungs were stunted but sometimes contained mature eggs. In high level experimental infections (10000 metacercariae or more) the death of all calves was due to haemorrhages in the liver tissue and the resultant profound anaemia (Boray, 1967b). In medium level infections (1000 to 5000 metacercariae) chronic fascioliasis and profound anaemia developed (Table XIX). The animals in these experiments were kept nematode free and diarrhoea, which is often described as common in fascioliasis, was never observed.

Table XIX shows some of the results of experimental infections in more than 50 cattle of different ages carried out in this laboratory (Boray, 1967b; Boray, unpublished data; Boray and Roseby, unpublished data). It has been shown that clinical disease could only be produced in young calves, and the resistance increased with the age of the animals. The role of nutrition in the resistance of

TABLE XIX

*Experimental infections with* F. hepatica *in cattle*

| No. of metacercariae given | Age of cattle at infection in months | No. of cattle | Mean no. of flukes recovered 18–20 weeks after infection | Mean % recovery | Clinical manifestation of disease |
|---|---|---|---|---|---|
| 1000 | 6½–8½ | 4 | — | — | Severe anaemia, high egg counts, 1 died, 3 recovered spontaneously. |
| | 16–18 | 2 | — | — | Nil, low egg counts, spontaneous recovery. |
| | 24 | 2 | — | — | Nil, low egg counts, spontaneous recovery. |
| 5000 | 4 | 10 | 1358 | 27·2 | 8 out of 10 had profound anaemia, all high egg counts. |
| | 6½–8½ | 6 | 1381 | 27·6 | 2 out of 6 had anaemia, 1 died, others normal. |
| | 17 | 4 | 620 | 12·4 | Nil |
| 10000 | 6 –8½ | 4 | 4671 | 46·7 | Profound anaemia, all animals died due to subacute or chronic fascioliasis. |
| | 24 | 2 | 512 | 5·1 | Nil |
| 20000 | 4½ | 2 | — | — | Both died of acute fascioliasis. |
| | 14 | 2 | — | — | Transient slight anaemia, low egg counts, spontaneous recovery. |

sheep against the disease was shown earlier (Tables XVII and XVIII), and Boray (1967b) stated that calves in good condition showed more resistance than poorer ones. It has been suggested that some breeds of cattle may be more or less susceptible to fascioliasis than others. A comparative experiment was carried out in this laboratory with Jersey and Hereford calves (Boray and Roseby, unpublished data) and some preliminary results are shown in Table XX. The experiment confirmed that clinical, chronic fascioliasis with anaemia

TABLE XX

*The results of infection with* 5000 F. hepatica *metacercariae in calves aged 4 months. Five calves in each group*

| Breed | Jersey | Hereford |
|---|---|---|
| Mean number of flukes recovered 18 weeks after infection | 1177 | 1078 |
| Average E.P.G. during the 17th week after infection (5 days) | 1223 | 1436 |
| Mean PCV 17 weeks after infection | 21 | 26 |
| Mean Hb concentration 17 weeks after infection | 6·8 | 8·7 |

may be produced in cattle aged 4 months with high level infections (5000 metacercariae each). There was no appreciable difference in egg counts and in the number of flukes recovered from the two breeds 18 weeks after infection, but the clinical symptoms, particularly anaemia, were more pronounced in the Jersey calves. This difference may simply be due to the smaller size and lower growth rate of the Jerseys. More work should be carried out to determine the possible variation in the resistance of different cattle breeds against *F. hepatica* infection, which could lead to selective breeding in an endemic district.

In susceptible young cattle the clinical symptoms and gross pathology of fascioliasis were similar to those found in sheep with more intensive fibrosis in the liver associated with calcification. The latter was never observed in sheep. In more resistant animals the lesions were similar to those described by Ross *et al.* (1966) and Dow *et al.* (1967a). The histopathology of fascioliasis in various hosts has a voluminous literature and is described in the reviews. Detailed descriptions of the histopathological changes in sheep and cattle were given recently by Dow *et al.* (1967a, b).

### C. DIAGNOSIS OF FASCIOLIASIS IN SHEEP AND CATTLE

Much attention has been given to the diagnosis of fascioliasis, and most references may be found in reviews (Taylor, 1964; Pantelouris, 1965; Sinclair, 1967). A general discussion of the problem was given by Honer (1967b). It is relatively easy to diagnose chronic fascioliasis, but the only safe diagnosis for

acute fascioliasis may be at necropsy, preceded by the clinical symptoms described before. In peracute cases, the liver is of normal size, with characteristic perforations and subcapsular haemorrhages. Peritonitis is always present. Haemorrhages are seen also in the peritoneum, omentum, kidneys, spleen, pancreas, diaphragm and lungs. Numerous very small (1–3 mm) flukes are found in the tissues.

In cases of acute and subacute fascioliasis the findings at necropsy are similar, but the liver is much more damaged and enlarged. The liver tissue is fibrotic and friable, and the liver and surrounding tissues are joined or covered by fibrin. Large amounts of blood-stained peritoneal fluid are present. Large necrotic areas are seen and these pathological lesions often extend to the lungs. Numerous flukes are present in the damaged tissues. Live flukes are often found in the peritoneum, spleen, pancreas and lungs. The size of the flukes varies (1–7 mm). These findings are often present in sheep in the field already infected with adult flukes. The size of immature flukes does not indicate the age of the infection because in heavy infections the growth of the fluke is retarded (Boray, 1967a; Section V A). Liver function tests were recently found to be of diagnostic value in acute and subacute fascioliasis (Sinclair, 1967; Boray, 1967a).

The differential diagnosis between black disease due to *Clostridium oedematiens* and acute fascioliasis is particularly difficult, and mortalities may occur from both. In black disease the carcase rapidly putrefies and subcutaneous oedema is seen. The liver is enlarged, dark grey or brown, with characteristic necrotic areas. Yellowish areas of about 1–3 cm in diameter surrounded by a hyperaemic zone may be seen. They are usually under the capsule of the diaphragmatic liver surface, but may be present anywhere. In cattle they are more difficult to find and are of linear shape. A history of vaccination against black disease usually excludes the presence of this disease. Other acute infections by clostridia (blackleg, malignant oedema and anthrax) may be differentiated by local lesions.

Specific immunological tests may be of value in acute fascioliasis and in human infections (Geyer, 1967, 1968; Tailliez, 1967; Sinclair, 1967).

Subclinical chronic fascioliasis may be diagnosed only by reduced productivity and unthriftiness. A simple and reliable diagnosis is the faecal examination for fluke eggs. Infections may coincide with other chronic diseases (cobalt or copper deficiency) or other helminth infections, such as haemonchiasis, with similar clinical symptoms.

Differentiation by faecal examination is simple. Techniques for faecal examination, ranging from a simple smear to elaborate quantitative methods, have been used to diagnose chronic infection with *Fasciola* spp. The aim is to concentrate the eggs from the voluminous faeces, and has been successfully achieved by flotation (Vajda, 1927), sedimentation (Benedek, 1943) or by fractional sieving (Willmott and Pester, 1952; Dorsman, 1956a). Many such methods with different procedures and with different flotation fluids have been described (Döbel, 1963).

There is need for a relatively accurate quantitative method which is simple and quick, and gives reproducible egg counts for studies on the biology of the parasite and for the evaluation of anthelmintic efficiency in the field.

Much attention was paid to the quantitative diagnosis of chronic fascioliasis in sheep in this laboratory, and a simple quantitative flotation technique with potassium mercuriiodide was described by Whitlock (1950), and the sedimentation technique (Benedek, 1943) modified for quantitative diagnosis by Boray and Pearson (1960).

Happich and Boray (1967, 1969a) reported the comparative recovery of *F. hepatica* eggs added to fluke-free sheep faeces by these two techniques. Both techniques were modified and described by Happich and Boray (1969a). Table XXI shows some of the results. It was shown that only 26·7% of samples

TABLE XXI

*Recovery of* F. hepatica *eggs by flotation and sedimentation techniques in 427 three-gram samples with known numbers of eggs*

| No. of eggs/g | Positive samples % | % recovery of eggs | Calculated E.P.G. Mean | Calculated E.P.G. % of actual E.P.G. |
|---|---|---|---|---|
| Flotation | | | | |
| 10 | 26·7 | 1·0 | 3·1 | 31·0 |
| 100 | 100·0 | 1·0 | 30·4 | 30·4 |
| 1000 | 100·0 | 1·1 | 342·4 | 34·2 |
| Sedimentation | | | | |
| 10 | 100·0 | 25·4 | 2·5 | 25·4 |
| 100 | 100·0 | 29·6 | 29·6 | 29·6 |
| 1000 | 100·0 | 32·1 | 320·7 | 32·1 |

with 10 E.P.G. were positive by the flotation method, but the eggs were detected in all samples by sedimentation. Only one or two eggs were recovered by flotation from 32 samples (43%) containing as many as 100 E.P.G. In positive samples, the average calculated E.P.G. was about one-third of the actual E.P.G. by both flotation and sedimentation.

Happich and Boray (1969a) showed that a flotation method (Whitlock, 1950) and a sedimentation technique (Boray and Pearson, 1960) may be used with reasonable accuracy if the faeces contain 1000 E.P.G. or more. However, in lighter infections it was shown that because of the dilution factor, the successful quantitative diagnosis with the flotation technique would often depend on the recovery of one or two eggs. In these cases, the use of the sedimentation technique is more accurate and sensitive. Cattle, particularly adults, usually have low egg counts because of their resistance to infection, and the sedimentation technique would be more reliable. The technique by Dorsman (1956a) is also very accurate, and its reliability was confirmed by recovering known numbers of eggs. However, it is very time-consuming for routine examinations. It has been found that in most flotation techniques distortion of eggs occurred due to the concentrated flotation fluid, and differentiation between *Fasciola*

spp. and paramphistomatid eggs was difficult. The sedimentation technique is simple. No chemicals are necessary, it can be modified for quick field diagnosis, and it is suitable for the quantitative diagnosis of both *Fasciola* spp. and paramphistomatid infections.

There is considerable variation in egg counts from day to day, particularly pronounced in cattle. A regular pattern of diurnal fluctuation of egg counts was described by Dorsman (1956b, 1962). Subsequently, the effect of various factors on the interpretation of egg counts in cattle was discussed by Honer (1965a, b, c, 1967a) using a centrifugation flotation technique with $ZnSO_4$ in 1 g samples in various groups of cattle. The regular fluctuation as described by Dorsman (1956b) was not confirmed by Honer (1965a), but the results obtained from smaller samples with a less reliable flotation technique may not be comparable with those of Dorsman (1956b, 1962). Irregular results by Hagens and Over (1966) could also be explained by technical difficulties. Dorsman (1967) carried out further experiments, removing 3 g samples from 300–350 g mixed faeces collected hourly or at each spontaneous defaecation, and confirmed his previous results. The peak egg output was observed in the early afternoon, and faecal collection for diagnostic purposes was recommended to be carried out about midday. He gave some evidence that diurnal fluctuation of egg production by *F. hepatica* may depend on normal rhythmic liver function of the host. Dorsman (1962) did not find regular fluctuation of egg output within a day in sheep. However, faecal collections in this laboratory have been regularly carried out in the early afternoon to reduce the many possible variations in egg counts.

### D. EGG PRODUCTION CAPACITY IN SHEEP AND CATTLE

The egg production capacity of *Fasciola* spp. greatly influences the epidemiological pattern of fascioliasis, and more knowledge of it may assist in the design of control of the disease by management. The results of egg counts may also be used for the estimation of the number of flukes in the sheep and thus assist in the prognosis of chronic fascioliasis. Happich and Boray (1969b) investigated the egg production capacity of *F. hepatica* in sheep, together with aspects of quantitative diagnosis of chronic fascioliasis. 276 Merino wethers aged 4 to 5 years, chronically infected with *F. hepatica* of known age, kept in pens and fed on a standard diet, were used for the experiment. Among the quantitative methods for the detection of fasciolid eggs, the fractional sieving of Willmott and Pester (1952) modified by Dorsman (1956a), and the sedimentation of Boray and Pearson (1960) are regarded as the most accurate. The sedimentation technique modified by Happich and Boray (1969a) was used for the studies because it is faster and simpler than the sieving technique. Total daily faeces of all sheep were collected and weighed, and egg counts were carried out during several days when the flukes were 13, 15, 19, 21 and 27 weeks old in some sheep. From most of the sheep, faeces were collected when the age of the fluke averaged 16 weeks.

The average daily egg production capacity of fluke was calculated from the mean eggs per gram (E.P.G.), the mean daily faecal production for the period,

and the number of flukes found at necropsy in each sheep. An average of 109 flukes in sheep increased egg production with increased age, reached the maximum egg output 17 weeks after infection, and persisted for at least 27 weeks (Table XXII). Early workers greatly underestimated the egg production capacity of *F. hepatica* (Hutyra and Marek, 1938). The daily egg production per fluke in a rabbit was about 1300 (Montgomerie, 1931). There is little information about the daily egg production of *F. hepatica* in sheep. Dixon (1964),

TABLE XXII

*Egg production of* F. hepatica *in seven artificially infected sheep with an average of* 109 *flukes in their livers*

| | Weeks after infection | | | | | |
|---|---|---|---|---|---|---|
| | 13 | 15 | 17 | 19 | 21 | 27 |
| Average no. of eggs/fluke/day | 10664 | 14718 | 21649 | 21203 | 24311 | 22465 |
| Average daily egg output per sheep | 1162460 | 1604264 | 2359819 | 2311156 | 2649900 | 2448775 |
| Average E.P.G. per fluke | 9·3 | 9·0 | 14·4 | 19·2 | 21·5 | 20·2 |

using the sedimentation technique, found it to average 1331 in light infections. If his figure was calculated according to the modification by Happich and Boray (1969a) it would be about 4000 eggs/day. Happich and Boray (1969b) showed that the daily egg output by each fluke aged 17 weeks or more in a medium infection averaged 21 000 to 24 000, very much in excess of Dixon's results. They confirmed the results in a further 97 sheep, and it was shown that the daily egg output per fluke depended on the number of flukes in the liver and varied from 4000 to 50 000 (Table XXIII).

The number of eggs shed by infected sheep is of great significance in the epidemiology of fascioliasis. A sheep with a light subclinical infection may

TABLE XXIII

*Egg production of* F. hepatica *in* 97 *sheep* 13–19 *weeks after infection*

| No. of sheep | Average no. of flukes | Average no. of eggs/fluke/day | Average daily egg output per sheep |
|---|---|---|---|
| 46 | 19 | 25099 | 476881 |
| 12 | 70 | 20015 | 600450 |
| 16 | 137 | 16881 | 2312697 |
| 13 | 237 | 12309 | 2917233 |
| 10 | 401 | 8833 | 3542033 |

contaminate the pastures with more than a half million eggs daily, and a medium infection may result in the daily shedding of 2½ to 3 million eggs. The duration of the egg output in the heaviest infections, however, would be limited by the death of the sheep (Boray, 1967a), which may have a reducing effect on the contamination of pastures and on the occurrence of the disease.

The estimation of the number of flukes present in sheep from egg counts is particularly important for the correct prognosis of chronic fascioliasis, and for anthelmintic and epidemiological studies. Calculations with 269 sheep showed that egg counts increased, but not proportionally with the increase in the number of flukes present, and the E.P.G. per fluke showed a negative regression on the fluke burden. Table XXIV shows an extract of data by Happich and Boray

TABLE XXIV

*The faecal egg count-fluke ratio in 269 sheep with chronic fascioliasis using the quantitative sedimentation technique*

| No. of sheep | No. of flukes | Manifestation of infection | Average E.P.G. per fluke |
|---|---|---|---|
| 195 | 1–100 | Subclinical chronic fascioliasis | 33 |
| 60 | 101–250 | Clinical chronic fascioliasis | 20 |
| 32 | >250 | Serious clinical chronic fascioliasis | 12 |

(1969b). The average E.P.G. per fluke has been recorded, together with the expected effect of the degree of infection on the host according to the results of experimental infections (Boray, 1967a; Section V B). The correlation of fluke burdens with egg counts may assist in a realistic prognosis of chronic fascioliasis in sheep. The method of estimation of fluke burden may be very useful in anthelmintic trials in sheep, because the egg output is persistent in this host. On the other hand, the patent period of *F. hepatica* and *F. gigantica* infection in cattle is limited (Alicata and Swanson, 1941; Dixon, 1964; Boray, 1967b), and after a short peak the egg production may diminish rapidly (Boray, 1967b) due to reduced egg production and/or elimination of flukes. Coyle (1958) did not find a regular relation between egg counts and *F. gigantica* numbers in cattle. A short-term estimation of fluke burden in young, susceptible calves may be possible with quantitative egg counts. However, use of this method as a quantitative diagnosis in anthelmintic trials is limited and may only be applied in controlled experiments with large numbers of animals.

### E. ACQUIRED RESISTANCE IN SHEEP AND CATTLE

There is evidence that the hosts of *F. hepatica* and *F. gigantica* respond with antibody production to infection with the parasite or to the introduction of antigens derived from it. The literature has been reviewed by Dawes and

Hughes (1964), Pantelouris (1965) and Sinclair (1967). There is no indication, however, that any method which does not cause appreciable damage to the host would offer protection against the disease.

1. *Sheep*

There is no field evidence of any kind of immunological resistance against *F. hepatica* in sheep. Boray (1967a), however, reported lower recoveries in heavier infections and retardation of growth due to the severe liver fibrosis. There was no evidence of acquired resistance against *F. hepatica* in sheep which had a chronic infection that was eliminated with an anthelmintic, and which were challenged with 200 or 4000 metacercariae. However, in repeated heavy infections, or if the infection was given in daily doses, the results indicated that the tissue reaction caused by the migrating flukes might hinder the establishment of flukes from subsequent infections. All the above experiments were carried out in adult sheep of similar condition which were fed on a standard diet, but the clinical effect of the infections varied considerably from sheep to sheep. Recent studies showed that lambs on a low plane of nutrition died earlier than controls fed on a better diet after heavy experimental infections, and the growth of flukes was more retarded in adult sheep on a high plane of nutrition compared with controls (Section V B).

Boray (1967b) showed that when the sheep were previously repeatedly infected with 1000 normal metaceracriae, and each infection was terminated with an anthelmintic 5 weeks after infection, the pathological changes were less serious after the challenge dose but the fluke burden was not reduced, and it seemed that fibrosis of the liver acted simply as a mechanical barrier. He concluded that this reaction did not reduce the number and the size of the fluke, but might have reduced the motility of the migrating fluke in the challenge infection. In further experiments (Boray, unpublished data), an infection with 1000 metacercariae was terminated 8 weeks after infection, when the tissue damage is more substantial, and challenged 9 days later with 1000 metacercariae in five sheep together with five controls. There was no difference in the fluke burden and the size of flukes in the two groups, but anaemia occurred earlier in the controls, 10 weeks after the challenge.

A small number of *F. hepatica* may live in the sheep permanently without any sign of detrimental effect either to host or parasite, and it seems that there is no specific reaction in the host acting against the parasite, and that the survival of the host and the parasite depends on the number of metacercariae ingested by the sheep. Previous repeated exposure of adult sheep to *F. hepatica* infections in the field may not prevent mortalities from subsequent infections, but adult sheep or sheep on better nutrition may tolerate reinfections better than lambs or sheep which experience a heavy first infection or are undernourished.

2. *Cattle*

It has been generally recognized that cattle are more resistant to both *F. hepatica* and *F. gigantica* than sheep. Hutyra and Marek (1926) stated that *F. hepatica* generally lives 9–12 months and only a few flukes were found 3–5

years after an initial infection. Similar observations were reported by Dixon (1964). Kotlan (1953) stated that mainly young cattle suffer from clinical fascioliasis due to *F. hepatica*, and adult animals exposed to repeated infections show a high degree of resistance to reinfection. Davtyan (1956) found that in cattle, *F. hepatica* was found to be less infective but more pathogenic than *F. gigantica*, and it was considered that the bovine is the specific host for *F. gigantica*. However, Alicata and Swanson (1941) found in Molokai Island, Hawaii, that most *F. gigantica* were eliminated from five steers infected with 800 metacercariae 16 months after infection, but two of the animals retained three and six flukes respectively 3 years after infection. Coyle (1958) suggested that *F. gigantica* may be more detrimental in cattle than *F. hepatica*. He found (Coyle, 1961) that the ventral lobes were more severely affected than the dorsal lobes of the liver in cattle infected with *F. gigantica*. It was assumed by Cohrs (1967) that young flukes may burrow directly into the left lobe from the abomasum. Coyle (1961) suggested that the presence of severe localized fibrosis and calcification is most likely to produce a physical barrier against reinfection.

Sewell (1966) produced death from subacute fascioliasis in two Zebu steers aged 2 years by giving 20 000 *F. gigantica* metacercariae. Infections with 675 and 1400 metacercariae produced fatal chronic disease in two cattle, but two others showed no appreciable clinical symptoms for 10 months after they were given 2600–5000 metacercariae. Keck and Supperer (1966) suggested that calcification of the bile ducts is responsible for the spontaneous recovery of cattle from chronic *F. hepatica* infection. They also showed in radiological studies that these changes may resolve completely about a year after the termination of the infection. Ross (1965, 1966a, b) confirmed Coyle's finding of a preferential migration of immature flukes, and stated that in heavier infections the majority of flukes were immobilized and eliminated during the migration phase by the tissue reaction, and in challenge infections this effect may occur earlier as a result of the previous changes in the liver. Most experiments, on a limited number of cattle of different breeds and often unstated condition and nutrition, showed that there was great individual variation from one animal to another in the response to infections with both *F. hepatica* and *F. gigantica*.

The age of cattle and their nutrition may have an appreciable influence on the resistance status of the host, and subsequently on the host reaction against the flukes (Section V B). Boray (1967b) showed that if an initial infection were removed or reduced by anthelmintic treatment, or if the infection were eliminated or reduced by spontaneous recovery, a high degree of acquired resistance developed in cattle. The resistance was manifested by lesser clinical symptoms, and in lower incidence of flukes, lower egg output and extremely short patency in the challenge infections. Boray (1967b) showed in another experiment that spontaneous recovery occurred in cattle after single infections, but recovery was more rapid in challenge infections. Further experiments were carried out in the laboratory on cattle kept on an adequate standard diet. (For results see Table XXV).

In 6–8-month-old calves the dose of 1000 metacercariae caused clinical chronic fascioliasis, with anaemia and high egg counts, but recovery was spontaneous. When challenged with 1000 metacercariae, together with

TABLE XXV

*Acquired resistance in cattle aged* 16–18 *months challenged with* 1000 F. hepatica *metacercariae each*

| Experimental Group | No. of cattle | No. of flukes recovered 20 weeks after challenge | % recovery | Mean length of flukes (mm) 20 weeks after challenge | Mean E.P.G. during 3 weeks of peak egg production period | Clinical symptoms |
|---|---|---|---|---|---|---|
| Previously non-infected controls | 2 | 64<br>119 | 6·4<br>11·9 | 13·8 (4·5–19·5)<br>14·9 (6·0–21·5) | 81 (49–115)<br>115 (90–160) | Nil |
| Dosed with 1000 metacercariae each 10 months before challenge and animals recovered spontaneously | 4 | 57<br>67<br>85<br>97 | 5·7<br>6·7<br>8·5<br>9·7 | 11·5 (4·0– 7·0)<br>12·1 (4·5–23·0)<br>7·2 (3·5–14·0)<br>8·2 (4·0–15·0) | 10 (2–16)<br>32 (12–52)<br>0·1 (0–0·5)<br>2·6 (0–7·5) | Nil |
| Result of infection of above group at first infection at the age of 6–8 months | 4 | — | — | — | 2043 (1290–2810)<br>1576 (640–2660)<br>1553 (1060–2060)<br>796 (560–1020) | Temporary severe anaemia |

controls, at 16–18 months of age, both groups showed high resistance and low recoveries. However, the development of flukes in the controls was more advanced and had higher egg counts. Both groups were free from any clinical symptoms of the disease, showing that the age of the host was a more important factor in the resistance.

The high resistance of adult cattle was further demonstrated when animals, recovered from previous heavy and medium infections, were challenged with a weekly dose of 500 metacercariae each for 18 weeks (total of 9000 MC), together with previously non-infected controls of the same age (Table XXVI). All animals showed high resistance and were free from clinical symptoms before they were killed 13 weeks after the last dose of metacercariae. Substantial liver damage was found at necropsy in all animals but the recovery rate of flukes was low. The flukes were larger and produced eggs in the controls, but in most of the "resistant" animals the infection did not become patent and the flukes were smaller.

It may be concluded that a high degree of tissue reaction and intensive fibrosis may be necessary to produce resistance to reinfection in younger cattle. This may be achieved by initial heavy infection resulting in extensive fibrosis of the liver tissue during the early migration phase, or by moderate infection resulting in chronic cholangitis, or continuous repeated infection which may be common in the field. However, because of the long tissue migration period of the parasite, resistance may develop in adult cattle from a single infection resulting in a spontaneous recovery. In these animals a delayed tissue reaction is responsible for the reduction of the number of flukes before they enter the bile ducts. In challenge infections the elimination of flukes may occur earlier in the already fibrotic liver. The preferential migration of young flukes into the ventral or left lobe, which was also observed in our experiments, together with hypertrophy of the right lobes, facilitate the formation of a host barrier even in moderate infections. At the same time, however, it permits the survival of the host by leaving enough liver tissues relatively undamaged. If some of the flukes reached the bile ducts, cholangitis, proliferation of the epithelium of the bile ducts and fibrosis of the surrounding tissue is present, and specifically in cattle the collagenous connective tissues produce a dystrophic calcification with the fibrosis proliferating into the parenchyma. Under these conditions the adult flukes may be eliminated simply by starvation. The resistance in cattle is due to the combination of an early and delayed tissue reaction and a chronic reaction forming mechanical barriers. The expression of "acquired self cure" (Ross, 1965), which should be preserved for a clearly immunological phenomenon, may not be justified.

It seems that in the field the development of severe fascioliasis in cattle may only occur in young animals, chiefly during their initial infection. Treatment of young cattle, during their first infection, with an anthelmintic efficient against both mature and immature fluke, would prevent serious clinical symptoms of the disease. The treated animals, however, may develop strong resistance to subsequent infections without severe liver damage. In contaminated pastures continuous low level infections may produce a strong protective acquired resistance, and evidence of this is presented above. This resistance may depend,

TABLE XXVI

*Acquired resistance to* F. hepatica *in Jersey × Zebu cattle aged 2–3 years challenged with 500 metacercariae (MC) weekly 18 weeks (9000 MC each)*

| Experimental group* | No. of flukes recovered 13 weeks after last challenge dose | % recovery | Mean length of flukes (mm) 13 weeks after last challenge dose (age of flukes 13–21 weeks) | Mean E.P.G. 10–13 weeks after last challenge |
|---|---|---|---|---|
| Previously infected with 20 000 MC and the animals recovered spontaneously | 35<br>497 | 0·4<br>5·5 | 7·0 (4·0–13·0)<br>7·6 (3·5–13·0) | 5·0<br>0·0 |
| Previously infected with 2 × 1000 MC and the animals recovered spontaneously | 29<br>111 | 0·3<br>1·2 | 7·3 (3·5–11·5)<br>7·2 (3·5–12·5) | 0·0<br>0·0 |
| Previously non-infected controls | 169<br>279 | 1·8<br>3·1 | 10·4 (5·0–23·0)<br>12·7 (3·5–27·0) | 113·0<br>145·5 |

* Two cows in each group. These animals showed no clinical symptoms and their haematology was normal.

however, on nutrition and on other concurrent diseases, such as ostertagiasis (Reid *et al.*, 1967), and consequently on the condition of the animals. On contaminated pastures adult cattle may maintain a high degree of resistance, but if reinfection does not take place for approximately 1 year, they may become susceptible again and may suffer from clinical or subclinical fascioliasis for a short period until spontaneous recovery occurs.

It has been generally believed that mixed grazing of cattle with sheep might increase the contamination of sheep pastures. It seems, however (Section V G), that chronically infected sheep would contribute more to contamination than cattle. Boray (1967b) suggested that resistant cattle, often preferentially grazing in potentially contaminated areas, may well reduce contamination and influence the epidemiology of the disease (see Section VIII).

Active and passive immunization have been attempted by many workers and the literature is summarized by Dawes (1964), Dawes and Hughes (1964), Pantelouris (1965) and Sinclair (1967). The first report on inactivation of fasciolid metacercariae (*F. gigantica*) with X-irradiation was given by Jarrett *et al.* (1959). They found that the exposure to 5000 r was sufficient. It was shown by Stewart, Boray and Pearson (1960, unpublished data; Anon., 1961) that, although 350 r was the lowest exposure to X-irradiation which affected *F. hepatica* metacercariae and produced lower pathogenicity in mice, a dose of 2500 to 3000 r gave the most satisfactory attenuation. This dose caused death of the young flukes shortly after migration through the liver had commenced, and Dawes (1964) indicated how this comes about by leucocytic invasion of the cuticle. The general observations were confirmed by Wikerhauser (1961), Hughes (1962) and Dawes (1964), but Wikerhauser (1961) found that some flukes survived and developed to maturity in rabbits if the X-irradiation was less than 20 000 r. The possiblity of vaccinating sheep and cattle against fascioliasis by the use of X-irradiated metacercariae has been under investigation in this laboratory.

Boray (1967b) infected a group of six adult Merino sheep with X-irradiated metacercariae (20 000 r) three times at 6-week intervals. They were challenged with 4000 metacercariae each, together with six non-vaccinated controls. There was no appreciable difference in the number of flukes established in the sheep from the challenge infection compared with controls. The vaccination of sheep did not appear to affect their survival time, and there was no difference in gross pathology between the two groups. It was observed, however, that anaemia developed later in the vaccinated sheep. Similar experiments were carried out in groups of three calves (Boray, 1967b). The calves in one group were infected three times with 3000 X-irradiated metacercariae (20 000 r) each and were challenged with 5000 normal metacercariae each, together with three non-vaccinated controls. Sixteen weeks after the challenge infection there was no appreciable difference in the number of flukes or in their mean length. The pathological lesions were more severe in the previously non-infected group, and one of the calves died of subacute fascioliasis and anaemia 10½ weeks after infection. None of the other animals showed obvious clinical symptoms of the disease. The viability of both the X-irradiated and normal metacercariae were tested in mice before each "vaccination" and before

challenge. Moreover, two additional sheep and two calves were killed 3 weeks after they were given 1000 or 3000 X-irradiated metacercariae each respectively. In the mice the young flukes affected by radiation were alive and still migrating for 2 weeks, but all were dead 3–4 weeks after infection. The findings were similar in the sheep and in the calves. There was some liver damage and fibrosis due to the limited migration of the X-irradiated fluke, but substantially more fibrosis was produced when normal metacercariae were used for sensitization (see above and Boray, 1967b).

The protective effect of these previous infections against challenge was in accord with the amount of fibrosis present during the sensitizing infection in cattle. It was shown, however, that previous heavy infection with X-irradiated metacercariae apparently did not affect the challenge infection in sheep. Tewari (1967, unpublished data) found that guinea-pigs which were given injections of whole fluke extracts, or which received intra-peritoneal injections of anti-*F. hepatica* guinea-pig serum, developed a small degree of resistance to challenge infections, but there was no increased resistance to infection with *F. hepatica* in guinea-pigs as a result of a previous infection which was terminated before challenge, or as a result of the administration of X-irradiated metacercariae. Possibly a cellular reaction around the parasite in the liver tissue would be mobilized earlier in some sensitized hosts, but there was no convincing evidence of immune reaction which would influence challenge infections in sheep and cattle (Boray, 1967b; Ross, 1967d). The cellular response to any liver damage due to mechanical or toxic lesions may result in a more severe fibrosis, and chronic fascioliasis in cattle is always accompanied by calcification of bile ducts, which rarely occurs in sheep. It seems that the higher resistance against *F. hepatica* and *F. gigantica* infection in cattle and possibly in man is due to a tissue reaction characteristic of these hosts.

### F. OTHER DEFINITIVE HOSTS

Many mammals other than sheep and cattle are susceptible to *F. hepatica* infection, but their susceptibility and the pathological changes caused by the parasite vary considerably from host to host. Some small laboratory animals may be used as models in fascioliasis research. Studies on some domesticated and wild animals are necessary to clarify their different roles in the epidemiology of the disease.

#### 1. *Mice*

The first report on the susceptibility of mice to *F. hepatica* was given by Shirai (1927). Krull (1933b) successfully infected the white-footed mouse with *F. hepatica* and Taylor and Parfitt (1957) described the albino mouse as suitable for viability tests of metacercariae. Mice proved to be very useful model animals for detailed studies on the development of *F. hepatica* in its definitive hosts. From the first reports by Hughes (1959) and Dawes (1961a, b) a series of publications appeared on various aspects of development of *F. hepatica* (Dawes and Hughes, 1964). They showed that a certain proportion of flukes developed to full maturity and eventually caused the death of the host. Detailed

studies on the host-parasite relationship between mice and *F. hepatica* were reported by Lang (1966, 1967, 1968) and Lang *et al.* (1967).

Since 1958 more than 2500 mice have been used for biological tests of metacercariae in this laboratory (Boray, 1963b; Section IV C). All these mice, in groups of 20, received a single metacercaria each. If the metaceriae were viable, 18 or more showed typical migratory tracks in their livers (90–100%) 2–3 weeks after infection, which shows a remarkable susceptibility to the early stage of migration. The question of the "wastage of potential flukes" was discussed by Dawes and Hughes (1964). Our results in mice suggest that there may be very high initial susceptibility to *F. hepatica* infection in many mammals, and the fate of the fluke will depend on the vigour and the nature of the tissue reaction, depending on the varying innate or acquired factors in the host tissues.

2. *Rats*

The suitability of the albino rat for screening tests on drugs was described by Lämmler (1959), and Thorpe and Broome (1962) used this host for immunological studies. Rats have been used in this laboratory for viability and infectivity tests of metacercariae (Boray, 1963a, b). The pathology and host-parasite relationship in experimental infections of rats with *F. hepatica* were studied by Thorpe (1965a, b). Thorpe (1965c), Boray *et al.* (1967a) and Tewari (1968) carried out controlled efficiency tests for anthelmintics using albino rats. Rats are highly susceptible to infection with *F. hepatica.* Thorpe (1965b) showed the occurrence of a competitive inhibition in the development of flukes in heavier infections. Boray *et al.* (1967a) and Tewari (1968) showed that appreciably more flukes were recovered from rats at 5 weeks than at 20 weeks after infection with 25 metacercariae each. Some flukes which fail to enter the main bile duct during their early migration may become eliminated by a local tissue reaction. In recent experiments Boray (unpublished data) showed that the small number of flukes which survived in the main bile duct of several rats after artificial infection with 25 metacercariae each, lived and produced viable eggs as long as the natural life of the host (2½ to 3 years).

3. *Guinea-pigs*

Guinea-pigs are highly susceptible to *F. hepatica* (Sinitsin, 1914; Shirai, 1927; Nöller and Schmid, 1927; Clunies Ross and McKay, 1929; Turner, 1930). Infection usually results in severe pathological changes, chiefly due to secondary bacterial infection, with consequent high mortality (Wagner, 1929; Lämmler, 1955; Boray, 1963b). The high susceptibility of guinea-pigs to infection was reported in accidental outbreaks of acute fascioliasis due to infected hay (Goebiowski, 1959; Stoican and Lescinschi, 1959), and feeding grass to guinea-pigs was used to test the contamination of pastures with metacercariae (Ross and O'Hagan, 1966).

Boray (1963b) considered that the guinea-pig may not be suitable for anthelmintic tests because of the high mortality due to the parasites and possibly to

secondary bacterial infections. Tewari (1969) carried out experiments on the guinea-pig with reference to its suitability for testing anthelmintics, and an attempt was made to prevent secondary bacterial infections associated with fascioliasis in this host. These studies confirmed that the guinea-pig is highly susceptible to infection with *F. hepatica*, and an infection with 25 metacercariae was lethal in 80–100% of animals. When the dose was reduced, the mortality decreased considerably but few parasites reached maturity. There were wide variations in the number of flukes recovered from the guinea-pigs. The prepatent period averaged 55 days irrespective of the size of the infective dose. A wide spectrum antibiotic did not offer any protection to the infected animals, and vaccination against *Clostridium oedematiens* had no effect on the mortality.

Krull and Jackson (1943) reported that the flukes did not mature in guinea-pigs, but the studies by Tewari (1969) showed that sexually mature flukes were found in the bile ducts, and the eggs passed out in the faeces were fully viable. The number of flukes recovered from the guinea-pigs decreased as the duration of the infection extended. The reduction of almost 50% 4–8 weeks after infection was due to a severe tissue reaction which destroyed some of the flukes that failed to enter the bile duct. The surviving flukes were in the enlarged main bile duct of the guinea-pigs 12 weeks after infection, but the number of flukes recovered was again reduced by more than 50% 12–16 weeks after infection. In several infected guinea-pigs kept longer than 16 weeks in this laboratory, a spontaneous recovery from the infection occurred. The epithelium of the extended bile ducts was not thicker than that of the rats in this chronic stage. It seems that the guinea-pig is the only host of *F. hepatica* in which spontaneous recovery occurs without the presence of a drastic mechanical barrier, such as calcification. It may prove to be the most useful host for further studies on the immunological aspects of fascioliasis. Preliminary studies by Tewari (1967, unpublished data) indicated that administration of cortisone decreased the host resistance against *F. hepatica* in guinea-pigs. It is possible that cortisone reduced inflammation and interfered with the tissue reaction, and thus reduced resistance. Similar observations were reported by Sinclair (1968) in sheep.

### 4. *Rabbits*

The rabbit was one of the first experimental animals used for artificial infections with *F. hepatica* and *F. gigantica*, and is susceptible at all ages. The use of the rabbit was reported by Lutz (1893), and it was used for testing the viability of *F. hepatica* metacercariae and for therapeutic studies (Marek, 1927; Nöller and Schmid, 1927; Clunies Ross and McKay, 1929). The suitability of the rabbit as an experimental host, and its use for various immunological, pathological and therapeutic studies with *F. hepatica*, has been reported (Urquhart, 1954; Urquhart *et al.*, 1954; Jennings *et al.*, 1955; Lämmler, 1955, 1956; Urquhart, 1956; Kimura, 1961; Kendall *et al.*, 1967). The use of rabbits for comparative pathological studies on *F. hepatica* and *F. gigantica* was described by Davtyan (1956).

The role of the rabbit as a reservoir host has been recognized for many years. The life span of *F. hepatica* in this host is as long as the life of the host itself.

However, in recent studies in this laboratory, passages of *F. hepatica* were maintained in rabbits. It was found that flukes produced eggs continuously for more than 3 years after artificial infections with 50 metacercariae, but in many cases some of the eggs were deformed and the viability of eggs was very low. The viability was particularly low if the infection was associated with a serious tissue reaction and fibrous perihepatitis. It was also found that eggs were more viable 12 weeks after infection than in older infections. After the 4th passage of *F. hepatica* through rabbits, further infection of snails was almost impossible, and not only the viability and hatchability, but the infectivity of miracidia was impaired. It is concluded that although rabbits are susceptible, may suffer from the disease (Taylor, 1964; Pantelouris, 1965) and can maintain the survival of *F. hepatica*, their role as reservoir hosts, without the presence of other hosts, may not be persistent. Because of their habits, their role in contamination of the pastures may also be greatly restricted. Evidence of this in the field was reported by Boray (1969; Section VIII A).

5. *Marsupials*

The common wombat (*Vombatus hirsutus*) occurs in S.E. Australia where liver fluke disease is endemic. Artificial infections of two wombats with 50 metacercariae each in this laboratory showed that a strong natural resistance through a very early host reaction eliminated the flukes before substantial tissue damage could occur, or before the flukes could become established.

Artificial infections of three great grey kangaroos (*Macropus giganticus*) with 50 viable metacercariae each resulted in low recovery of flukes (17%). They were in restricted sections of the bile ducts 15 weeks after infection, but signs of previous fibrosis and perihepatitis were apparent. The fluke eggs hatched after a normal incubation period and were infective to snails. Similar infections resulted in only one to two flukes in the bile ducts of the Tammar wallaby (*Protemnodon eugenii*) and they were in small localized ampulla-form sections of the bile ducts, resembling light infections in pigs. However, fluke eggs were present which hatched normally and were infective to snails.

Two adults and one young common possum (*Trichosurus vulpecula*) were infected artificially with 50 metacercariae. Normal development occurred similarly to that in sheep and the recovery rate was 44%. The infection produced chronic fascioliasis associated with severe anaemia. The fluke eggs were of normal shape, hatched normally and were infective to snails.

The common wombat and the Tammar wallaby possess a degree of natural resistance, and a large proportion of flukes may be eliminated early during their migration. Their role as a reservoir host is negligible. The great grey kangaroo, though less susceptible than other hosts, may harbour adult flukes in the bile ducts. However, this host is relatively rare in endemic areas and its grazing habits may keep it away from marshy snail habitats. Its role as a reservoir host may not be important compared with the more susceptible domestic animals. The common possum may be a very good host for pasture contamination, but its role may be largely restricted by its low population in most grazing districts.

6. *Pigs*

Amongst domestic animals the most common hosts are sheep and cattle, and in certain countries, goats and buffaloes. There is little information on less important domestic hosts, but some attention has been paid to fascioliasis in pigs. Kotlan (1953) stated that the disease may occur in grazing pigs; the fibrosis of liver tissue may be negligible, and usually chronic cholangitis is found at necropsy and is localized to small sections of ampulla-form encapsulated areas of the bile ducts. Kovacs and Nemeseri (1957) reported that clinical fascioliasis amongst grazing pigs is very common in certain years in Hungary when the infection is high in sheep and cattle. They refer to some previous publications and to successful treatment of the disease. Kovacs and Nemeseri (1957) suggested that clinical fascioliasis may be a predisposing factor for other parasitic or bacterial infections by lowering the natural resistance of animals. However, early descriptions (Bugge and Müller, 1928; Kotlan, 1953) and the author's personal experience suggest that the disease may be subclinical as a rule, and that most economic loss is due to the condemnation of livers.

Clinical significance of *F. hepatica* infection may only be present or suspected when the resistance of pigs is low due to malnutrition or a concurrent disease. The infection was recognized in lactating sows in poor condition with severe *Hyostrongylus rubidus* infection (Kovacs and Boray, 1956), and in some of the sows the extended bile ducts were localized and contained 150–200 mature *F. hepatica*. Ross *et al.* (1967a) found low *F. hepatica* egg counts in two sows suffering from clinical hyostrongylosis, and a low level artificial infection with metacercariae was eliminated by an early tissue reaction in a small number of young pigs, possibly in good condition, in the laboratory.

7. *Horses and donkeys*

*F. hepatica* infection occurs in horses and donkeys everywhere in the world where they are allowed to graze pastures and the disease is endemic. They are susceptible, and both subacute fascioliasis (Kralj *et al.*, 1960) and chronic fascioliasis (Collins, 1961; Mijatovic and Herceg, 1962) have been recognized. The description of the pathology of subacute changes indicates that at the early stage, the disease may be similar to that in sheep, but the fibrous perihepatitis and peritonitis were more pronounced than in sheep. Chronic infection causes cholangitis and serious interstitial fibrosis leading to cirrhosis without calcification. There seems to be a disproportionally severe fibrosis due to relatively small number of flukes usually recovered from the bile ducts. Without experimental infections it is very difficult to define the resistance status of the horse. This host may react more vigorously than other hosts to relatively small numbers of invading flukes, or the resultant tissue reaction may reduce the number of flukes in the liver tissue or in the bile ducts.

There is no report of *F. hepatica* infection in horses in Australia. However, a group of Shetland ponies grazing heavily contaminated pastures were observed for 2 years. They showed no clinical symptoms of fascioliasis and 12 of the 25 animals had very low egg counts (Boray, unpublished data). Most other ponies on the same property were grazing heavily contaminated pastures

for several years without apparent clinical symptoms. Accumulative infection, which is very common in sheep, is unlikely to occur in the horse. It seems that the disease in horses is of little importance because of husbandry methods. Grazing animals may not be exposed to heavy infection due to their selective grazing habits, and it is possible that vigorous delayed tissue reaction may destroy the majority of flukes if the animals become infected, and the life span of adult flukes may not be very long.

8. *Man*

Reports on human fascioliasis were summarized by Dawes and Hughes (1964). There is considerable tissue reaction and calcification in the bile ducts due to the presence of even a small number of flukes, and as in cattle, a spontaneous recovery from the infection is common.

## G. HOST-PARASITE RELATIONSHIPS

Ross (1967c) divided the more common hosts for *F. hepatica* into three groups, based on low resistance, medium resistance and high level resistance. In view of the evidence presented in this chapter, his conclusions require considerable amendment (see Tables XV and XIX). Although some evidence of a higher resistance status was found in sheep with better nutrition, the present results basically agree with the conclusions of Ross (1967c), who stated, however, that during the immature migration phase, inhibition of development did not occur either in light or in heavy infections. Boray (1967a) and the present evidence showed that the common and most important feature of heavy infection in sheep is the retardation of development during tissue migration due to fibrosis, causing a delayed subacute fascioliasis in the field (Section VIII B). This reaction, however, does not eliminate the flukes.

The conclusions by Ross (1967c) concerning cattle may be true in adult and well fed animals, or in animals with previous experience of infection. However, it was shown in the present results that in calves aged up to 6–8 months, clinical chronic or acute fascioliasis and deaths may be produced by increased doses of metacercariae. Spontaneous recovery occurred from light infections in all animals and from heavy infections in some young and all adult cattle, or after challenge infections. Pigs may be highly resistant under normal conditions, but the statement of complete inhibition (Ross, 1967c) may be an oversimplification. Fascioliasis of pigs under special environmental conditions could cause some concern.

Based on our experience and on a survey of the literature, an attempt has been made to classify most hosts of *Fasciola* spp. into groups depending on their resistance (Table XXVII). It seems that resistance in a purely immunological sense does not exist in *F. hepatica* infection. There is a group of animals where the tissues are not suitable for the parasite. These have a natural resistance and an early tissue reaction eliminates the parasites, with relatively low pathogenic effects on the host. If a limited number of flukes reached the bile ducts the section of the bile duct usually became encapsulated by a chronic

TABLE XXVII
*Hosts of* F. hepatica

| Early resistance | Delayed resistance | Low resistance |
|---|---|---|
| **Sus scrofa* | **Bos taurus* | **Ovis aries* |
| *Sus cristatus* | *Bos indicus* | *Ovis aries musimon* |
| *Cricetus cricetus* | *Bubalus bubalis* | **Capra hircus* |
| **Mesocricetus auratus* | **Cavia porcellus* | **Oryctolagus cuniculus* |
| *Canis familiaris* | *Homo sapiens* | **Lepus europeus* |
| *Felis domestica* | **Equus caballus* | *Lepus californicus* |
| **Vombatus hirsutus* | *Equus burchelli* | *Sylvilagus floridanus* |
| **Protemnodon eugenii* | *Equus asinus* | **Mus musculus* |
| | **Cervus elaphus* | **Rattus rattus* |
| | *Dama dama* | *Rattus norvegicus* |
| | *Capreolus capreolus* | *Peromyscus leucopus* |
| | **Macropus giganticus* | *Castor fiber* |
| | | *Myocastor coypus* |
| | | *Citellus citellus* |
| | | *Sciurus vulgaris* |
| | | **Trichosurus vulpecula* |

The author had personal experience with hosts marked * in connection with *F. hepatica* and/or *F. gigantica*

reaction. The infection in this group is self-limiting without harming the host (Early Resistance Group).

The second group has a basically mechanical resistance acquired during the first few weeks of a single infection or during challenge infections. A delayed host reaction controls the parasites during the later phase of tissue migration, and if some flukes arrive in the bile duct, a chronic reaction would eliminate them by imposing a mechanical barrier to blood sucking. In this group the disease is self-limiting, but may cause severe pathogenic lesions and mortalities may occur, particularly in young or debilitated animals (Delayed Resistance Group).

The third group has low resistance; there is a severe early and delayed tissue reaction, but without sufficient fibrous tissue, and cellular reaction to immobilize and eliminate the parasites. In the chronic state in the bile ducts, there is no calcification and light infections may survive as long as the host (Low Resistance Group). In this group the disease is highly pathogenic in both the acute and the chronic phases.

There is some overlapping between the groups, mainly due to the effect of age and nutrition of the hosts and concurrent diseases influencing the resistance status of the hosts, but there is a general trend which shows how host reaction may influence the epidemiology of the disease, and probably indicates some new methods for its control (Section VIII).

It seems that there is a very unbalanced host-parasite relationship between

*Fasciola* spp. and the most common domesticated hosts, and it may be futile to discuss the question of which is the most successful host-parasite system. In some hosts the parasite will be eliminated; in others the parasite will be destroyed together with the host, depending on the level of infection and on the physical condition of the host. In both systems the disease is self-limiting. The survival, the flourishing existence and distribution of the fasciolid species may be supported chiefly by bovine and ovine hosts, assisted by human interference. Under natural circumstances the most effective controlling factor of the disease may be the death of the heavily infected intermediate host (Section VII B) or the domesticated definitive host, and the natural resistance and selective grazing habits of some domestic and wild animals.

The survival of the parasite and the host is necessary for continuation of the life cycle. Without the detrimental influence of man, resistant cattle would graze the low-lying districts and could possibly benefit from the "legendary" effect of liver damage on their metabolism. Sheep would have a distinct affinity for dry pastures by natural selection, and the wild animals possessing natural resistance would not be affected.

## VI. Chemotherapy of Fascioliasis

The number of compounds tested against *Fasciola* spp. has increased substantially in recent years. This trend reflects the continuous demand for more effective anthelmintics, which should be cheap, easy to apply to large numbers of animals, non-toxic and highly effective against both mature and immature flukes. There are numerous contributions on different aspects of experimental chemotherapy of fascioliasis, and reviews of the literature may be found in recent publications by Dorsman (1962), Kendall and Parfitt (1962), Boray (1963b), Lämmler (1963, 1964a, b), Leiper (1963), Lienert (1963), Standen (1963), Gibson (1965, 1967), Boray and Happich (1966b, 1968) and Boray *et al.* (1967a, b). The present section will deal chiefly with some experimental work carried out in Australia, paying particular attention to the efficiency of drugs against immature flukes.

### A. Screening in laboratory animals

The albino rat is used most extensively for primary screening because of its small size and easy handling. Its use for comparative screening was first described by Lämmler (1959). Lämmler (1963) and Thorpe (1965b) concluded that *F. hepatica* at a comparable age was more sensitive to drugs in rats than in ruminants. Boray *et al.* (1967a) carried out comparative trials on the anthelmintic efficiency of nine compounds, efficient against *F. hepatica* in ruminants, by means of the controlled test. The efficiency of the drugs was tested at several dose rates in 635 rats, given as single oral doses 4, 6, 8, 10, 12 or 19 weeks after infection. The results are summarized in Fig. 9. Anthelmintic activity varied, depending on the age of the flukes and on the pathological damage to the liver caused by the parasites, and it was recommended that the controlled test should be used with sufficient number of rats given the maximum tolerated dose of the drugs at the most suitable time after infection. In rats the most suitable

age of infection for anthelmintics was found to be 4 weeks for immature and 19 weeks for mature flukes.

There was evidence that the activity of some compounds was hindered by serious fibrosis 6 and 8 weeks after infection. *F. hepatica* infection, particularly at approximately 8 weeks, greatly increased the susceptibility of rats to poisoning with some compounds. High efficiency was obtained with most compounds 19 weeks after infection, when all flukes were in the main bile duct and the serious liver damage was resolved. However, it was suggested that if the screening of prospective compounds was carried out at the beginning of the patent periods, 6–8 weeks after infection, the results could be misleading because flukes were shown to be least susceptible to anthelmintics during this period. The most striking result of the experiments was that the efficiency of "Hilomid" at 90 mg/kg (three times the efficient dose in sheep) was slight or nil, 4, 12 and even 19 weeks after infection. When the dose was increased to 120 mg/kg, and higher efficiency might have been expected, the compound was ineffective 4 and 19 weeks after infection, although most other drugs showed their highest efficiency at 19 weeks. The results indicate that the efficiency of "Hilomid" in sheep might not have been demonstrated by a routine screening in rats. Boray *et al.* (1967a) therefore considered that rats are less suitable for screening potential anthelmintics against this parasite than sheep. They suggested, however, that the controlled test with sufficient numbers of rats might give useful information, if the maximum tolerated dose of the compound was administered when the immature and mature flukes appeared to be most susceptible to anthelmintic treatment, i.e. 4 and about 19 weeks after infection.

Tewari (1968), in some additional studies in this laboratory, confirmed the high efficiency in rats of hexachloroethane against flukes aged 4 weeks, and also against fully mature *F. hepatica*, in controlled trials, as reported by Thorpe (1965b). Boray and Happich (1968) found clioxanide, given by intraruminal injection, to be almost 100% efficient in sheep against flukes aged 4 weeks at the dose rate of 135 mg/kg body weight, and 93% efficient against flukes aged 12 weeks at 15 mg/kg body weight. Tewari (1968) found that clioxanide had no efficiency in rats at dose rates as high as 130 or 200 mg/kg body weight against flukes at the times they are considered to be most susceptible to treatment. These results are included in Fig. 9. Clioxanide, like Hilomid, is also a halogenated salicylanilid, and it is suggested that these types of compounds may not be efficient against *F. hepatica* in rats. The albino rat is less suitable than sheep for screening potential compounds against *F. hepatica* in ruminants.

A third halogenated salicylanilid, 3,5-dibromo-4'-chloro-3'(p-chlorophenoxy) salicylanilid*, which was found to be highly efficient in sheep, was inefficient in rats in experiments carried out in this laboratory. Inefficiency of halogenated salicylanilids in rats may be due to their administration as a single oral dose. Therefore, rats harbouring adult *F. hepatica* were fed a 0·5% food mixture of Hilomid and Clioxanide for 7 days. The daily consumption by the rats was 144 mg/kg and 211 mg/kg Hilomid and Clioxanide respectively, but the treatment was inefficient. The efficiency of halogenated

* Experimental compound MK464, Merck, Sharp & Dohme (Aust.) Ltd., Sydney.

salicylanilids, and possibly of other drugs, may depend on the specific ruminant metabolism rather than on the method of administration.

Happich (1967, unpublished data) infected 50 mice with ten metacercariae each in this laboratory. Treatments with Hetol, Hilomid, nitroxynil and hexachlorophene at two dose rates of each drug, found to be highly efficient in ruminants, were given to 40 mice 3 weeks after infection. The mice were killed 7 days after treatment, together with ten untreated infected controls. The results showed that doses usually tolerated by other hosts were toxic to infected mice, and if lower dose rates were employed the drugs showed no efficiency against

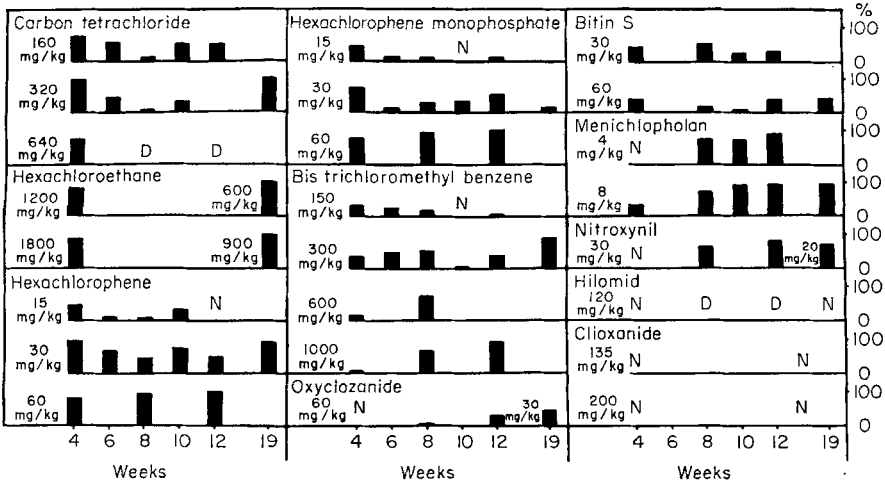


FIG. 9. Anthelmintic efficiency of various compounds against *F. hepatica* in albino rats.

flukes aged 3 weeks. Happich concluded that mice may not be suitable for screening potential compounds for ruminants. He recovered an average of five flukes per mouse (50%) from the controls 4 weeks after infection.

Mice were not susceptible to infection with *F. gigantica* (Anon., 1959). However, the successful infection of mice with this species was reported by Boray (1963b) and Standen (1963). Both authors found that the development of *F. gigantica* in mice is slower than that of *F. hepatica*. However, Standen (1963) reported that the liver damage was considerable after *F. gigantica* infection. Tewari (1969) carried out studies on the suitability of guinea-pigs for screening anthelmintics against *F. hepatica*. He reported that Hilomid and nitroxynil showed some evidence of activity in this host. However, the extensive liver damage in heavy infections, the very low recovery in lighter infections, and the strong indication that the infection of this host terminates much earlier than in other hosts, suggests that guinea-pigs are not suitable for screening anthelmintics.

Lämmler (1956) found the rabbit suitable for chemotherapeutical studies. However, he later provided evidence (1964b) that the minimum efficient dose of many compounds was appreciably higher in rabbits than in ruminants.

The above evidence indicates that screening potential anthelmintics for ruminants in small laboratory hosts is an economical compromise. Possibly the activity of some compounds for ruminants would not be detected by this technique, and it is preferable to use sheep.

### B. THE APPLICATION OF STANDARDIZED TECHNIQUES

Many research laboratories are currently engaged in the evaluation of the increasing number of new chemical compounds. This calls for the use of standardized techniques to give comparable and reproducible results for both efficiency and toxicity. The present Section gives a short summary of such laboratory experiments, on the comparative efficiency and acute toxicity of most drugs tested against immature and mature *F. hepatica*, carried out in more than 1000 artificially infected Merino sheep in this laboratory.

#### 1. *Standardized Chemotherapeutical Test*

(a) *Experimental animals.* Anthelmintic efficiency depends on the host, its age and condition, the number of flukes in the liver, the duration of the infection, and the pathological changes produced. Merino wethers aged 4–5 years were used. The animals were purchased from the same district free from *F. hepatica* infection. All sheep were kept in a fluke-free paddock with adequate pasture, and were vaccinated against black disease. Each sheep was infected with 200 *F. hepatica* metacercariae, producing an average infection of approximately 100 flukes in the liver.

(b) *Metacercariae.* The environmental conditions during the development of the flukes and during the storage after encystment affected the infectivity and pathogenicity of the metacercariae. The metacercariae which were used for all experimental infections were produced by the standard technique mentioned in Section IV C. The snail, *Lymnaea tomentosa*, was maintained under standard laboratory conditions for many years, kept at a constant temperature and fed with a standard diet. The metacercariae were released and stored by a standard technique. Before infection they were tested for viability, counted individually under a stereomicroscope, and were given to the animals in gelatine capsules.

(c) *Treatment.* A uniform method of administration was employed because activity may depend on the site of absorption. All drenches formulated as a suspension were given either by intrarumenal injection or in gelatine capsules. In very few cases, when the volume was too large, the material was administered through a long stomach tube into the rumen. However, some drugs, e.g. menichlopholan, may be more efficient if absorption occurs from the abomasum or small intestine (Federmann, 1963, personal communication; Boray *et al.*, 1969). Some drugs, such as clioxanide, are more efficient if metabolized in the rumen, and often fail if the drench by-passes the rumen through the oesophageal groove, particularly when an increased dose rate is given against subacute fascioliasis (Boray and Roseby, 1969). It is advisable,

therefore, that all drugs should be administered uniformly into the rumen, but experimental groups of sheep should be included in which the drug should be injected into the abomasum or given after induction of the oesophageal groove reflex. The subcutaneous injections were given in the wool-free area on the side of the sternum, where the degree of absorption and any local reaction could be observed. All sheep were divided into groups of five according to their body weight approximately 4 or 5 weeks after infection. Some of the groups (20–25% of the whole experimental group) were left untreated as controls.

(d) *Recovery of flukes.* All sheep, treated or untreated, were killed 14 weeks after infection and the livers were recovered immediately. All flukes were removed from the extended bile ducts, gall bladder and *ductus choledochus*, and each liver was then cut into small pieces. The pieces were squeezed by moderate pressure in physiological saline, the material was left for a few hours, decanted and the remainder of the flukes were recovered and counted. The percentage of fluke-burden reduction was calculated from the mean number of flukes recovered from the treated and control sheep. A particular dose rate of the drugs was usually regarded as "highly efficient" if the reduction of flukes was 90% or more.

2. *Standardized acute toxicity test*

Most of the toxicological tests for anthelmintics were carried out previously in fluke-free animals, or in animals having usually a light fluke burden. The animals were occasionally exposed to different stress factors, such as starvation, various diets, or varied temperature. Since the anthelmintics tested for toxicity are intended to be used against sheep infected with *F. hepatica*, infection with the parasite would be the most obvious stress likely to affect the sheep at treatment. Artificially infected animals were used in the present experiments in order to standardize this stress. Boray (1967a) showed that the critical period in the life of the sheep infected with 4000 metacercariae or more was approximately 8 weeks after infection. Infections with 1000–2000 metacercariae caused considerable liver damage at about this time, but the sheep did not show obvious clinical symptoms. Therefore, in toxicity tests for anthelmintics intended for use against immature flukes, an 8-week-old infection with 1000 metacercariae seemed to be the most appropriate stress factor for sheep.

In the standardized toxicity tests, Merino wethers aged 4–5 years, which previously were not exposed to *F. hepatica* infection, were vaccinated against Black Disease. The sheep were infected with 1000 metacercariae each, and were kept in a fluke-free paddock with natural pasture. Six weeks after infection the sheep were moved into pens and were continuously fed on 50% lucerne chaff and 50% wheaten chaff *ad lib.* Drinking water was provided by an automatic device. The selected dose rates of the drugs were administered as described for the efficiency trials, 8 weeks after infection. The sheep were observed for clinical symptoms of toxicity for several weeks after treatment. The sheep were weighed before treatment, every second day for 2 weeks after treatment and weekly thereafter. In each experimental group five to ten sheep were left untreated as

controls, observed together with the treated animals and killed 16 weeks after infection; the flukes were recovered from their livers and counted.

Liver function tests were carried out in some sheep from each group, by determining the retention of bromosulfalein (BSP) 20 min after i.v. injection of 5 mg/kg of the dye before treatment, and 24 h and 48 h after treatment. The pathological changes were recorded at necropsy if the sheep died. Some sheep in each experiment, treated with toxic doses of the drug, were killed and the pathological changes were also recorded at necropsy. Boray and Happich (1967) showed that hexachlorophene, Hilomid and clioxanide were appreciably better tolerated by fluke-free sheep than by sheep infected with 1000 metacercariae 8 weeks after infection. Some of their results of the comparative tests are presented in Table XXVIII. Acute toxicity tests carried out on fluke-

TABLE XXVIII

*Comparative toxicity of some anthelmintics in fluke-free sheep and in sheep infected with* 1000 *metacercariae* 8 *weeks before treatment*

| Drug | Dose rate mg/kg | No. of sheep | Infection | Mortality |
|---|---|---|---|---|
| Hexachlorophene | 75 | 3 × 10 | Fluke-free | 0–20% |
| | | 10 | Infected | 80% |
| Clioxanide | 200 | 5 | Fluke-free | 0% |
| | | 6 | Infected | 50% |
| | 150 | 5 | Fluke-free | 0% |
| | | 5 | Infected | 40% |
| Hilomid | 90 | 10 | Fluke-free | 0% |
| | | 10 | Infected | 20% |

free sheep should precede the standardized acute toxicity test. These tests may give some indication of the dose rates which are likely to be tolerated by infected sheep. To save infected sheep, it is advisable to begin with groups of five sheep at the higher dose rates tolerated by fluke-free sheep (pilot groups). The dose rate then may be adjusted, depending on the clinical effect of the treatments in these smaller groups. The maximum dose rate which was tolerated by 20 or more infected sheep without serious clinical symptoms was regarded as the Maximum Tolerated Dose.

Lämmler (1964b) discussed the comparative value and toxicity of some compounds in the treatment of fascioliasis, and the use of the Chemotherapeutical Index = Maximum Tolerated Dose mg/kg divided by the Minimum Highly Efficient dose mg/kg. For the establishment of a statistically valid Chemotherapeutical Index, $DL_5\%$ figures (lethal dose to 5% of animals) should be used as maximum tolerated dose, but an impracticable large number of infected experimental animals would be needed. Boray *et al.* (1967b) stated that it is almost impossible to establish an accurate Chemotherapeutical Index for the drugs because of the many factors which can affect the drug tolerance of animals under different environmental conditions. Furthermore, it is essential

to estimate the approximate Chemotherapeutical Index for infections with immature and mature flukes separately. They attempted to provide more exact data, and established the "Efficiency Spectrum". Boray and Happich (1968) revised the Efficiency Spectrum, which showed the range of age of fluke in which the treatment results in 90–99% reduction ($DC_{90}$–99%). The "Safety Index", which is the maximum tolerated dose evaluated with the standardized acute toxicity test divided by the dose actually used, was assessed for 11 compounds currently available or under test against *F. hepatica*. In a review Lämmler (1967) accepted this expression of therapeutical efficiency and safety, particularly in connection with the treatment of immature *F. hepatica* infection, and discussed the differences between the establishment of a Chemotherapeutical Index and Safety Index described above.

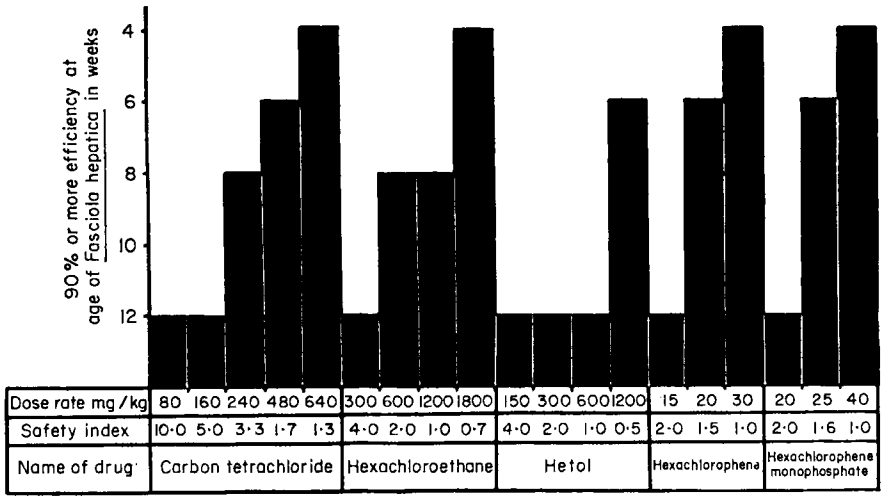


FIG. 10. The efficiency spectrum of some anthelmintics against *F. hepatica* in sheep.

The efficiency of some anthelmintics, evaluated earlier with the standardized technique, was tested by Boray *et al.* (1969) in sheep infected with 4000 or 1000 metacercariae each. It was shown that the drugs tested at dose rates comparable to those used in light infections produced comparable efficiency against heavier infections of corresponding age. Confirmation of comparable efficiency in an outbreak of acute fascioliasis in the field was obtained by Happich *et al.* (1967). The standardized chemotherapeutical test is suitable to predict potential anthelmintic efficiency against acute clinical fascioliasis. Figures 10 and 11 show the Efficiency Spectrum of 12 anthelmintics against *F. hepatica* in sheep based on the combined results of standardized chemotherapeutic studies by Boray *et al.* (1965, 1967b), Boray and Happich (1966b, 1967, 1968), Boray *et al.* (1969) and Boray and Roseby (unpublished data).

All drugs were efficient against immature *F. hepatica* as early as 4 weeks after infection at high, potentially toxic doses, and all drugs showed a progressive increase in efficiency as the dose rate and the time between infection and treatment increased. The increasing susceptibility of flukes to anthelmintics with their increasing age may be due to their locality, feeding, physiological development and metabolism (see Section IV F).

Boray (1967a) showed that fatal liver damage and retardation of the growth of flukes, which affects anthelmintic efficiency, usually occurred from the 6–8 weeks after heavy artificial infections of sheep. Mortality from the chronic disease occurred from the 16th week after infection with 1000–2000 metacercariae. Egg production by flukes did not commence in sheep earlier than 9 weeks after infections with 200, and only 13 weeks after infections with

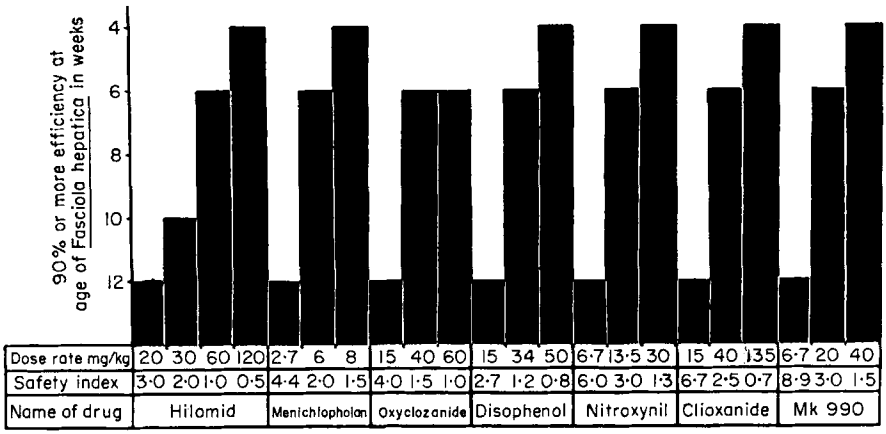


FIG. 11. The efficiency spectrum of some newer anthelmintics against *F. hepatica* in sheep.

2000 metacercariae each. Therefore, the critical period of the disease for curative treatment of acute and subacute fascioliasis, and for the prevention of pasture contamination, is about 6 weeks after infection. The other critical period is 12 weeks, just before the clinical symptoms of chronic fascioliasis occur and before egg production by the fluke reaches its peak. The selection of the most suitable drug against acute or chronic fascioliasis could be made on the basis of the minimum dose rate with an efficiency of 90% or more, and the safety indices at these two critical periods of the infection in sheep (Table XXIX). The results suggest that some improvement in safety has been achieved by some of the newer drugs, such as menichlopholan, nitroxynil, clioxanide and MK990 at dose rates highly efficient against immature flukes, compared with others. Their correct use may prove successful in the curative and preventive treatment of acute and chronic fascioliasis, with a reasonable margin of safety. Table XXIX shows that carbon tetrachloride, the most commonly used

drug against fascioliasis, has the highest safety index at the dose rate efficient against flukes aged 12 weeks, and it seems relatively safe at the dose rate efficient against flukes aged 6 weeks. However, heavy losses may occur occasionally in some flocks of sheep, and the occurrence of these losses is unpredictable in most cases (Seddon, 1967). A considerable amount of work has been devoted to the toxicity of carbon tetrachloride in Australia. The influence of many factors such as nutrition, environmental temperature and the method of administration has been described. The information given by Gallagher (1960, 1961, 1962, 1964), Gallagher *et al.* (1962), Kondos and McClymont (1961, 1965, 1966, 1967), Setchell (1961, 1962) and Boray (1965) may assist the safer application of the drug. However, experience with the newer drugs showed that such losses are unlikely to occur in the field if they are applied at the recommended dose rates, and their use may be preferred to carbon tetrachloride.

The importance of absorption, or possibly ruminal metabolism on the efficiency of drugs was discussed above. One of the new halogenated salicylanilid compounds, clioxanide, at the dose rate of 40 mg/kg, which was highly efficient against immature flukes when the drug was given intraruminally, was recently found to be inefficient against flukes aged 6 weeks when injected into the abomasum. When the larger dose rate (40 mg/kg) was given as a drench the drug failed in the treatment of immature flukes aged 6 weeks in a number of sheep (Boray and Roseby, 1969). In the definitive treatment of acute fascioliasis or in preventive treatment when immature fluke infection may be present, parenteral nitroxynil or coated menichlopholan may be preferred to clioxanide. Tests have been carried out in this laboratory (Boray and Pearson, 1960; Gallagher *et al.*, 1965; Boray and Happich, unpublished data) on the efficiency and safety of some other drugs*, such as Freon 112, Bitin-S, Bithionol, "DS-6", MK186 and MK464. However, efficiency of these drugs against either immature or mature *F. hepatica* was not achieved at safe dose rates, and their use for the treatment of fascioliasis is not recommended.

Innate and external factors may influence the efficiency and safety of anthelmintics when used in the field. Although the standardized techniques provide more reliable, comparable and reproducible information on both the efficiency and safety of anthelmintics, the safety of their regular application should also be determined in controlled field experiments (Happich *et al.*, 1967).

* Freon 112 (tetrachlorodifluoroethane): E.I. du Pont de Nemours & Co., Delaware, U.S.A.

Bitin-S (2,2′-sulfinyl-*bis* (3,5-dichlorophenil): Tanabe Pharmaceuticals, Osaka, Japan.

Bithionol (2,2′-thio-*bis*(4,6-dichlorophenol), Actamer, Lorothidol: Sterling Pharmaceuticals Pty. Ltd., Sydney, N.S.W., Australia.

"DS-6" (Bis-(2-hydroxy-3-nitro-5-chlorophenyl)-sulfide): E.R. Squibb and Sons Pty. Ltd., Melbourne, Vic., Australia.

"MK 186" (3,5-dibromo-3′-chloro-4′-(*p*-chloro-phenoxy) salicylanilid): Merck, Sharp & Dohme (Aust.) Ltd., Sydney, N.S.W., Australia.

"MK 464" (3,5-dibromo-4′-chloro-3′-(*p*-chlorophenoxy) salicylanilid), Merck, Sharp & Dohme (Aust.) Ltd., Sydney, N.S.W., Australia.

TABLE XXIX

*Comparative anthelmintic efficiency and safety of drugs suitable for the treatment of fascioliasis in sheep*

| | Drug | Maximum tolerated dose (mg/kg) | Infection aged 6 weeks: 90% or more efficient dose (mg/kg) | Infection aged 6 weeks: Safety index | Infection aged 12 weeks: 90% or more efficient Dose (mg/kg) | Infection aged 12 weeks: Safety Index |
|---|---|---|---|---|---|---|
| 1 | Carbon tetrachloride | 800 (0·5 ml/kg) | 480·0 (0·3 ml/kg) | 1·7 | 80·0 (0·05 ml/kg) | 10·0 |
| 2 | Hexachloroethane | 1200 | 1800·0 | 0·7 | 300·0 | 4·0 |
| 3 | Hetol | 600 | 1200·0 | 0·5 | 150·0 | 4·0 |
| 4 | Hexachlorophene | 30 | 20·0 | 1·5 | 15·0 | 2·0 |
| 5 | Hexachlorophene monophosphate | 40 | 25·0 | 1·6 | 20·0 | 2·0 |
| 6 | Hilomid | 60 | 60·0 | 1·0 | 20·0 | 3·0 |
| 7 | Menichlopholan | 12 | 6·0 | 2·0 | 2·7 | 4·4 |
| 8 | Oxyclozanide | 60 | 40·0 | 1·5 | 15·0 | 4·0 |
| 9 | Disophenol | 40 | 34·0 | 1·2 | 15·0 | 2·7 |
| 10 | Nitroxynil | 40 | 13·5 | 3·0 | 6·7 | 6·0 |
| 11 | Clioxanide | 100 | 40·0 | 2·5 | 15·0 | 6·7 |
| 12 | MK990 | 60 | 20·0 | 3·0 | 6·7 | 8·9 |

1 AR Grade
2 Avlothane: I.C.I. Australia & New Zealand Ltd., Melbourne, Vic., Australia.
3 Hetol (1,4-bis trichloromethylbenzene): Farbwerke Hoechst A.G., Frankfurt, Germany.
4 Hexachlorophene (2,2′-methylene-*bis* (3,4,6-trichlorophenol)), wettable powder: Farbenfabriken Bayer, Leverkusen, Germany.
5 Hexachlorophene Monophosphate (Hepadist): N.V. Nederlandsche Combinatie voor Chemische Industrie, Netherlands.
6 Hilomid (equal mixture of 3,5-dibromosalicylic acid-4′-bromanalid and 5-bromosalicylic acid-4′-bromanilid): Astra Pharmaceuticals, Sweden.
7 Menichlopholan (2,2′-dihydroxy-3,3′-dinitro-5,5′dichlorodiphenyl). Bilevon M: Farbenfabriken Bayer, Leverkusen, Germany.
8 Oxyclozanide (3,3′-5,5′-6-pentachloro-2,2′-dihydroxy-benzanilide). Zanil: I.C.I. Australia & New Zealand, Ltd., Melbourne, Vic., Australia.
9 Disophenol (2,6-diiodo-4-nitrophenol): Cyanamid (DHA) Pty. Ltd., Sydney, N.S.W., Australia.
10 Nitroxynil (N-methylglucamine salt of 4-cyano-2-iodo-6-nitrophenol); Trodax (May & Baker Ltd.) is the eglumine salt of nitroxynil: May & Baker Ltd., Dagenham, Essex, England.
11 Clioxanide (2-acetoxy-4′-chloro-3,5-diiodobenzanilide); Tremerad: Parke Davis & Co., Caringbah, N.S.W., Australia.
12 MK990 (3,5-diiodo-3′chloro-4′-(*p*-chlorophenoxy) salicylanilide): Merck, Sharp & Dohme (Aust.) Pty. Ltd., Sydney, N.S.W., Australia.

## VII. Control of Intermediate Snail Host

### A. Physical and chemical control

The essential role of snails in the transmission of all trematodes means that their elimination would result also in the elimination of the diseases caused by these parasites. The application of intensive agricultural methods may have reduced the extent of the habitats suitable for lymnaeid snails serving as intermediate hosts for *Fasciola* spp. but at the same time, particularly in some parts of Europe, it restricted the grazing stock to wet pastures which were unsuitable for cultivation, or increased the stocking rate on existing pastures, as in Australia. The great expansion of irrigation projects provides the snails with ideal habitats in many parts of the world, including Australia. Some snail habitats may be reduced by physical means, such as proper drainage, particularly underground tile drainage which could become an economical measure with improved technology. Building dams on low lying marshy pastures may reduce snail habitats and increase grazing areas (Gordon *et al.*, 1959). Chemical destruction of snails seemed to be the simplest solution of the problem, and copper sulphate has been recommended for snail control for almost 50 years. As a result of the enormous effort to control human schistosomiasis, a large number of new compounds, highly efficient at economical concentrations, were developed for the chemical destruction of snails. The problem of chemical snail control in Australia was discussed by Gordon *et al.* (1959).

The efficiency of many compounds which were found to be highly lethal to schistosome-bearing snails and of some potential molluscicides, were tested in this laboratory against *L. tomentosa* with a simple technique especially suitable for the amphibious intermediate hosts of *F. hepatica*. In additional tests, the toxic effect of the chemicals against the snail eggs was also investigated. In order to achieve continuous immersion for the required period, sterile plastic Petri dishes 9 cm in diameter were used. Each concentration of the compound and each exposure time required a separate Petri dish. Snails were washed with de-ionized water and placed on to a filter paper where the amount of water collected on the shell and between the shell and the body was absorbed. Ten snails were then placed in each Petri dish. The prepared solutions were poured into the Petri dishes containing the snails and the dishes were filled up in such a manner that when the lid was placed on the Petri dish it touched the solution and formed a thin air pocket. The water did not flow out of the dishes because of the water-repellent properties of the plastic surface. The air pockets contained sufficient air for 24 h and the snails could not leave the solution. When the required time expired, the solution was removed, the snails were thoroughly washed with de-ionized water and the solution was replaced by de-ionized water. The snails were examined microscopically and the dead ones removed, but the survivors were observed for a further 2 or more days. A control group for each exposure time was placed in Petri dishes with only de-ionized water, and at the end of the period a similar procedure was carried out.

Egg masses were immersed in the Petri dishes containing the solutions, removed and washed after the exposure period and examined microscopically. If the embryos survived the treatment, they were observed until they hatched. The efficiency of numerous compounds such as 12 different herbicides and also

neguvon, dinitro-*o*-cyclohexyl phenol, diazinon, malathion, dithiazanine iodide, the delta and gamma isomers of benzene hexachloride, bithionol and many others were tested with the above method. These compounds were not efficient at economical concentrations and the results will not be discussed in detail.

The high molluscicide efficiency of sodium pentachlorophenate (NaPCP), "Bayluscide"* (5,2′-dichloro-4′-nitrosalicyclic anilide) and "Frescon"† (N-tritylmorpholine) was reported by McMullen *et al.* (1948), Gönnert and Schraufstätter (1959) and Boyce *et al.* (1966) respectively, and was confirmed by others. Table XXX shows the high efficiency of "Bayluscide" and NaPCP

TABLE XXX

*Efficiency of molluscicides against adult* L. tomentosa *and eggs*

| Chemical compound | Snails | | | | | | Eggs | |
|---|---|---|---|---|---|---|---|---|
| | $LC_{90}$ ppm Exposure in hours | | | $LC_{100}$ ppm Exposure in hours | | | $LC_{90-100}$ ppm Exposure in hours | |
| | 1 | 5 | 18 | 1 | 5 | 18 | 1 | 18 |
| Sodium pentachlorophenate | 0·8 | 0·8 | 0·4 | 1·0 | 0·8 | 0·5 | 1·0 | 0·5 |
| "Bayluscide" | 0·7 | 0·5 | 0·08 | 1·0 | 0·7 | 0·1 | 1·0 | 0·5 |
| N-tritylmorpholine | 5·0 | >3·0 | >3·0 | >5·0 | >5·0 | 5·0<br>3·0* | >10·0 | >10·0 |
| Copper sulphate | — | — | 1·5 | 40·0 | 20·0 | 2·0 | 10·0 | 5·0 |

* Approximately 2-week-old snails

against *L. tomentosa* compared with the effect of copper sulphate. The results with N-tritylmorpholine were disappointing, possibly due to the relatively low pH (5) of the water. When the experiments were repeated with NaPCP and "Bayluscide" using water from the most common habitats in Australia as the solvent, NaPCP was more efficient than "Bayluscide". It was found that "Bayluscide" precipitated rapidly in the water from natural snail habitats. The pH of the water was 6–6·5, but when buffered to pH 8 a more satisfactory solution resulted. In laboratory tests it was found that the concentration of both NaPCP and "Bayluscide" rapidly diminished if the solutions were exposed to ultraviolet light. These results were confirmed in field pools when about 90% of the original concentration was lost after 16 h, which suggested that higher concentrations were necessary to kill snails in the field than in the laboratory tests.

Field trials were carried out using similar techniques to those described by Gordon *et al.* (1959), Enigk and Düwel (1960) and Ollerenshaw (1962, and personal communication). It was found that if concentrations of 2–5 p.p.m. were used as high volume sprays in experimental areas which contained springs, slowly flowing streams or channels, and dams, both "Bayluscide" and NaPCP

* Farbenfabriken Bayer, Leverkusen, Germany.

† Shell Chemical (Aust.) Pty. Ltd., Melbourne, Australia.

virtually eliminated the populations of *L. tomentosa*. The areas were thoroughly examined weekly after the treatments and small numbers of snails reappeared usually 3 to 6 months after treatments, and thereafter rapid recolonization occurred. Similar habitats were sprayed with N-tritylmorpholine until concentrations reached approximately 5–8 p.p.m. All snails were apparently eliminated but 4 weeks later rapid recolonization occurred, obviously from the egg masses which survived the treatment (see Table XXX). The great potential of reproduction, resistance and dispersal of lymnaeid snails has been discussed (Section II).

The ideal molluscicide should kill snails and their eggs at a low concentration, and should be harmless to mammals and fishes. It is particularly important that the molluscicide when used for the control of amphibious snails should persist in the soil for several days or weeks at a toxic concentration. The use of the available molluscicides, particularly the ovicides, NaPCP and "Bayluscide", against the intermediate hosts of *F. hepatica* as an additional measure, together with chemotherapy may be very useful if they are applied regularly after a careful survey (see Section VIII). Depending on the climatic conditions, two or three treatments a year would be essential to achieve satisfactory snail control in Australia and in tropical countries, and one or two treatments in Europe. Occasional treatment is inefficient and wasteful, and there is no evidence to suggest that a single treatment would have a persistent effect.

### B. BIOLOGICAL CONTROL

There are many reports in the literature on the biological control of schistosome-bearing snails, but no substantial attempt has been made to control the intermediate host of *Fasciola* spp. by biological means. The problem of biological control was discussed in detail and the literature was reviewed by Berg (1964). The influence of the biological environment on *Lymnaea tomentosa* was summarized by Boray (1964a) in Australia. He found that some plants, bacteria, fungi, algae and nematode parasites may reduce reproduction or the growth of snails under special circumstances, but none of them seems to act efficiently enough to cope with the rapid rate of reproduction of snails. Predatory birds have been mentioned as a means of biological control in many early papers (Cobb, 1897, 1904). Birds may consume many snails in certain habitats but this does not seem to reduce populations, and there is no experimental evidence of their efficiency in controlling fascioliasis.

Berg (1953) found that the larvae of sciomyzids are efficient killers of snails, and an attempt was made to use them for biological control. The sciomyzid, *Sepedon macropus*, was introduced to five islands in Hawaii, but no evidence of its effect on the intermediate host of *F. gigantica* has yet emerged (Berg, 1964). Berg collected larvae and flies in 1961 from N.S.W. and South Australia. Boray (1964a) reported that at Hampton, N.S.W., larvae resembling sciomyzids had been seen from time to time and the presence of one species, and the ability of its larvae to kill snails, was confirmed. Larval sciomyzids were feeding on snails in a shallow drain at the Hampton experimental area, and they were identified as specimens of *Dichaetophora hendeli* and *D. biroi*; both species

fed readily on species of planorbids as well as *L. tomentosa*. The large, final instar larvae of *D. hendeli* consumed an average of 1·0 snail/larva/day, *D. biroi* consumed 3·7 snails/larva/day. The average snail consumption of *D. hendeli* remained fairly constant during growth, while that of *D. biroi* showed a steady increase from the first to last instars with a slight drop prior to pupation.

Larvae reared in the laboratory suffered a very high mortality; *D. biroi* was a little hardier than *D. hendeli*, probably as a result of inefficient rearing techniques. The scarcity of larvae in the field, compared with the numbers of eggs which are laid, suggests high larval mortality even under natural conditions, particularly when their predators (certain species of dragonflies) were

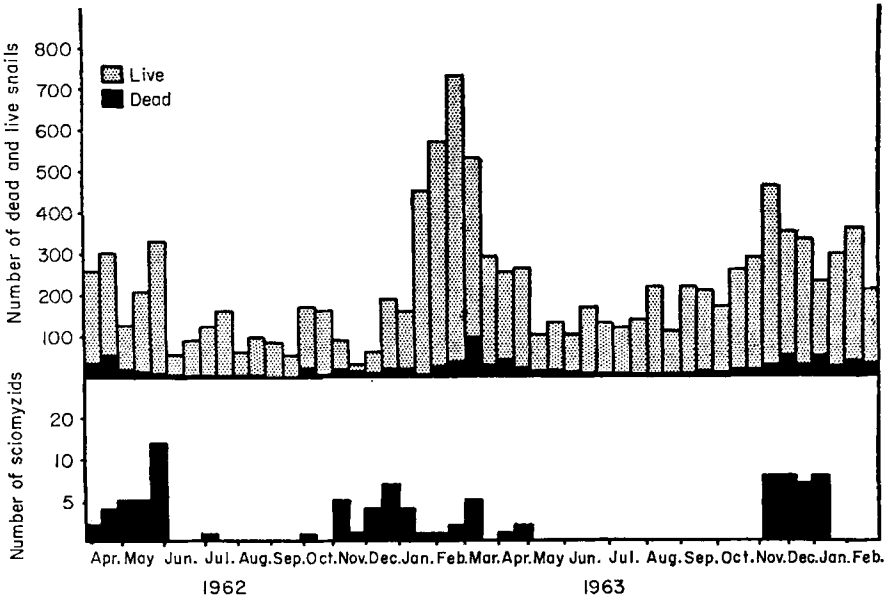


FIG. 12. Seasonal variations of *L. tomentosa* populations and of their predators *Dichaetophora hendeli* and *D. biroi* (Central Tableland, N.S.W.).

present. Fortnightly records of the number of sciomyzid larvae and of dead *L. tomentosa* found at the experimental area were made for almost 2 years commencing April 1962 (Fig. 12). In the periods when most sciomyzids were recorded, the largest numbers of dead snails were found. The numbers of larvae and pupae recorded were very much smaller than the numbers of snails, and there was no inverse relationship between numbers of live snails and sciomyzids. Falls in total snail numbers were adequately explained by the drying up of some of the observation sites. Thus, the slight increase in mortality (as indicated by the numbers of empty shells) which appeared to be due to the presence of sciomyzids, had little effect on the population as a whole in this locality, and is unlikely to influence the infestation rate of stock with *F. hepatica*. Lynch (1965a) reported the presence of *D. biroi* from South Australia. He found that the fly may be an important predator of *L. tomentosa* in certain circumstances,

but stated that the appearance of the flies at various habitats is unpredictable; they appear to be poor at dispersing, and the larvae could not attack snails which were submerged in water. It seems that the presence of sciomyzid larvae is only incidental in snail habitats. Their number increases when the number of snails serving as food increases, and decreases when the number of snails declines due to other factors. Their role as agents of biological control may therefore be insignificant.

Boray (1964a) emphasized the importance of trematode infection of snails. The larval stages of trematodes live as true parasites within the snail's body, and may cause severe pathological changes. Infection with *F. hepatica* causes severe damage to the snails and mortality may occur from acute (infection phase), subacute (developmental phase) and chronic (accumulation phase) infections. The death of many infected snails may be one of the controlling factors of the intensity of fascioliasis in livestock. However, under natural conditions the death of the snail host often occurs when the life cycle of *F. hepatica* has been completed, and the result of the pathogenic effect of *F. hepatica* in the snails may be favourable for the completion of the life cycle by forcing the snails to leave deeper water.

The first report on the antagonistic interaction between echinostome larvae and other trematodes was given by Boray (1964a), who noted that infection by many species of Echinostomatidae is common in *L. tomentosa*, inhibiting *F. hepatica* infection and causing lesser patholgical effects in the snails. The presence of echinostomes may influence the epidemiology of fascioliasis. Subsequently, an antagonistic interaction between schistosome and echinostome larvae was reported by Lie (1966). He suggested that the rediae of echinostomes in *Biomphalaria glabrata* could consume the sporocysts of schistosomes that also infect the snails, and Boray (1967c) concurred. His laboratory experiments indicated that antagonistic action against *F. hepatica* occurred only when the snails contained rediae, and not when encysted echinostome cercariae derived from another snail was the only stage present. The laboratory experiments were confirmed by field observations (Boray, 1967c): mixed infections with echinostome and *F. hepatica* larvae were extremely rare, and *F. hepatica* infection of snails may be absent in areas where echinostome infection is heavy. It is possible, therefore, that echinostome infection of lymnaeids may serve as a biological control against infections with *Fasciola* spp.

Backlund (1949) and Khalil (1961) considered that *Chaetogaster limnaei* (Naididae, Oligochaeta) play some part in limiting the distribution of trematodes by catching miracidia and consuming cercariae. Their incidence amongst *L. tomentosa* populations and the ability of the worms to prevent the infection of snails with miracidia was observed in this laboratory, and Boray (1964a) suggested that the presence of this worm may be a very important factor in certain areas, as it may provide some protection against miracidia.

It has been shown that many members of the biological environment of the intermediate host may act to some degree in keeping the balance between hosts and parasites, but an efficient agent for the biological control of fascioliasis is yet to be found.

## VIII. Epidemiology and Control of Fascioliasis

### A. EPIDEMIOLOGY

The primary and most essential factors which determine the incidence of *F. hepatica* infection are the presence of the intermediate host, *L. tomentosa*, sufficient moisture and suitable temperature. However, the occurrence of fascioliasis, its effect on the hosts, and the continuous propagation of the parasite itself, depend on a wide variety of secondary factors—biological, topographical and, to a substantial extent, human. These factors will determine whether the parasite produces subclinical or clinical fascioliasis with serious epidemics and mortality. The various factors involved in the epidemiology of the disease, with reference to many earlier contributions to the problem, were discussed by Ollerenshaw (1959, 1967) in Britain and Gordon (1955), Osborne (1962), Boray (1963a, 1967d, 1969) and Boray *et al.* (1967c) in Australia.

The efficient control of the disease depends on the correct application of various curative and preventive measures integrated into improved farm management. The strategic application of these methods requires information on the seasonal variation of the infection in the most important parts of Australia. This information can be derived from previous studies on the ecology of snails, and of the free-living and other stages of the parasite. However, because of the many factors involved in the epidemiology of fascioliasis, it was considered that the use of tracer animals would provide direct supporting evidence for the validity of this information. The results of such investigations were reported by Boray *et al.* (1967c) and Boray (1969).

Two districts in N.S.W., which are typical of many infected areas of S.E. Australia, were selected, one in the Central Tableland and the other in the Murrumbidgee Irrigation Area. The observations were carried out from 1961 to 1966. A total of 560 Merino wethers aged 4–5 years were used as tracer animals in groups of ten. The sheep were obtained from a fluke-free district. They were vaccinated against black disease and were regularly treated against nematode infections. The contamination was maintained by chronically infected sheep for more than a year in an area of 5 acres on the Central Tableland. The area included dams joined by several small streams and marshy depressions and springs containing the snail, *L. tomentosa.*

In January 1962, when the whole property surrounding the area was free from sheep or cattle but numerous rabbits were present, the infected sheep were removed from the paddock. Groups of ten tracer sheep were then introduced successively every 6 weeks for a period of 27 months. In May 1964, at the end of autumn, the experimental paddock was recontaminated by the introduction of ten chronically infected sheep. Further groups of ten tracer sheep were then introduced successively every 6 weeks for 2 years. After the exposure periods, all tracer sheep were removed to a fluke-free paddock and were killed 13–14 weeks after removal from the infected area. The flukes from the livers were recovered and counted. Data on local average monthly temperatures and total monthly rainfall were also recorded for the whole experimental period (Fig. 13).

Every 6 weeks 50–300 *L. tomentosa* snails were collected, and were examined for the presence of *F. hepatica* larvae. In the irrigation area the contamination of a 5-acre irrigated paddock had been maintained by infected dairy cows before the experiment commenced.

From the 8th of June 1964, ten sheep with chronic *F. hepatica* infection were continuously kept on the paddock. Groups of ten tracer sheep were introduced successively every 6 weeks thereafter for 2 years. After the exposure periods all

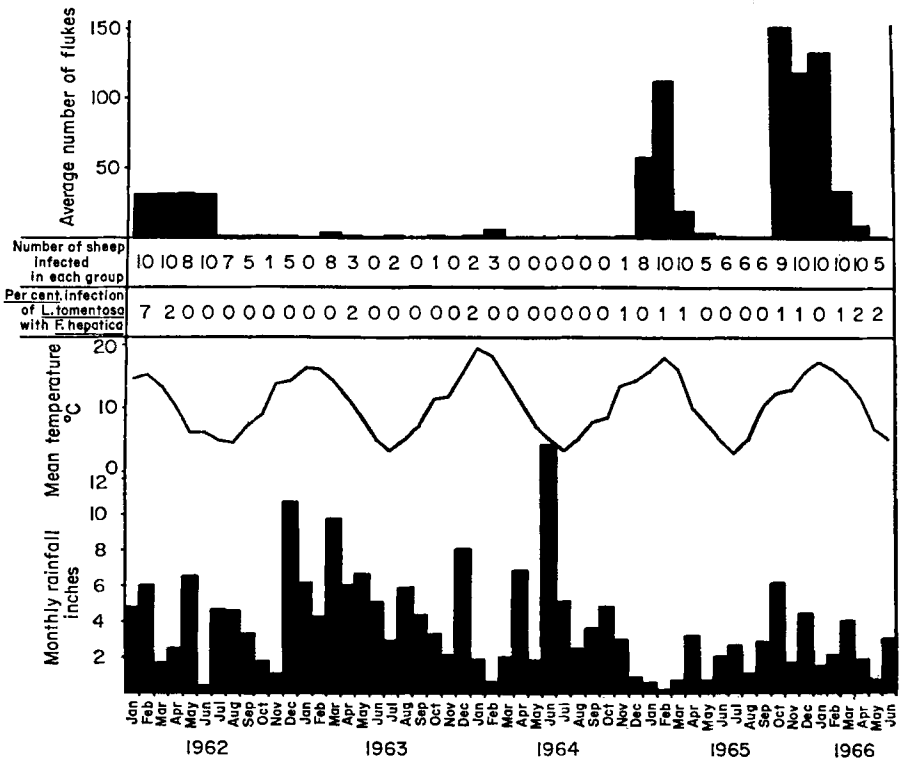


FIG. 13. The seasonal infection of tracer sheep and snails at a Central Tableland area in N.S.W. (Reproduced with permission from Boray, 1969.)

tracer sheep were removed to a fluke-free paddock and were killed 13–14 weeks later. The flukes from the livers were recovered and counted. Snails collected from the area were examined in the laboratory for *F. hepatica* infection. The results are recorded in Fig. 14. Meteorological data were collected from official stations in the near vicinity of both experimental areas, and from reports issued by the Commonwealth Bureau of Meteorology. The epidemiological studies using tracer sheep were carried out during a period of 5 years on the Central Tableland and 2 years in the irrigation area. Rainfall had been above average and conditions were considered favourable for the development of clinical fascioliasis; these conditions may be representative of at least 75% of the

endemic areas of Australia. The results of these studies serve as strong supporting evidence for creating epidemiological models for various districts.

Boray (1967d) stated that the temperature is much more favourable for the propagation of *F. hepatica* in Australia than in Europe. Reproduction of snails takes place throughout the year (Boray, 1963a, 1964b) and the development of eggs and larvae of the parasite is continuous for about 7½ to 9 months. There

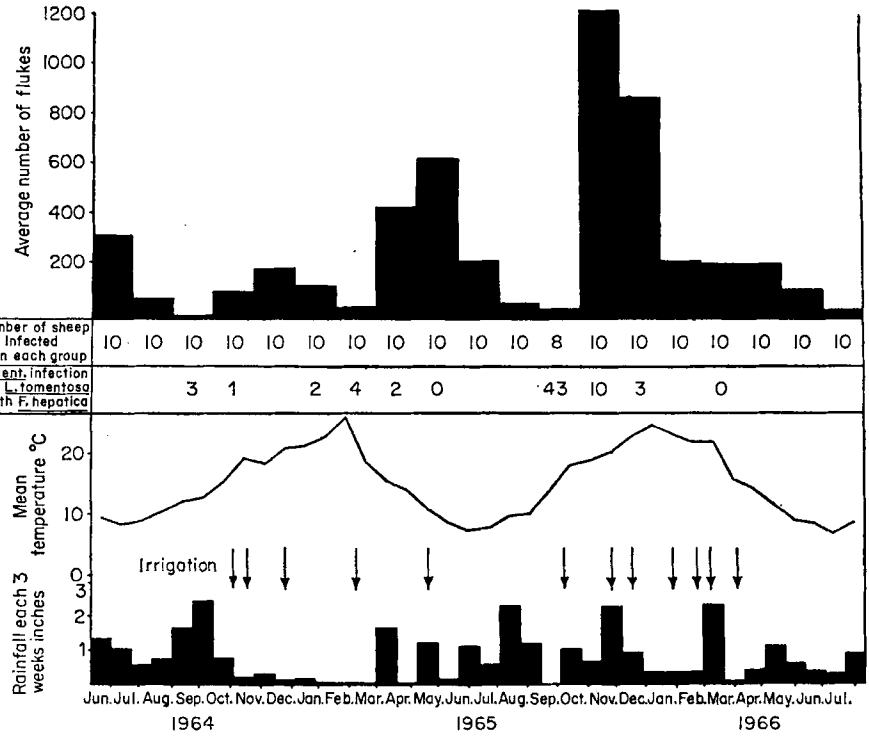


FIG. 14. The seasonal infection of tracer sheep and snails at an Irrigation Area, N.S.W. (Reproduced with permission from Boray, 1969.)

are some permanent snail habitats where the development of infective metacercariae depends only on appropriate temperature, but suitable conditions are only temporary on most pastures. The successful propagation of the parasite in these temporary habitats depends chiefly on rainfall or the alternation of wet and dry periods, but is also influenced by non-climatic factors, such as the quality of pasture and the grazing habits of animals. The total amount of rainfall in the various districts of Australia may vary from a slight drizzle lasting several weeks to tropical downpours for short periods causing local or general floods. Although general predictions can be made on the basis of the prevailing climatic conditions, the methods for forecasting heavier infections from year to year, as described by Ollerenshaw and Rowlands (1959), may be

very difficult to apply separately to different districts in Australia with varying local conditions.

The effect of suitable conditions during the different life cycle stages of *F. hepatica* on the epidemiology of fascioliasis was discussed by Ollerenshaw (1959) in relation to conditions in the United Kingdom. Discussion of these conditions, including some new aspects of epidemiology, was extended to subtropical and tropical countries by Boray (1967d). It is relatively easy to make general recommendations for the strategic application of various control measures, based on temperature and the presence of moisture, in Europe and Britain. Table XXXI was constructed to show the general effect of climate on the seasonal occurrence of the disease and illustrates that the warmer the climate the more difficult it is to plan successful strategic control (Boray, 1967d). He emphasized the important effect of biological, climatic, topographical and human factors on the epidemiology of fascioliasis (Table XXXII).

The critical temperature for the life cycle of *F. hepatica* is 10°C. Below this temperature there is no activity, or very little, in the larval development of the parasite and in the multiplication of snails (Clunies Ross and McKay, 1929; Rowcliffe and Ollerenshaw, 1960; Boray, 1963a, 1964b). On the Tableland the most important period of infection was during spring, summer and autumn. The results of the 5th year (Fig. 13) showed that if contamination with eggs was continuous during the previous season, heavy infections could occur in mid-spring. This infection was due to the emergence of cercariae from over-wintering snails which had become infected during the previous autumn, but in which larval development was retarded by low temperartue. In the irrigation area infection may occur throughout the year and be reduced only for a short period during the end of winter and early spring due to low temperature (Fig. 14).

Stimulation of cercaria-emergence by sudden physical changes, and the accumulation of cercariae in the snails during a dormant period (such as aestivation), was observed both in the field and in the laboratory (Boray, 1963a, b). The results of the experiments in the irrigation area (Boray *et al.*, 1967c; Boray, 1969) provide evidence that peak infections occurred after irrigation. In the presence of water, most of the accumulated cercariae were liberated from the snails, which previously were in a dormant or semi-dormant state (Fig. 14). The only time other than winter when a drastic reduction of infection occurred was in February and March 1965, when there was very little rain and no irrigation during the previous 12 weeks. Irrigation at the end of this period was followed by a high peak of infection. High infections in spring may be enhanced by the sudden temperature changes stimulating massive emission of cercariae. The difference between early morning and midday temperature in spring may be as much as 20°C. The results showed that although the size and contamination of both paddocks were similar, virtually all tracer sheep on the irrigated paddock became infected during the whole period, but on the Tablelands a proportion of sheep in most groups did not become infected. This may be explained by many factors, but on the Tablelands snail habitats were more localized and mostly permanent, and therefore sheep which preferred to graze on dry pasture were able to avoid fluke-infected herbage. On the irrigated

TABLE XXXI

*Seasonal occurrence of fascioliasis*

| | | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Species | Number of generations of cercariae per year |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Eastern, Northern and Central Europe; hill pastures of Scotland; Northern Asia; N. America | Development | . | . | . | . | + | ++ | ++ | ++ | ++ | . | . | . | *F. hepatica* | ½–1 |
| | Infection | . | . | . | . | . | . | . | ++ | ++ | ++ | . | . | | |
| | Disease | + | . | . | . | . | . | . | . | . | ++ | ++ | ++ | | |
| Britain; Ireland; West Coast of Europe; subtropics; highlands of tropical Asia | Development | . | . | . | . | + | ++ | ++ | ++ | ++ | ++ | . | . | *F. hepatica* | 2 |
| | Infection | + | + | . | . | + | + | + | ++ | ++ | ++ | ++ | + | | |
| | Disease | ++ | ++ | ++ | ++ | . | . | . | + | + | ++ | ++ | ++ | | |
| Subtropics of Australia; highlands of tropical countries ; Southern hemisphere | Development | ++ | ++ | ++ | ++ | + | . | . | . | ++ | ++ | ++ | ++ | *F. hepatica* and/or *F. gigantica* | 4 |
| | Infection | ++ | ++ | ++ | ++ | + | + | + | . | + | ++ | ++ | ++ | | |
| | Disease | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | + | + | ++ | | |
| Tropics | Development | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | *F. gigantica* | 6 |
| | Infection | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | | |
| | Disease | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | | |

pasture there was not only massive emission immediately after irrigation, but passive dispersal of cercariae throughout the area could also have occurred.

Clunies Ross and McKay (1929) showed that in summer, metacercariae lived for only 2 days when exposed to sunshine and for 17 days when in the shade. Ono *et al.* (1954) found that metacercariae lived for 10 days on grass dried at 25–32°C. The survival of metacercariae under different environmental conditions was studied by Boray and Enigk (1964), and the relevant literature was reviewed (Section IV D). It was shown that metacercariae may survive for long periods at low temperature provided sufficient moisture is present. They were shown to be very susceptible to desiccation and to temperatures higher than 25°C. The results of Boray *et al.* (1967c) seem to indicate that metacercariae may have a short life on herbage, and infection seemed to occur from newly produced metacercariae in the field. This was shown by the sudden reduction of infection during winter when the temperature would, theoretically, be most suitable for the survival of metacercariae, and the grazing of wet areas was intensive due to dry conditions. The destruction of metacercariae was probably due to the great diurnal variation of temperature and the long periods of sunshine during all seasons. Metacercariae may survive longer in winter in the dominantly winter rainfall areas of Australia (Fig. 15, Type B), but the survival of metacercariae on the herbage during winter may be more important in Britain (Ollerenshaw, 1959) and in Holland (Jansen, 1964).

Daily egg production of flukes was calculated by Happich and Boray (1969b) (see Section V C); daily egg production per fluke decreased with the increase of fluke burden, but chronic, moderate infections could result in a relatively constant and persistent daily egg output of 2 million eggs per sheep. On the other hand, the patent period of *Fasciola* infection in cattle is limited (Alicata and Swanson, 1941; Dixon, 1964; Boray, 1967b), and after a short peak, egg production may diminish rapidly and the animals resist reinfection (Boray, 1967b). Boray (1967b) also suggested that in sheep pastures, cattle may have a beneficial effect by ingestion and destruction of numerous metacercariae which would otherwise be available to sheep. He also showed that high egg output in cattle lasts for only a few weeks. Calculated on the mean egg count of 5 E.P.G. which is usual in Australian herds, the daily egg output per beast would not be more than approximately 50 000–100 000. This calculation is supported by Hagens and Over (1966). This daily egg output in cattle is about 20 times less than that in sheep. Moreover, with normal stocking rates, approximately seven to ten times more sheep would be grazing on a similar pasture area. This calculation shows that egg output by chronically infected sheep is about 140–200 times higher than that by chronically infected cattle, and suggests that a subclinical infection of sheep which may last for a long time may be the most important contributing factor in the contamination of pastures in Australia.

The results of the present experiments show that the intensity of infection increased and the seasonal pattern changed gradually from the time when chronically infected sheep were introduced and were present continuously. In the second phase of the experiment on the Tablelands (Fig. 13), it is clear that infection did not occur before the end of summer (January 1964) because

TABLE XXXII

*The various factors influencing the life cycle phases of* Fasciola *spp.*

| Favourable factors | Life cycle phases | Unfavourable factors |
|---|---|---|
| *Biological* | 1 | *Biological* |
| High output of eggs 1 | Presence and survival of eggs | Resistance in final hosts 1, 2, 7 |
| Long life span 1 | | Grazing habits of hosts 1, 6 |
| Multiple hosts 1, 7 | | Short life of miracidia 3 |
| Reaction of final hosts 1, 2, 3 | 2 | Absence of snails 3 |
| Grazing habits of hosts 1, 6 | Development of eggs | Presence of snail predators 3 |
| High reproduction of snails 3 | 3 | Presence of other trematodes 4 |
| High susceptibility of snails 3,4 | Presence and infection of snails | Resistance of snail hosts 3, 4 |
| Active and passive dispersal 3, 5, 6 | | *Climatic* |
| Aestivation and hibernation 3, 4 | 4 Larval development | Excessive temperature 3, 4, 5 |
| Parthenogenetic multiplication 4, 5 | | Lower than 10°C 2, 4 |
| Resistance of cercariae 5 | 5 | Wet season 6 |
| Passive dispersal of cercariae 5, 6 | Emission, encystment and availability of cercariae | Lasting drought 2, 3, 4, 5 |
| *Climatic* | | *Topographic* |
| Higher than 10°C 2, 4 | | Restricted wet areas, deep gullies, creeks 3, 5, 6 |
| Alternating low and high temperatures 5 | 6 | Faster water flow, flood 3, 5 |
| Wet season 1, 2, 3, 4, 5 | Ingestion of metacercariae by final host | Stagnant water 3, 4 |
| | | *Human* |
| | | Physical and chemical snail control 3, 4 |

TABLE XXXII (*continued*)

| Favourable factors | Life cycle phases | Unfavourable factors |
|---|---|---|
| *Climatic (continued)* | | *Human (continued)* |
| Temporary drought 6 | | Regular treatment with drugs highly efficient against immature flukes 1, 2, 7 |
| Alternating wet and dry (irrigation) 5, 6 | | Correct stocking rate 6, 7 |
| *Topographic* | | Keeping stock out of habitats 1, 6 |
| Permanently wet areas, springs, irrigation channels 2, 3, 4, 5 | | Pasture and stock rotational control 1, 6 |
| Slow water flow 3, 4 | 7 | |
| *Human* | Development and survival in final host | |
| Curative treatment with dose rates inefficient against immature flukes 1, 7 | | |
| Inefficient drainage of potential snail habitats 1, 2, 3, 4, 5, 6 | | |
| High stocking rate 6, 7 | | |

there was no contamination during the previous year. With continuous contamination, however, the level of infection during the next fluke season was generally much higher, and the peak of infection occurred from the middle of spring in October. The suggestion that sheep play a more important part in pasture contamination than cattle is supported by strong circumstantial evidence from the results of the experiment in the irrigation area. Figure 14 shows that the intensity of infection increased gradually from season to season as the source of contamination was changed from a herd of dairy cows to chronically infected sheep.

It was reported (Boray, 1967c; Section V F) that the viability of *F. hepatica* eggs was lower in rabbits than in sheep, and that it was further reduced through rabbit passages. Although rabbits are susceptible hosts and can maintain the survival of the parasite for a limited period, their role in contamination of pastures is restricted. Evidence for this was shown in an experiment (Boray, 1969) on the Tablelands (Fig. 13, 1962–64); the infection of tracer sheep was negligible when contamination of the pasture was maintained only by rabbits. Wombats,

which were present on the experimental paddock, were found to have a strong, innate resistance against *F. hepatica* infection (Boray, 1967c; Section V F).

The percentage of snails found infected with *F. hepatica* in the Tablelands was very low during the whole period (Fig. 13), and was of little value for predicting the intensity of infection in sheep. This was probably due to topographical conditions resulting in fluctuations in local snail populations (Boray, 1963a; Section II). The method may only be useful to indicate that the parasite is present in the area. However, in the irrigation area where generally heavier infections occurred, higher infections in sheep could be predicted from higher infection rates in snails (Fig. 14). This was particularly striking when 43% of snails were infected with *F. hepatica* 6 weeks before the highest infection of the tracer sheep occurred, and caused mortality from acute and subacute fascioliasis. Although the method may only serve to indicate the presence of *F. hepatica* in most Australian districts, exceptionally heavy infections resulting in deaths from acute fascioliasis may be predicted several weeks before the outbreak.

*Classification of endemic districts*

Figure 15 shows that the endemic areas of Australia may be classified into climate-types, depending on temperature and seasonal distribution of rainfall, based on the average figures issued by the Commonwealth Bureau of Meteorology over 20–30 years for many stations with similar characteristics.

(a) *Type A.* Type A has a yearly average rainfall of 26 in; this is irregular, but on the average equally distributed throughout the year. This climatic type comprises the Central and S. Tablelands of N.S.W. and part of their N., W. and E. slopes, the N.E. highlands of Victoria with their river valleys and the coastal area S.W. of Geelong, and south of the line between Launceston and Herrick in E. Tasmania.

(b) *Type B.* Type B has a yearly average rainfall of 28 in, most of which falls during late autumn, winter and early spring. This type comprises central and S.W. Victoria and the E. part of the N. coast of Tasmania with Flinders and Cape Barren Islands. The small endemic areas of South Australia are also classified in this type. The epidemiology of fascioliasis in the Lake Alexandrina area of South Australia depends on many factors other than seasonal climatic changes (Ewers, 1958; Boray, 1964a; Lynch, 1965a).

(c) *Type C.* The average yearly rainfall of Type C is 30 in; it is irregular, but more rain generally falls during spring, summer and early autumn. This type comprises the N. Tablelands of N.S.W. and parts of the slopes and the southern endemic area of Queensland, including the Stanthorpe and Warwick districts.

(d) *Type D.* This climatic type consists of the Irrigation Areas of the Murrumbidgee and Murray Rivers of N.S.W. and all irrigation districts of N. Victoria. Type D has an average yearly rainfall of 17 in, with slightly more rain falling during winter and early spring. This rainfall would not be sufficient to maintain fluke infection, but it is supplemented by irrigation, usually from September to April, with intervals of 10 days or longer. Fascioliasis in irrigation areas is an increasing problem, but it is usually limited to certain paddocks with inadequate drainage and does not necessarily occur on all properties. The

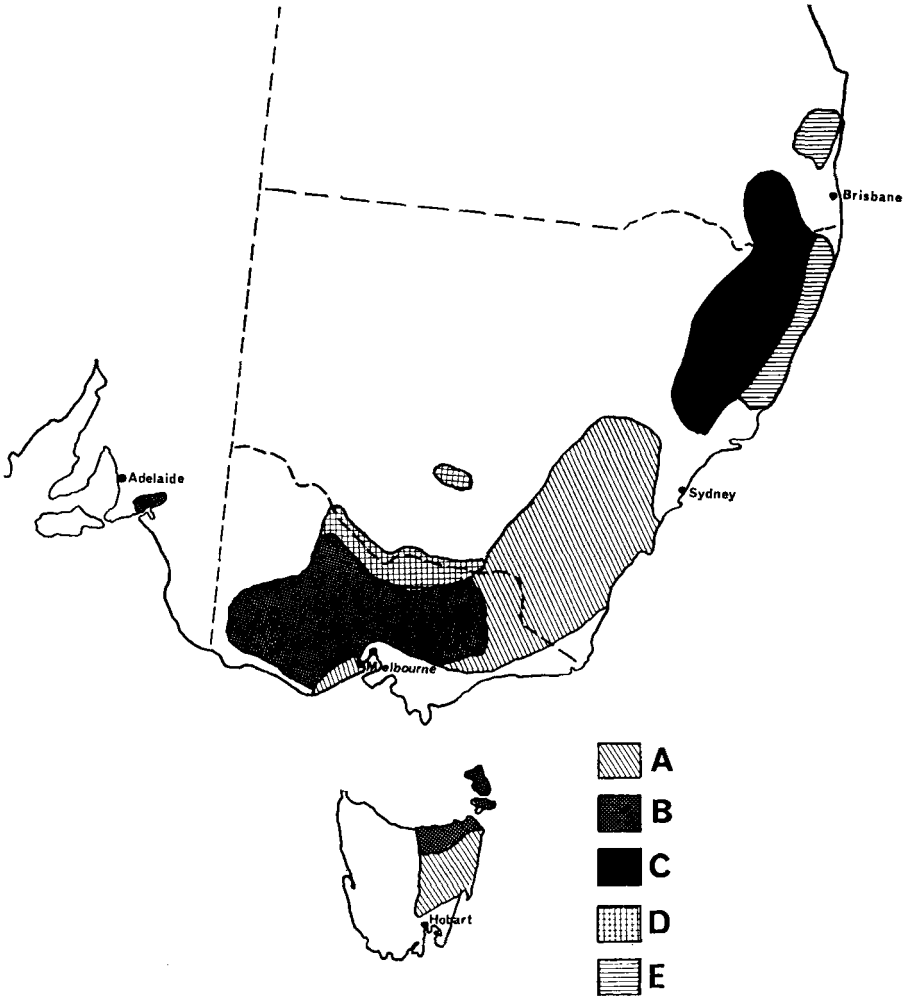


FIG. 15. Climatic classification of endemic areas of S.E. Australia. (Boray, 1969).

temperature is slightly higher than in Types A, B and C, and the inactive period due to low temperature is restricted to the 3 winter months.

(e) *Type E.* The N. coast of N.S.W. and the endemic area on the Maleny Range, Gympie, north of Brisbane, belong to this type with an average yearly rainfall of 40 in. The rainfall is lowest in winter, increasing to a peak in summer. The temperature is suitable for the life-cycle of *F. hepatica* throughout the whole year. This area is grazed mainly by cattle, and the low infection rate of the animals in Queensland causes little concern (Dixon, 1963). Higher infections and mortality, particularly amongst young cattle, occur on the N. coast of N.S.W. around Casino and Lismore.

(f) *Type F.* This additional type is not marked on Fig. 15 because it represents a very small part of the endemic areas on the higher ranges of the Snowy Mountains in N.S.W., such as Kiandra, and some parts of the Lake District, such as the Miena district, in Tasmania. The yearly average rainfall is 46 in; higher falls occur during winter, some of it as snow. The conditions are similar to some parts of central Europe. The temperature is suitable for the larval development of *F. hepatica* for a period of only 3 or 4 months a year in summer.

Types A, B and C are the most extensive endemic areas in Australia. In these areas the development of all larval stages of *F. hepatica* is very slow, or negligible, from May to September due to average temperatures lower than 10°C.

## B. CONTROL

The efficient control of fascioliasis depends on the correct and integrated application of:

1. Reduction of the number of parasites in the host by regular anthelmintic treatment.
2. Reduction of the number of intermediate host snails by physical, chemical and biological means.
3. Reduction of the chances of infection by farm management.

There are various favourable and unfavourable factors which influence the successful completion of the life cycle of the parasite, and directly or indirectly influence the effectiveness of control measures (see Table XXXII).

In Fig. 16 the expected activity of various stages of the life cycle is shown, together with general recommendations for the appropriate time for control measures for each climatic type. The expected activity of *F. hepatica* and of the intermediate host snails, for each individual climatic type, has been constructed from the existing knowledge on the ecology of the parasite based on the literature, and on observations carried out in this laboratory.

The recommendations are made on a preventive rather than on a curative basis. In the models, treatments are recommended for each climatic area, usually 6–8 weeks after the probable massive intake of metacercariae, to prevent gross hepatic lesions and to kill the flukes before their sexual development in order to prevent contamination of pastures. The preventive winter treatment is usually recommended about 12 weeks after the last probable intake of metacercariae, before chronic symptoms develop and massive egg production commences.

### 1. *Anthelmintics*

The preventive treatment in late winter is an essential part of control to reduce contamination with eggs before the active spring period. The treatment should be carried out in late winter when most of the flukes acquired during late autumn become mature. According to Fig. 16, and to epidemiological studies (Boray *et al.*, 1967c; Boray, 1969; Figs 13 and 14), the emergence of cercariae could be substantial during spring from snails infected in autumn.

Approximately 8 weeks after this period, a treatment should be carried out to reduce the number of immature flukes which may cause severe pathological damage to the liver at that time. After this period, cercariae would be developed in the snails infected in the spring, and production of cercariae would be continuous until the end of autumn. Therefore, initially in heavily infected areas, additional treatments may be necessary in February and April, and in June. This general treatment pattern is necessary when the anthelmintics used

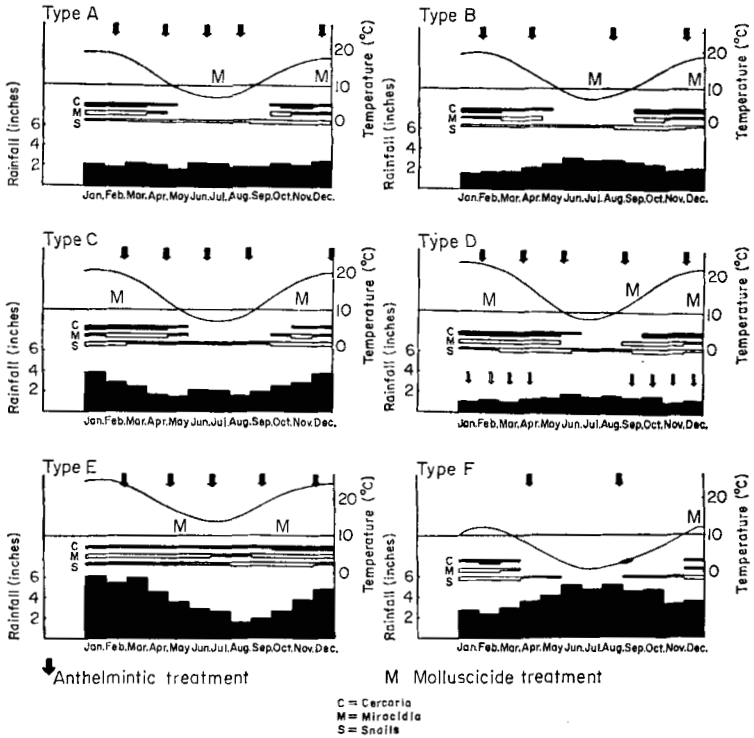


FIG. 16. Expected larval activity of *F. hepatica* and control recommendations for various climatic areas. (After Boray, 1969.)

are, at their normally recommended dose rates, efficient against mature flukes only (5–10 ml of double strength carbon tetrachloride, 2–4 ml $CCl_4$ per sheep, hexachloroethane at 300 mg/kg, or oxyclozanide (Zanil) at 15 mg/kg). The use of 2 ml $CCl_4$ is an ideal means of maintaining subclinical fascioliasis in sheep, thus contaminating the pastures while still exposing the animals to unpredictable $CCl_4$ poisoning.

Flukes in a wider age range may be killed with clioxanide (Tremerad), menichlopholan (Bilevon) or nitroxynil (Trodax) at the average dose rates of 20, 4 and 10 mg/kg respectively as recommended by the manufacturers (Boray and Happich, 1968; Happich *et al.*, 1967), or with MK990. The efficiency of the

latter against immature fluke was reported above (Section VI B). Subsequently, treatment in February, and in some districts (Types A and B) in June, may be omitted. These drugs are better tolerated at dose rates which are highly efficient against flukes aged 6 weeks, and if these drugs are used, the number of treatments per year could be reduced with confidence. Conditions in Type A climate may be regarded as average for most endemic areas. However, changes from the above pattern have to be made, depending on the different seasonal distribution of rainfall. The usual dry weather at the end of summer in Type B areas would reduce snail activity and production of cercariae in autumn, and infection could rapidly diminish in early winter. Consequently treatment in June would not be necessary but because of the moist winter, spring infection may be more important. Therefore, the summer treatment should be carried out in early December, or even in late November. Likewise the second summer and autumn treatments should be slightly earlier than in Type A districts. In Type C districts, particularly in the New England area, the relatively dry and cold winter would reduce the activity of snails and fluke larvae, resulting in lower and later spring infection, which in turn would also delay the summer infection. Most of the cercariae would be produced from the middle of summer to the end of autumn. Consequently, after the late August treatment, all other treatments should be delayed until the end of February and April. An additional treatment is necessary in late June to prevent outbreaks of subacute fascioliasis during winter in sheep which became infected during late autumn. The frequent occurrence of this condition in winter was reported by Osborne (1958, 1962). Increased grazing of the more permanent snail habitats during the dry winter may contribute to the fluke burden of sheep, but the epidemiological studies showed that metacercariae may not survive very long in Australian habitats, and subacute fascioliasis is less likely to occur when the winter is dry. Boray (1967a) showed that in heavy infection the growth of flukes was retarded, and flukes 2–6 mm long were up to 12 weeks old. Osborne (1958, 1962) described outbreaks in the New England district and found that 2 weeks after treatment with carbon tetrachloride, flukes were about 6 mm long. Boray (1967a) reported that in experimentally infected sheep the length of flukes from 8 to 12 weeks was 1–16 mm, and without treatment the sheep died of subacute fascioliasis between 8 and 12 weeks after infection. The sheep described by Osborne were treated with carbon tetrachloride; all the larger flukes were possibly killed, thus partially relieving the liver from some damage and delaying clinical manifestation of the disease. The majority of retarded flukes could have survived and with prolonged tissue migration caused further haemorrhages and death, three or four months after the bulk of metacercariae were ingested. If drugs at dose rates highly efficient against immature flukes were used in late April and June, losses such as these could possibly be prevented.

Similar delayed outbreaks occur frequently in Europe, where subacute fascioliasis may cause death 4 months after sheep have been housed in winter. Treatment with as little as 1 ml carbon tetrachloride per sheep is common practice in some European countries and in Britain. The low efficiency of this treatment, coupled with a usually low plane of nutrition, may perpetuate this delayed type of subacute fascioliasis.

On irrigation areas (Type D), the frequent and regular alternation between wet and dry periods maintained by irrigation is an ideal environment for the propagation of snails and for the larval development of *F. hepatica.* The temperature is higher and larval development is intensive and continuous, except for a short break in winter. Activity is very high at the end of autumn due to increased egg-contamination. Sheep may become infected from early October to the end of June; therefore the preventive winter treatment should be delayed until early September, and frequent treatments with dose rates highly efficient against immature flukes should thereafter be employed in December, February, April and June. The data for this model are supported by the epidemiological studies and by the observations of Buckley (1961).

In the subtropical Type E, conditions are very favourable for continuous larval development, with an expected peak in the production of cercariae in October, November and December. Not many sheep are grazed in such areas, but their treatment pattern would be similar to that of Type D. In the small area of Type F, alpine climatic conditions restrict the activity of *F. hepatica* to a short period in summer and early autumn, and two treatments a year may be adequate. Naturally, these recommendations would be valid only for an average year, and modifications may have to be made according to local climatic and pasture conditions. At a favourable stocking rate on improved pasture, sheep may not be exposed to infection due to their selective grazing habit, but during drought when shortage of food drives the sheep to permanent snail habitats, heavy infections may occur (Rose, 1938; Osborne, 1958). Regular drenching of all stock may reduce egg contamination and less frequent treatments may be sufficient to control the disease in subsequent years.

If the recommendations were rigidly followed, the parasite would be efficiently controlled but many unnecessary treatments would be involved. The correlation between egg counts and fluke infection in the chronic stage was found to be reliable in sheep (Happich and Boray, 1969b). The use of this correlation, and the correct evaluation of data on the clinical and post mortem pathology of fascioliasis (Boray, 1967a), may assist considerably in the diagnosis and prognosis of both acute and chronic fascioliasis and facilitate the control of the disease more economically.

*Treatment of cattle.* It has been generally recognized that cattle are more resistant to *F. hepatica* than sheep. Under normal conditions, mainly young calves show clinical symptoms of fascioliasis, but the life span of the parasite is limited, spontaneous recovery occurs and previously infected animals resist reinfection (Hutyra and Marek, 1938; Dixon, 1964; Keck and Supperer, 1966; Ross, 1966b; Boray, 1967b). Boray (1967b) showed that if an initial infection was removed, reduced by anthelmintic treatment or eliminated by spontaneous recovery, a high degree of acquired resistance developed in cattle. This resistance was manifested by lesser clinical symptoms, lower recovery of flukes, lower egg output and extremely short patency in the challenge infection. Recent experiments showed that high resistance was maintained in animals receiving 500 metacercariae weekly for 18 weeks (Section V E).

This information suggests that treatment of cattle, particularly young cattle,

twice a year (August and April) would be sufficient in most climatic types, but an additional treatment may be necessary in January in Types D and E. Particular attention should be paid to the treatment of young calves, cattle introduced for the first time to a contaminated pasture and animals in poor condition due to other diseases or malnutrition. The treated animals would maintain strong resistance to subsequent infections for many months, without severe clinical symptoms. One of the most important factors which may increase pasture contamination is the present method of anthelmintic treatment, particularly with carbon tetrachloride and other drugs, at dose rates inefficient against immature flukes. Methods for anthelmintic treatment should be revised by graziers, and this will soon be possible by the improved safety achieved in some recently developed anthelmintics.

2. *Snail control*

The high reproduction rate of *L. tomentosa*, survival under various unfavourable conditions and active and passive migration were reported by Boray (1963a). The high biotic potential of snails makes their eradication unlikely either by chemical or biological means. It can be achieved only by the complete elimination of all suitable habitats by underground or tile drainage. Improvement of existing drainage systems and their maintenance, or the building of dams at marshy places may reduce snail habitats. This rather costly physical control may have a limited application under Australian conditions for some time. Gordon *et al.* (1959) and Boray (1967c) showed that efficient control may be achieved by using highly efficient molluscicides in the field, but snails usually reappeared 3–6 months after treatment and thereafter rapid recolonization occurred (Section VII A). Because snails can aestivate for long periods (Boray, 1963a), the presence of sufficient water at the habitats is essential when molluscicides are used. At present sodium pentachlorophenate is the most suitable molluscicide for Australian conditions (Boray, 1967c; Section VII A). The best method for treatment is that described by Gordon *et al.* (1959), using equipment for producing a high volume spray. Figure 16 indicates the most suitable times for molluscicide control for each climatic type, based on our present knowledge of the ecology of the snails and on the climatic conditions.

For Types A and B one treatment in mid-winter would reduce the number of snails before their rapid reproduction in the spring, and a second treatment in early summer would kill the progeny of the survivors. At both these times the snails may be harbouring numerous, developing fluke larvae, and their elimination would reduce contamination with metacercariae. For Type C, September is a more suitable time for the first treatment because of the delayed activity due to the dry winter. The second treatment should be carried out at the end of summer. In Type D (irrigation areas), molluscicide treatment should coincide with irrigation, killing the snails before most of the accumulated cercariae are released in September (or first irrigation), December and February. The most suitable period for treatment in Type E may be in October and May when the habitats have sufficient water but are not flooded. Most habitats in Type F may be unsuitable for molluscicide treatment, but one treatment in December may be beneficial.

Many Australian properties are topographically unsuitable, or make it very difficult to apply molluscicides, and their effective use requires specialist advice, co-operation with neighbours and expensive labour. However, in some cases chemical snail control may be the most effective and simple measure.

3. *Black disease* (*infectious necrotic hepatitis*)

The time usually recommended for vaccination against this disease is November or December (Seddon, 1967). However, Osborne (1962) showed that black disease and acute fascioliasis often occur in winter on the N. Tablelands. The occurrence of black disease in winter may be correlated with the conditions resulting in delayed subacute fascioliasis, as discussed above. The present studies showed that after a short winter break, heavy infections with *F. hepatica* may occur as early as October in most endemic areas, and suggest that black disease could occur throughout practically the whole year. Vaccination of adult sheep against the disease should be carried out in September, as a rule; lambs can be vaccinated at any time of the year as soon as grazing of flukey-pastures is commenced.

4. *Pasture-rotation control*

Reduction of the chances of infection by farm management has been receiving attention for a long time, particularly prior to 1926 when the first effective treatment against the parasite was discovered. Long before the life cycle of *F. hepatica* was known, it was common practice amongst European shepherds to avoid moist pastures at certain times of the year because of the popular belief that certain aquatic plants were associated with the infection. Cobb (1897, 1904) gave a vivid description of methods to reduce snail habitats and prevent infection in Australia. He also described a sound concept of rotational control, based on his observations that fluke are more common in sheep than in other animals. Osborne (1967) suggested a rotational control programme of grazing for a minimum of 10 weeks on fluke-free pastures and a maximum of 9 weeks on fluke-infected pastures. Drenching would be necessary before moving to fluke-infected areas (five treatments in 2 years). This method would, theoretically, reduce infection considerably, provided that each anthelmintic treatment was carried out at dose rates highly efficient against flukes younger than 10 weeks (Boray and Happich, 1968). Boray (1969) suggested that to prevent contamination with fluke eggs, the minimum grazing time on fluke-free pastures should be about 12 weeks or more, treatment should be carried out 2 weeks before moving into fluke-infected pastures, and attention should be paid to seasonal variations of infection.

It was suggested by Osborne (1967) that cattle should be grazed in fluke-free paddocks. It is, of course, desirable to keep cattle away from infected areas. Boray (1967b) suggested that resistant adult cattle may be grazed safely on fluke-infected pastures, and mixed grazing may have a beneficial effect by reducing the infection in sheep. It may be more economical if cattle were moved with sheep according to the rotation programme. Cattle may also serve a useful purpose on marshy land by controlling the overgrowth of vegetation, thus reducing silting and expansion of the area. It is desirable, however, that calves

under one year old should not be exposed to fluke infection because they are more susceptible to clinical fascioliasis (Boray, 1967b; Section V). The selection of the correct method and the efficiency of rotational control may depend on many factors and vary from property to property. In some properties snail habitats form only a very small proportion of the whole area, often confined to a single paddock; in others, however, many temporary and permanent snail habitats are present, and division between fluke-free and fluke-infected paddocks by fencing may require a major investment. Based on our epidemiological studies, on the life-cycle of *F. hepatica* and on the pathogenesis of fascioliasis in sheep and cattle, a scheme for rotational control has been proposed to suit most conditions in Australia (Boray, 1969; Fig. 17).

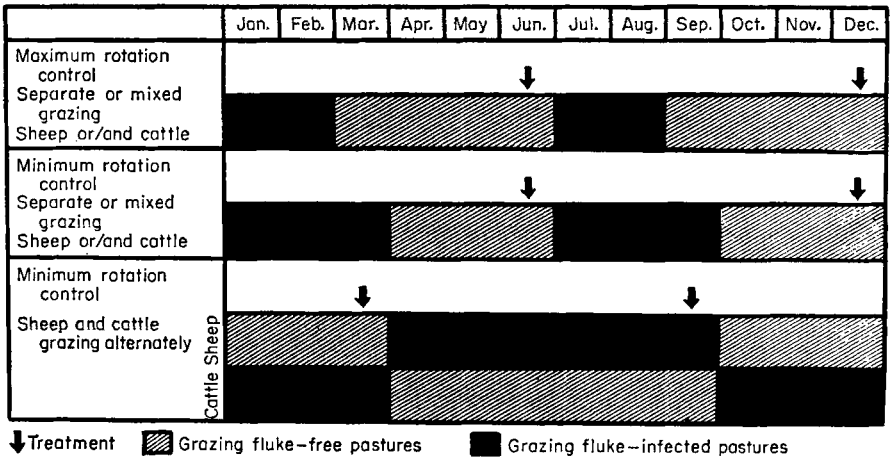


FIG. 17. Pasture and stock rotational control of fascioliasis. (Reproduced with permission from Boray, 1969.)

A programme has been designed as a maximum rotational control for properties grazing only sheep or cattle and for properties grazing both, where snail habitats are limited to a small portion of the whole area. Minimum rotational control programmes for both separate and mixed grazing are suggested for properties with extensive snail habitats.

(a) *Maximum rotational control—separate or mixed grazing.* The programme should commence in September after efficient winter treatment of all stock of the property. The animals should graze fluke-free areas in the spring, when most cercariae would be present on the pasture from autumn infection of snails. Treatment should be carried out in the middle of December two weeks before moving in January to fluke-infected paddocks, to eliminate the small number of flukes from a winter infection which might have survived the previous treatment. The animals would then be moved to the fluke-infected paddocks in January. The area would have a negligible number of metacercariae because it was without contamination during spring. The possible egg output by a few surviving flukes may infect the snails, but the stock would be moved into fluke-

free pastures in March, before the completion of larval development. Drenching against flukes from the summer infection should be carried out in the middle of June two weeks before moving to the fluke-infected area again. This scheme would require only two treatments annually, integrated into normal farm management at set dates, well before late autumn or early spring lambing. On the Northern Tablelands, winter grazing would be on the marshy areas, possibly the only areas where some pasture is available. The treatments would be carried out when all flukes had reached maturity and 100% efficiency could be achieved. After the 2nd year, if rotation is carried out as suggested, one treatment a year in June may be sufficient under normal conditions.

(b) *Minimum rotational control—separate or mixed grazing.* On some properties, 4 months spelling of paddocks may not be possible. The minimum rotational control for these areas would be similar to the maximum rotational control, but grazing on fluke-infected paddocks would be extended to September and March. The programme should commence, as above, in the spring. Two treatments per year are essential from year to year, and the treatments should be carried out with dose rates of anthelmintics efficient against immature flukes, because some infection would occur towards the end of each grazing period on the infected pastures.

(c) *Minimum rotational control—sheep and cattle grazing alternately.* This programme should also commence in spring after efficient anthelmintic treatment of all stock. Sheep should graze the fluke-free area from October to the end of March, and cattle the infected area during the same period. Two weeks before moving the sheep to fluke-infected areas in the middle of March, they should be treated to reduce contamination. The cattle should also be treated at the same time to reduce the number of flukes and so prevent clinical fascioliasis before they are moved to fluke-free paddocks. Sheep would graze the fluke-infected areas and cattle the fluke-free area from April to September. In September, 2 weeks before the next exchange of grazing area, the anthelmintic treatment should be repeated. With this system, the more resistant cattle would graze the fluke-infected areas during the period when infection would be most likely to occur. They would produce less contamination than sheep, most flukes would be eliminated by spontaneous recovery and enthalmintic treatment, but the animals would not lose their resistance because of their regular exposure to infection for 6 months each year. Anthelmintic treatment of sheep should be carried out twice a year, but after the 2nd year treatment of cattle may be necessary only once a year in March.

Boray (1969) discussed many aspects of the complex epidemiology of fascioliasis. It was evident, however, that continuous and co-ordinated application of all available measures most suited to each different climatic and topographic condition, may provide control for the disease which would be highly successful and economic. It is to be hoped that these concepts will be tested in the field.

ACKNOWLEDGEMENTS

The author is grateful to the Editors of *Zeitschrift für Tropenmedizin und Parasitologie* for permission to use three figures from Symons and Boray

(1968), and to the Editor of the *Australian Veterinary Journal* for permission to use five figures from Boray (1969). Dr. D. F. Stewart, Associate Chief of the Division of Animal Health, C.S.I.R.O., kindly assisted with the manuscript. Many results reported in the present review were based on experimental work carried out in this laboratory. The author is indebted to the staff of McMaster Laboratory for their conscientious and able assistance. The particular assistance given by members of the staff of this laboratory with the bibliography, photography and typing of the present review is gratefully acknowledged.

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# The Epidemiology and Control of Some Nematode Infections of Grazing Animals

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## I. Introduction

One feature of the life histories of nematode parasites of grazing animals is that they do not multiply in the host. The eggs or larvae produced by the adult worms pass out of the host and after some free living development become infective and capable of resuming a parasitic way of life. Every adult parasite has therefore been separately picked up by the host and every larva on the pasture is derived from an egg laid by a worm in the host. This has often been pointed out, but the more important implications are often overlooked.

Population increase of a parasite which completes its life cycle within the same host tends to follow a constant pattern. Characteristically the increase is exponential during the early stages of the infection while the host offers an ideal environment. Subsequently, when the host becomes resistant and

represents a less suitable environment, the rate of increase declines to zero and the population then rapidly decreases.

The nematodes with which we are concerned here may not increase in number according to this pattern; the form of their population increase is determined by conditions in the external environment and the extent and manner of the host's contact with it, and is almost infinitely variable. Infection does not of itself produce standardised or predictable pathological or immunological results, and an infected host is not necessarily diseased.

In spite of this fundamental difference, attempts have been made to interpret the epidemiology of nematode infections in the same terms as that of any other infectious disease, the host and the outside world together being seen as a uniformly favourable environment. This commonly held view was well expressed by Cameron (1932) in the following terms.

> If an animal with, say, ten worms is confined to a limited space of ground, in a very short space of time that piece of ground will be contaminated by droppings containing, say, 10000 eggs, each of which, in due course, will give rise to the same number of larvae. The animal has to graze over the limited area of ground and must eat all the grass; accordingly practically all the larvae are swallowed and in a few months the number of worms in the host has been increased by several thousand. In a few months more, this has been multiplied by the thousand or so larvae produced by each of this new generation—and so on. Of course not all the larvae hatch, not all become mature, and not all are swallowed or develop in the host, but the rate of increase is in geometrical rather than arithmetical progression.

A given increase in population can arise either through the occurrence of many generations, each producing a relatively small increment, or by a few generations each yielding a large increment. If the number of generations is small the initial population might have a profound effect on the numbers finally present, but if the increase involves many generations the initial population is relatively less important.

Life cycles which entail the need for each generation of worms to undergo a period of development in the external environment may assist the parasite in colonising hew hosts, but also involve the hazard that the free living forms may perish before they find their way to a suitable host in which they can resume their development. We are not surprised to find, therefore, that parasitic nematodes are extremely prolific, e.g. one female *Haemonchus contortus* laying between 5000 and 10000 eggs per day, or one female *Dictyocaulus viviparus* laying 2000 per day. Some effects of high fecundity linked with heavy mortality of free living forms were considered by Michel and Ollerenshaw (1963), who argued that a small decrease in environmental pressure would lead to a disproportionately large increase in the number of survivors. For instance, if it were normal for only one individual out of every 1000 to survive, a decrease of 1% in mortality would result in a 900% increase in the number of survivors. A certain instability in populations of infective larvae was therefore anticipated. Sudden unpredictable population increases of the cattle lungworm do indeed occur. Otherwise the free living stages develop more slowly and their mortality is less, where this instability is not so marked. Here, however,

the failure of host resistance can lead to dramatic increases in worm populations.

Taylor (1943) believed that worm burdens in lambs would show

> . . . an increase by geometric progression which, as the weeks go by, results in the development of a heavy infestation of both the lambs and the pasture. The rate of increase is so great, in fact, that were it not for the development of a resistance to the parasitic worms it would be quite impossible to maintain lambs on our agricultural pastures, for they would all die of acute parasitic gastritis. Partly as a result of their growth to maturity, however, and partly as a result of the infections acquired in early life, the lambs begin to build up a resistance and an increasingly smaller percentage of acquired larvae develop to maturity. This resistance continues to develop; fewer and fewer worms reach maturity; egg production by the worms is inhibited to an increasingly greater extent; a comparatively rapid expulsion of the worms follows as the resistance of the lamb becomes properly established and the infection falls to the comparatively low level of that carried by healthy adult sheep.

Taylor's thesis is illustrated in Fig. 1.

Crofton (1955) gave supporting evidence relating to trichostrongylid worms, noting that the faecal egg counts of lambs in lowland flocks increased logarithmically to a peak where, he thought, further increase for 2–3 weeks would have resulted in counts exceeding a certain level. Crofton interpreted his egg counts as reflecting a process of increasing pasture contamination and reinfection involving numerous generations. He discussed some of the implications, assuming that the rate of free living development was uniformly rapid and that the larvae remained infective for only a short time. Crofton felt that these conditions may have been met during the period of his observations, and his

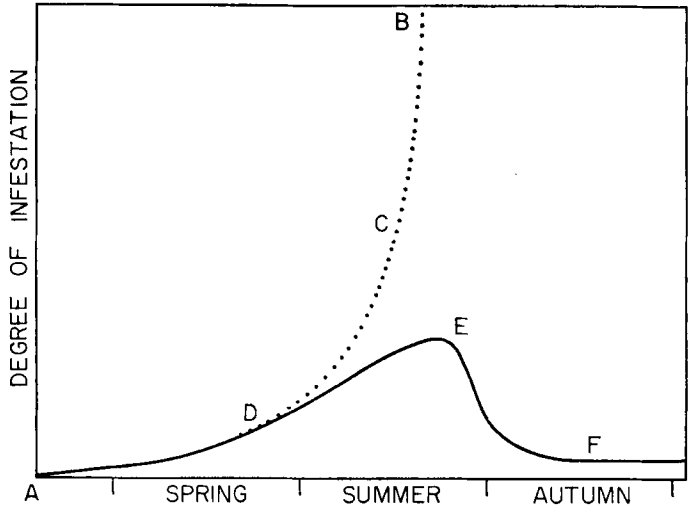


FIG. 1. The hypothetical course of worm burdens in lambs, reproduced with permission from Taylor (1943), showing a logarithmic increase which is first moderated and then terminated by the growing resistance of the host.

conclusions were generally accepted. They are well expressed by Thomas (1959): "Development during spring and summer is rapid, the infective stage being reached under favourable conditions in 5–15 days and the life cycle may be completed in 4 weeks. Under these conditions the host can harbour a number of generations of any one of a number of species and the build up of the worm burden is due to repeated reinfection". The number of generations has not been estimated but in another context Crofton *et al.* (1965) have suggested that in the U.S.A. *Haemonchus contortus* has completed 500 generations in the last 60 years, i.e. eight to nine per annum.

This picture of a logarithmic increase in worm burdens due to a succession of ever greater generations of the parasites, continuing to a crisis when the host becomes immune and worm burdens decrease dramatically, can no longer be accepted. It does not apply to infections of *Dictyocaulus* and of *Nematodirus* spp. and recent work indicates that it does not fit the case of other species of gastro-intestinal nematodes. These developments are discussed in the present review.

The form of population increase is influenced by conditions in the external environment and by the development of host resistance. Where the external environment provides uniform conditions throughout the year, the course of an infection depends only on the reactions of the host. Such a situation was observed by Donald (1964) in Fiji. At whatever time of year calves were first turned out, the course of infections of gastro-intestinal worms was the same.

If susceptible hosts appear at one time of year, as where lambs are born or calves turned out for the first time in the spring, a seasonal pattern is imposed on worm infections (Tetley, 1941). A climate which is not uniform throughout the year also contributes to an annual rhythm in worm infestations. Climatic factors determine whether and how rapidly free living development can take place, what proportion of individuals succeeds in becoming infective and for how long they survive. The nutritional status of the grazing animal and hence its resistance to infection is also subject to seasonal influences.

Two factors, temperature and moisture, are of particular relevance to the free living phase of the life cycle, and attempts have been made to relate the seasonal occurrence of outbreaks to climatic changes on the basis that certain minima of mean temperature and of rainfall are required. Gordon (1948) in Australia proposed the use of so called bioclimatographs for this purpose. In these, mean values for temperature and rainfall are plotted one against the other for every month of the year and the resulting pattern superimposed on arbitrarily chosen limits. Gordon selected these limits very shrewdly and was able to define the areas where different species were likely to predominate and the seasons in which outbreaks were to be expected. As pointed out by several authors, however, not only does the selection of appropriate limits owe more to art than to science, but bioclimatographs for the same area in different years may be very different and they have been less successfully related to the incidence of disease in a particular year. The approach is not adequate if any degree of precision is required. Levine (1963) has proposed the use of records of temperature and soil moisture status to define periods during which particular species can be transmitted.

The concept of "outbreak weather" is inherent in work of this kind, but may be more appropriate to those situations where the lack of moisture is the chief limiting factor and where a heavy shower of rain may suddenly and dramatically create the conditions in which infection can be transmitted. It is less appropriate perhaps where temperature is the limiting factor because it is improbable that a sufficiently sharp increase in temperature would occur out of season to speed development very greatly. With the exception perhaps of *Nematodirus battus* and *N. filicollis*, where a stimulus of this kind may lead to a mass hatching of eggs, the concept of outbreak weather is less valuable in

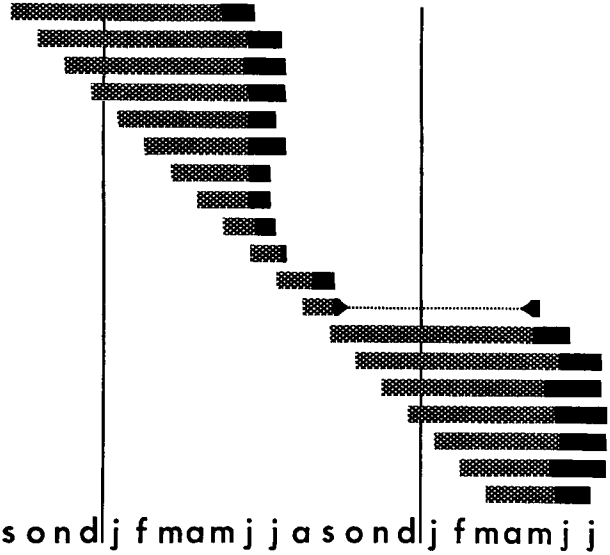


FIG. 2. The time taken by eggs of *Nematodirus helvetianus* in fresh faeces exposed in different months to complete their free living development and hatch from the egg. The black band indicates in each case the period during which hatching occurred. (Drawn from data presented by Rose, 1966.)

Britain than one of changes in worm populations being linked to the calendar. In Britain the transmission of nematode infection is largely dominated by seasonal changes in temperature. In most cases the season during which free living development can proceed at something approaching its optimum rate is very short. This is well illustrated by Fig. 2, which shows the time taken for the free living development of *Nematodirus helvetianus* to be completed out of doors and indicates when the first and last eggs were observed to hatch. The period during which development can be completed is very short indeed, and all the eggs dropped in 9 months of the year and some of those in the 10th month all hatched within the same period of 6 weeks. Clearly, the generation interval is not fixed but depends on the time of year at which free living development begins. The number of generations that can be completed in the

year is therefore likely to be smaller than might be concluded by dividing the minimum generation interval into the period during which development is not impossible—a deplorably common method of calculation.

Worm burdens harmful to the host are nearly always picked up over a short period of time; they are the consequence of the exposure of insufficiently resistant animals to heavily infested grazing, as will be shown. This situation may come about in two ways: (1) The pasture may be contaminated by one group of animals and, when heavily infested, grazed by a second susceptible group. This may be regarded as the simple transmission of infection from one group to another. It can occur in all nematode infections and in some it is the normal way in which outbreaks of disease are caused. (2) A group of animals becoming lightly infected at first, may contaminate the pasture and become reinfected from it. This is known as autoinfestation. How many generations of the parasite are involved until a pasture infestation sufficient to cause disease is built up depends on the generation interval and the rate at which host resistance develops. Both factors have been subjects of recent study.

## II. Host Resistance

It was once supposed that when the host had acquired a certain experience of infection it became resistant, threw off its worms and henceforth was refractory to further infection. Stoll (1929) described what appeared to be such a sequence of events in two lambs which were exposed to infection with *Haemonchus contortus*. They became heavily infected but after some time their faecal egg counts fell rapidly to very low levels and the experimental administration of numerous infective larvae failed to increase it. Stoll termed the phenomenon "self-cure and protection".

The notion of a certain degree of infection being needed before an immune response is elicited has been developed by Dineen (1963) who, drawing a parallel with the immunology of homograft reactions, considered that if the worm burden remains below a threshold level the presence of the worms is tolerated. If the threshold is exceeded, however, the host responds, mechanisms of resistance come into play, and the worm burden is reduced. Thereafter a lower threshold operates. This threshold would be high where host and parasite are well adapted to each other. With Sprent (1959) and Damian (1962), Dineen considered that in the course of the evolution of a host-parasite association the difference between the antigens of host and parasite will have decreased, so that unless a large number of worms is present, the host will not respond to their presence. This view does apply to some expressions of resistance but it is not universally applicable. Mechanisms which do not depend on such thresholds are of considerable epidemiological importance.

Resistance to helminths consists of a number of mechanisms which should be regarded as entirely separate, both in the manner of their causation and in the effect produced on the worms (Michel, 1968a). It has come to be recognised that the "self-cure and protection" of Stoll are separable, that one is not inevitably accompanied by the other. Of the separate mechanisms of resistance four are of particular interest.

### A. RESISTANCE TO THE ESTABLISHMENT OF WORMS

Where reference is commonly made to immunity to helminths, a resistance to the establishment of worms is meant. This is the "protection" envisaged by Stoll (1929). A difficulty of definition occurs, however, for there is considerable variation between different species of worms in the stage against which this resistance is expressed. It may not be easy to draw a distinction between failure of worms to become established and their premature expulsion. Thus, while Stewart (1958) claimed to have recovered third stage larvae from the faeces of resistant calves when these were infected with *Cooperia punctata*, Herlich (1963) reported that *Trichostrongylus colubriformis* is expelled from the resistant host 2–3½ days after infection, and *H. contortus*, according to Brambell *et al.* (1964), is eliminated from the resistant host 9 days after infection. Consideration must also be given to species which perform an extended migration through the host's body. Thus *Dictyocaulus filaria* migrates normally in the resistant host as far as the mesenteric lymph glands and persists there until the 7th day after infection, but it fails to reach the lungs (Michel, 1956). *Nippostrongylus brasiliensis* may be destroyed in the lungs of a resistant rat (Porter, 1935) or, if immunisation has been more effective, will not proceed further than the skin (Sarles and Taliaferro, 1936). A certain latitude in definition is needed to encompass this range and it is proposed that the terms "protection" or "resistance to establishment" be used for any phenomenon whereby the worms are prevented from reaching that stage at which their development is characteristically inhibited (see below).

Different species of nematodes vary in the point of development at which they are attacked in a host resistant to their establishment, and the experience of infection required to elicit this response also varies widely. Relatively little experience of infection is required to make calves resistant to the establishment of *Dictyocaulus viviparus* and this resistance develops very quickly. Michel (1962) found that only 11 days after infection with a single moderate dose of larvae, calves were resistant to reinfection, only one-fifth as many worms becoming established in them as in susceptible control calves (see Fig. 3).

*Nematodirus* spp. also appear to stimulate the rapid development of protective immunity (Gibson and Everett, 1963). In infections of *Ostertagia ostertagi*, on the other hand, this form of resistance appears only after very prolonged experience of infection. Even large experimental infections resulting from a single administration of larvae do not affect the establishment of a subsequent infection. When larvae are administered daily, the percentage which becomes established does not decrease for some months (Michel, 1963).

*Haemonchus contortus* in sheep presents a rather confused picture because different breeds of sheep react differently. While British sheep can become refractory after they have reached a certain age (Manton *et al.*, 1962; Urquhart *et al.*, 1966), it appears that Australian Merinos do not readily acquire this form of resistance even when adult.

The experience of infection needed to evoke resistance to the establishment of worms and the speed with which it can be acquired have important

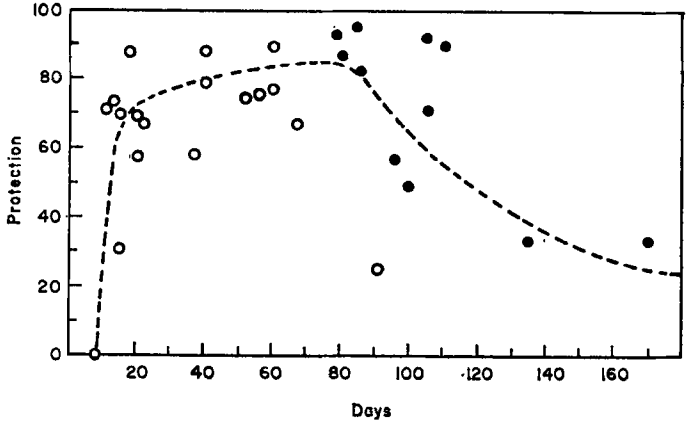


FIG. 3. The development of resistance to the establishment of *Dictyocaulus viviparus* in calves immunised on day 0 with 3500 larvae and subsequently challenged with 35000 larvae.

$$\text{Protection} = 100\left(1 - \frac{\text{worms of challenge infection recovered from immunised calf}}{\text{worms of challenge infection recovered from susceptible control calf}}\right)$$

Cases in which worms of the immunising infection were still present at the time of challenge are plotted as open circles, those in which they were no longer present as filled circles. (Reproduced with permission from Michel, 1962.)

epidemiological consequences which will be discussed in detail in later sections. They also influence the relative importance of other resistance mechanisms, e.g. if, as in *Dictyocaulus viviparus* in calves, the host becomes refractory to reinfection very quickly, it is difficult to demonstrate how the development of worms in a resistant host would be affected.

### B. SELF-CURE AND OTHER FORMS OF WORM LOSS

The first use of the term self-cure to denote a phenomenon separate from protection was by Gordon (1948), who referred to dramatic decreases in faecal worm egg counts, due chiefly to *Haemonchus contortus*, which occurred simultaneously in nearly all the sheep of many flocks in one area. Stewart (1950) showed that this phenomenon was almost certainly due to massive reinfection, a possibility envisaged by Tetley (1949). Stewart found that giving a large number of larvae to a sheep with suitable previous experience of infection led to the rapid expulsion of the adult worms present. The phenomenon could be produced with several species. In *Haemonchus contortus* the newly administered worms became established but in *Trichostrongylus colubriformis*, to which the host becomes more readily resistant, this did not often happen. Stewart (1953) provided evidence that an allergic response was involved in the expulsion of worms. Michel (1952a) studied a similar phenomenon in infections of *Trichostrongylus retortaeformis* in rabbits. He distinguished between a gradual decrease in worm numbers seen in small infections and reflected in

faecal egg counts declining, apparently exponentially, over a period of many months, and an abrupt decrease whose occurrence appeared to demand the presence of a sufficient biomass of worms (Fig. 4). This abrupt loss of worms could be elicited equally by superimposing a very large infection on a pre-existing small one, whereupon the original worms, but not the new ones, were removed within a week, as by giving a single large infection to a previously worm free rabbit.

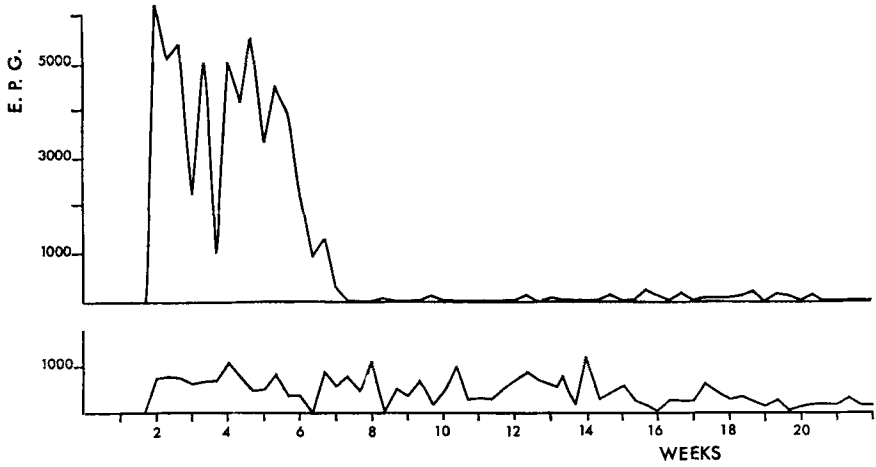


FIG. 4. The two common forms of decrease in worm burdens illustrated by the faecal egg counts of two rabbits infected at the beginning of the first week. The lower graph refers to a rabbit which received 300 larvae, the upper to one that received 3000 larvae.

There seemed every justification, therefore, for regarding the phenomenon as one and the same, whether the necessary stimulus to elicit it resulted from one administration of worms or from two. On the other hand a distinction between the abrupt loss of worms and the more gradual exponential loss seemed entirely valid. The significance of this gradual loss has only recently been recognised, although many years have passed since Sarles (1929) fitted curves of the form $y=a(b)^{-x}$ to egg count data from dogs experimentally infected with *Ancylostoma caninum* and an infection of *Trichostrongylus calcaratus* in the rabbit. Winfield (1933) commented on a similar finding with *Heterakis spumosa* in rats, but thereafter no further interest was taken in the characteristics of curves of declining egg counts, although such curves continued to be published in very large numbers. If, as was assumed, these egg count curves reflected a similar pattern of decrease in worm numbers, this would have profound implications. If the rate at which worm numbers decreased were proportional to the number of worms present, and this is what a logarithmically decreasing population curve must mean, this phenomenon would represent a powerful mechanism for the regulation of worm burdens. The worm burden of an animal exposed to new infection at a constant rate would remain at a level at which the appropriate rate of worm loss balanced the new

infection that was being acquired. Michel (1967a) concluded that populations of *Ostertagia ostertagi* in calves were regulated by a mechanism of this kind, although his data suggested that the rate of loss was proportional to the square of the number present. More extensive experimentation has, however,

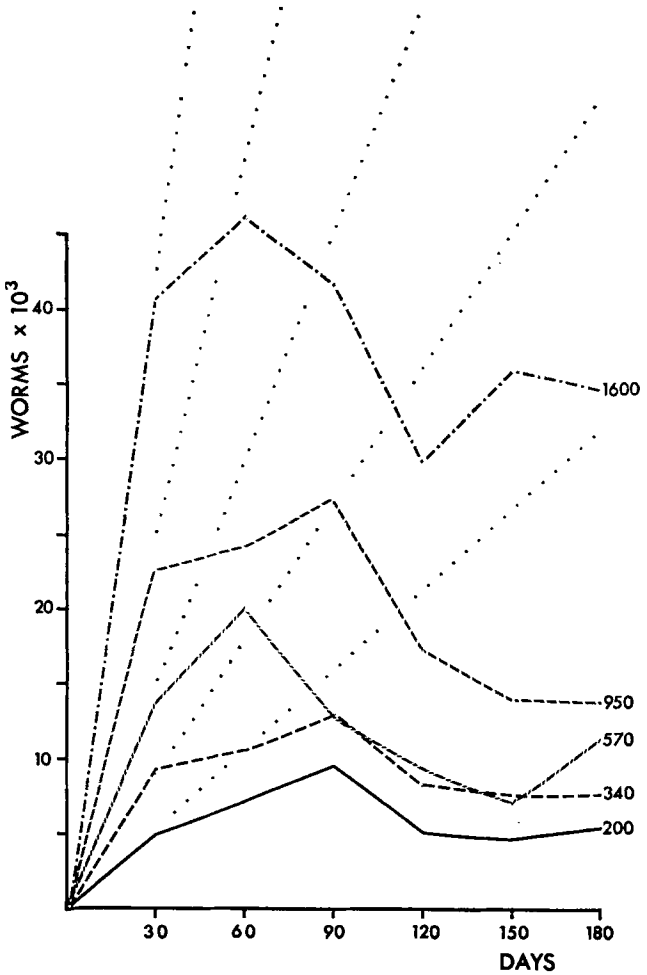


FIG. 5. The worm burdens of five groups of calves which received, respectively, 200, 340, 570, 950 and 1600 larvae of *Ostertagia ostertagi* daily.

shown a direct relation between worm numbers and the rate of loss. Figure 5 shows the worm burdens of five groups of calves which were infected daily throughout the experiment, the calves of each group receiving a different number of larvae. Figure 6, in which the mean worm burden of each group over a period of 5 months is plotted against the infection rate, shows that a linear relationship exists. It is clear that the regulation of worm numbers does

not depend on a mechanism of the kind visualised by Dineen, in which the host tolerates the presence of a certain number of worms but reacts when this is exceeded and reduces the burden to a lower level. Some consequences of this mechanism for the regulations of worm burden must be considered. The infection rate determines the size of the worm burden, not the rate of its increase. Since the consumption of herbage by a grazing animal may be regarded as very roughly constant, and the worm burden depends on the rate at which new larvae are ingested, the worm burden will be directly related to the number of larvae per unit weight of the herbage. This can be measured.

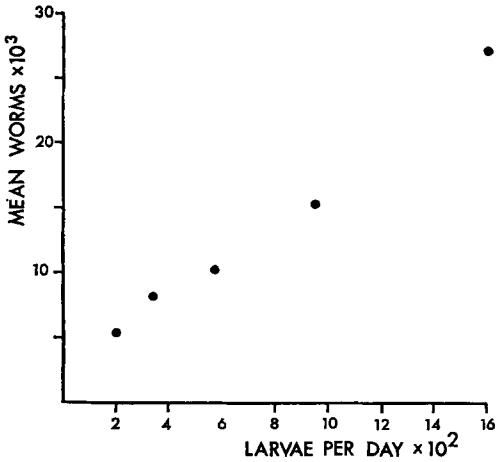


FIG. 6. The relationship between infection rate (larvae administered per day) and burdens of *Ostertagia ostertagi*.

That the worm burden is regulated by a loss and replacement of worms, means that the worms present at one point in time are not the same worms as were present a month earlier or will be present a month later. Michel (1963) deduced this from the finding that in infections of *O. ostertagi* in calves exposed to daily infection, the worms became smaller as the infection progressed. Dineen and Wagland (1966) arrived at the same conclusion regarding infections of *Nematodirus spathiger* because of changes in the ratio of male to female worms. The worm burden of an animal exposed to constant infection should be visualised as in a state of fairly rapid turnover, a circumstance that has a bearing on the use of anthelmintics, the effect of which cannot be to destroy an accumulation of worms steadily built up over a long period but merely to remove worms that have developed to maturity over a short period and will as promptly be replaced. It is a further consequence of the occurrence of a rapid turnover of worms that those present at any time have all developed recently, and their appearance gives an indication of the present state of resistance of the host. Worms that have developed in a resistant host are smaller in size and may show morphological abnormalities (Michel, 1967a; Keith, 1967).

C. EFFECT OF RESISTANCE ON FECUNDITY OF WORMS

It has been known for some time that in the resistant host the egg output of the worms is depressed (McCoy, 1931; Chandler, 1932). Stewart and Gordon (1958) demonstrated this phenomenon in infections of *T. colubriformis* in sheep, and Gibson (1952) published data concerning lambs experimentally infected with *T. axei* which, at a late stage in the infection, passed very few worm eggs while carrying large numbers of adult worms. Tetley (1935) regarded the egg output of *Nematodirus* spp. to be a good measure of the resistance of the host. Michel (1963) found that female *O. ostertagi* recovered

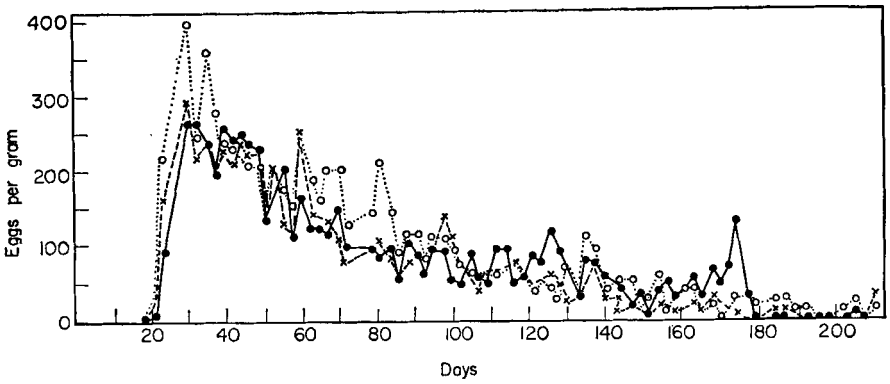


FIG. 7. Mean faecal egg counts of three groups of calves infected daily with 500 (●———●), 1000 (×– – – –×) and 1500 (○.....○) larvae of *Ostertagia ostertagi*, respectively, and carrying appropriately different worm burdens. (Reproduced with permission from Michel, 1967b.)

from calves which were infected daily, contained progressively fewer eggs as time went on. It has now been shown (Michel, 1967b) that in infections of this parasite, egg output is regulated in a very interesting way. The faecal egg count of groups of animals carrying different numbers of worms was found to be the same (see Fig. 7). This suggested that the total of eggs produced per unit of time by the entire population was limited, and that the environment of the worms could only sustain the production of a limited number of worm eggs. This limit was substantially below the potential maximum output of the females. Just how much less could be demonstrated by reducing the host's resistance by the administration of cortisone, a treatment which in one experiment increased the egg output sixfold. There is some evidence that the limitation of egg output depends on an immunological phenomenon. This rests not only on experiments with immunosupressants but also on the finding that in debilitated animals in which the other manifestations of resistance are not in evidence, the limitation of egg output also breaks down.

It will be seen from Fig. 7 not only that the peak of mean faecal egg counts reached by groups of calves carrying different numbers of worms was the same

but that the counts declined from the peak according to the same curve. The decrease was logarithmic and this meant that total egg output at one time bore a constant relation to total egg output at a second point of time preceding the first by a fixed interval. This implies that the regulation of egg output is self-contained, and indeed egg counts follow the same stereotyped pattern whatever changes, subject to a certain minimum level, occur in worm numbers.

These results obtained with *Ostertagia ostertagi* in cattle cannot be transferred without modification to other species of host and parasite. However, the same pattern, a rapid increase to a peak followed by a logarithmic decrease, occurs in a very large number of cases, among them *Haemonchus contortus*, *Bunostomum phlebotomum* and *Oesophagostomum radiatum* in calves (Mayhew, 1941, 1948, 1950), *Cooperia oncophora* in calves (Peterson, 1957), *Nematodirus* spp. in sheep (Crofton, 1955) and even the small lungworm *Cystocaulus nigrescens* in sheep (Akopyan, 1956). Decreases in faecal egg count according to this pattern are difficult to interpret. Not only are worm burdens liable to decrease in this way, and the regulation of worm egg output likely to produce the same effect, but even where the operation of host resistance can be excluded, the fecundity of single females may decline according to this pattern (Graham, 1938).

That faecal worm egg output may follow a standardised pattern has an important implication to epidemiology, for the contamination of the pasture will not be closely related to the worm burdens of the grazing animals. The view formerly held—that as worm burdens increased so also did pasture contamination—is clearly incompatible with this finding. Further, differences in the initial infestation on the pasture to which calves are first exposed will not be fully reflected in the number of eggs which, in time, they put out onto the pastures.

### D. THE INHIBITION OF DEVELOPMENT

A tendency for the development of worms to be arrested at a particular stage is seen in many species of nematodes. The precise point at which development is stopped appears to be characteristic of each species. *Trichostrongylus retortaeformis* is arrested in the late third stage (Michel, 1952b), *Ostertagia* in the early fourth stage (Threlkeld and Johnson, 1948), *Cooperia curticei* in the late fourth stage (Sommerville, 1960), and *Dictyocaulus viviparus* in the early fifth stage. In many cases the phenomenon is a consequence of the acquired resistance of the host. For example, a larger proportion of *Dictyocaulus viviparus* is inhibited in a previously infected calf than in a susceptible calf (Michel, 1955a) and the same is true of infections of *T. retortaeformis* in rabbits (Michel, 1952b). The phenomenon is more marked in hosts that have been infected on several occasions than in those infected once only. This has been shown in numerous cases, among them *Haemonchus placei* (Roberts, 1957), *Cooperia curticei* (Andrews, 1939) and *Ostertagia ostertagi* (Threlkeld and Johnson, 1948). In some cases such as *Ostertagia circumcincta* (Dunsmore, 1960), *Cooperia oncophora* (Herlich, 1965) and *Graphidium strigosum* (Martin *et al.*, 1957), a far larger proportion of a large infection acquired on a single

occasion is inhibited than of a small infection. The age of the host and its suitability can also be implicated, Gibson (1959) having shown that the development of *Nematodirus* was inhibited in old but not in young lambs, while Scott (1928) demonstrated that the proportion of *Ancylostoma caninum* which was arrested at the third stage increased along the series puppy–dog–kitten–cat.

While all this evidence suggests that the response of the host to infection is involved, the condition of the larvae may also play a part. Itagaki (1928) reported that when conditions for the free living development of *Ascaridia galli* had been unfavourable, there was some inhibition of development, but when conditions were good development proceeded without interruption. Similarly Michel and MacKenzie *et al.* (1965) found that larvae of *D. viviparus* from some cultures but not from others were inhibited in susceptible hosts, and Anderson *et al.* (1965a, b) have shown that *O. ostertagi* acquired from the pasture in autumn and winter are liable to be inhibited even in susceptible calves while those picked up from the pasture in spring and summer are not. Michel (1967a) has found that in groups of calves experimentally infected with *O. ostertagi* larvae every day at different rates, a constant proportion of the larvae administered accumulated in the host as early fourth stage larvae. This suggests that the immunity of the host was not involved. Recently Anderson *et al.* (1967) have claimed that different strains of this parasite vary in the extent to which they are liable to be inhibited.

The position is illuminated by the views of Madsen (1962), who suggested that the migratory behaviour of nematodes is variable, may be altered if large numbers of larvae are given, and depends on the condition of the larvae and the state of resistance, in its broadest sense, of the host. It is doubtful whether inhibition of development is always associated with abnormal migratory behaviour, but it seems that a particular point in the development of the parasite may be seen as an obstacle or barrier and the resistance, either innate or acquired, of the host may determine the height of that barrier while the conditions of the larvae may influence their ability to surmount it.

It was once believed that the inhibition of development was an irreversible process but it is now generally accepted that inhibited forms are capable of resuming their development. This has been demonstrated for *Trichonema* spp. (Gibson, 1953), *Trichostrongylus retortaeformis* (see Michel, 1952a), *Ostertagia ortertagi* (see Martin *et al.*, 1957) and *O. circumcincta* (see Dunsmore, 1963).

The resumption of development can be on a massive scale. Winter ostertagiasis provides an example in which large numbers of inhibited forms develop at once and produce outbreaks of disease of quite sudden onset which may occur several months after the cattle are removed from a source of infection (Martin *et al.*, 1957; Anderson *et al.*, 1965b). The so called "spring rise" in the worm burden of sheep is another example. In neither case has the cause, either of the inhibition or of its subsequent failure, been elucidated, but it is unlikely that the resumption of development results from the removal of precisely those factors which were the primary cause of the inhibition. This would be entirely consistent with the views of Madsen (1962). Normally the inhibited

forms resume their development in small numbers, replacing such worms as are lost or are removed by anthelmintic treatment. The normal turnover of adult worms may therefore take place in animals which are no longer exposed to infection, the process being supplied from a reserve of inhibited forms. There is some evidence that populations of inhibited forms decline at a constant rate (Scott, 1928; Michel, 1952b). If all the decrease in number were attributable to development, a constant rate of population decrease would mean that the numbers resuming their development per unit time depended directly on the number present. Michel (1963) sought to interpret his results with *O. ostertagi* on this basis, but subsequent work has shown that, in *O. ostertagi* infections at least, this theory is not tenable.

A number of workers have shown that the removal of adult worms stimulates the development of inhibited form. The most clear-cut result is that of Dunsmore (1963), who by means of anthelmintic treatment removed some of the adult worms from lambs carrying both adult and inhibited forms and found that the number of adults increased while the number of inhibited forms decreased. What may have been a similar phenomenon was reported by Gibson (1953), who treated horses periodically with the anthelmintic phenothiazine while withholding them from further infection. Treatment was followed by the appearance of adult *Trichonema* in the faeces and a sharp decline in faecal egg count. After a time, however, the faecal egg count rose again. This sequence of events was repeated every time anthelmintic treatment was given. As the horses were maintained so as to prevent accidental infection, it was concluded that worms removed by phenothiazine were replaced by the development of formerly inhibited forms. Gibson suggested that it was the removal of adults which stimulated inhibited forms to develop. This explanation may be correct, but if there were a constant turnover of worms, the observed phenomena would still follow anthelmintic treatment even if the development of formerly inhibited worms were not affected by the presence of adult worms.

There is, however, other evidence of a connection between the presence of adult worms and the inhibition of development. Roberts (1957, *in litt.*) observed an effect of removing adult *H. placei* from calves by anthelmintic treatment on the development of arrested fourth stage larvae, and Michel (1963) observed that while adult worms were regularly removed from calves infected daily with *O. ostertagi* by means of an anthelmintic that affected adults only, no inhibited forms accumulated. As soon as anthelmintic treatment stopped, however, the number of inhibited forms present began to increase sharply. Removal of adult *Ostertagia* from calves already carrying large burdens of inhibited forms has not been found to produce a striking effect but the regular removal of adults for a period tends to increase the rate at which the burden of inhibited forms is depleted. There is also some evidence that in infections of *T. retortaeformis,* inhibited forms develop batch-wise, each batch growing to maturity and being removed by self-cure to be followed by the next.

Consideration of a large series of worm counts of calves naturally infected with *O. ostertagi* has shown that there is a close correlation between the number of adult worms, and of developing worms in the late fourth stage,

confirming that a turnover of worms was proceeding, and that the number of adults was determined by the numbers developing. However, there was not so good a correlation between the number of inhibited forms and the number of late fourth stage larvae, indicating that the inhibited forms did not resume their development at a rate related to the number present. Within any group of calves of similar history, burdens of adult worms were closely similar, but the numbers of inhibited forms varied widely. These findings may suggest that development of some inhibited forms is permitted as soon as the burden of adult worms falls below a critical level, a strange but conceivable mechanism whereby the number of inhibited forms which resume their development determines and is partly regulated by the number of adult worms.

It is clear, then, that the development of worms may be arrested and that in consequence large numbers of immature forms may accumulate. These inhibited forms are generally unaffected by immune mechanisms resulting in the spontaneous elimination of adult worms, and they are singularly resistant to anthelmintics. The inhibited forms can resume their development and normally do so in modest numbers to replace worms removed by anthelmintic treatment or spontaneously lost, thereby maintaining a turnover of worms even in animals which no longer have access to infection.

## III. The Free Living Stages

The pasture may be regarded as a vehicle for the transmission of worm infection from one animal to another and studies of the bionomics of the free living stages are only relevant to epidemiology in so far as they illuminate the process of transmission. This is complex. It consists of development to the infective stage, migration of the larvae out of the faeces and to a position on the herbage where they will be available to the grazing animal, and their survival for sufficiently long to ensure successful contact with a susceptible host.

A complex process such as this may be studied at a variety of levels. At one extreme it may be broken down to its ultimate components and the effect of single factors on the organism isolated. To deduce from even a very detailed knowledge of the components how populations of larvae on the pasture will fluctuate demands an understanding of the interactions and relative importance of those components. These are difficult to assess and are often incorrectly estimated. At the other extreme is a more empirical approach according to which the process is studied as a whole and the relationship between certain inputs and outputs worked out without a detailed understanding of the mechanisms involved.

Many workers have favoured a fundamental or intermediate approach and it is difficult to relate their results to epidemiological problems. Further, much research work has been planned on the mistaken assumption that it was relevant to the design of control measures. Thus undue attention was paid to extremes and not enough to the mode. Minimum times for hatching and development and maximum times for survival may, in practice, prove unimportant.

### A. DEVELOPMENT

The nematodes dealt with in this review do not have intermediate hosts. They are passed in the faeces of the host as eggs or, in the case of *Dictyocaulus viviparus*, as first stage larvae. These develop to an infective third stage. *Nematodirus* spp. develop to this infective third stage within the egg shell but other trichostrongylids hatch as first stage larvae, which feed on coliform bacteria in the faeces, moult to form a second stage larva with a similar way of life and finally moult again to give a third stage larva. The second moult is incomplete in that the third cuticle is formed within the second which is retained. *Dictyocaulus viviparus* undergoes the same development without feeding. It tends to retain both the first and the second cuticle. That the infective larva has more than one cuticle has aroused considerable interest. It is widely assumed that this provides protection for what is a resting stage, and in the case of trichostrongylid larvae this is probably true. Some workers attach great importance to the process of completing the second ecdysis and finally casting the outer cuticle. They see it as a central act in the change from a free living to a parasitic existence (Rogers, 1962) and suggest that differences between species in the stimulus which elicits exsheathment may provide a basis for host specificity (Whitlock, 1966).

Temperature and moisture are important limiting factors to hatching and development. Both have been studied in some detail but mainly with regard to *Haemonchus contortus*. Discussion of the effects of temperature has been dominated by the minimum temperature at which development could occur. Ransom (1906) evaluated this critical temperature as 4·4–7·8 °C, but Dinaburg (1944) maintained that no development occurs at temperatures below 18 °C. This temperature, which was considerably higher than that determined by other workers, was not quickly rejected because it was used by Gordon (1948) with some success in predicting the geographical and seasonal incidence of outbreaks. That the critical temperature for development should have been confused with Gordon's arbitrary limit for the occurrence of outbreaks illustrates the common failure to distinguish between the minimum and the mode. Silverman and Campbell (1958) have finally established that development can occur at a temperature of 7·2 °C. Crofton (1965) has worked out the effect of temperature on the minimum time taken for eggs of a number of species to hatch and has shown that the lowest temperature at which any hatching occurs is 4 °C for *Ostertagia circumcincta*, 6 °C for *Chabertia ovina*, 8–9 °C for *Trichostrongylus axei* and *T. vitrinus*, 9 °C for *Haemonchus contortus*, 15 °C for *Bunostomum trigonocephalum* and 16 °C for *Cooperia curticei* and *C. oncophora*. These figures should not, however, be regarded as fixed or absolute values. They will be influenced by other factors such as moisture and the availability of oxygen. Moreover, Crofton *et al.* (1965) have shown that different strains of the same species may have different critical temperatures for development. Thus, the lowest temperature at which *Haemonchus* eggs from New York and Kentucky would hatch was 4–5 °C higher than that required by *Haemonchus* eggs from Bristol. Similar differences were shown by eggs of *O. circumcincta* from these sources (Crofton and Whitlock, 1965).

The lowest temperature at which development is possible will be highly relevant to the epidemiology of disease only in so far as it gives a general indication of the reactions of a nematode to temperature. Ciordia and Bizzell (1963) have studied the relationship between temperature and the time taken by *Cooperia punctata, C. oncophora, Ostertagia ostertagi, Trichostrongylus axei* and *T. colubriformis* to develop to the infective stage. Their work has been criticised by Crofton (1963) as necessarily imprecise on the grounds that they found no significant difference between these species, but the results, some of which are summarised in Fig. 8, are nonetheless of interest.

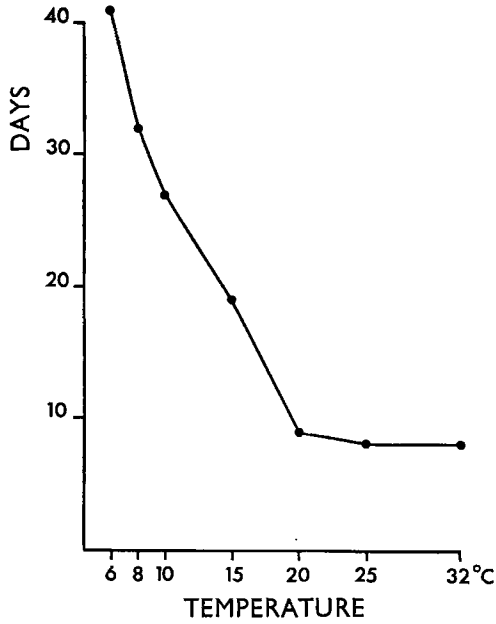


FIG. 8. The effect of temperature on the time taken by trichostrongylid nematodes to develop to the infective stage. (Drawn from data presented by Ciordia and Bizzell, 1963.)

A distinction must be drawn between the minimum temperature at which development is possible, the temperature at which it proceeds at the greatest rate, and the optimum temperature. Temperature exercises an effect not only on the rate of development but also on the proportion of the larvae which successfully reach the infective stage (Fig. 9). Clearly, it is possible to determine an optimum temperature at which the greatest production of infective larvae occurs. Critical work shows that such optimal temperatures for different species are not the same, a point illustrated in Fig. 10 by curves for *Ostertagia circumcincta, Haemonchus contortus* and *Cooperia curticei* (Crofton, 1963).

There is considerable variation in the rate at which different eggs in the same culture develop and intermittent states of active and inhibited growth occur. The use of minimum times is therefore misleading and Silverman and Campbell

(1958) considered not only the minimum time for each step in development but also the median. The difference is shown in Fig. 11, which relates to development of *H. contortus* eggs to the pre-hatch stage. Silverman and Campbell concluded that lack of oxygen inhibited development and presumably for this reason eggs failed to develop in faeces saturated with water. Desiccation was fatal to the pre-infective stages unless at the "embryonated egg stage", an observation that had also been made by Veglia (1915) and Furman (1944a). If the faeces dried out so rapidly that the eggs could not develop to this stage, all perished. However, if the embryonated egg stage was

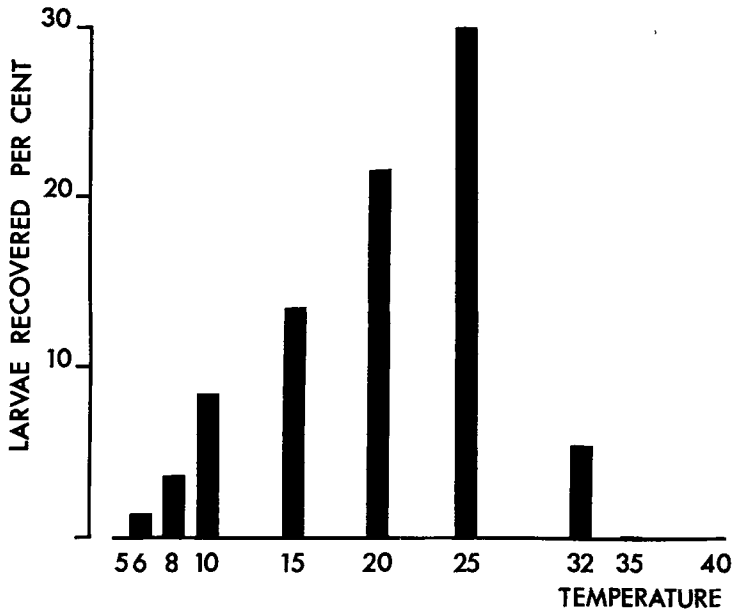


FIG. 9. The percentage of larvae developing to the infective stage at different temperatures. (Drawn from data presented by Ciordia and Bizzell, 1963.)

reached before the faeces dried, and there was a tendency for development to be arrested at that stage, they could survive for some months and hatched very quickly when the faeces were moistened.

Interactions between temperature and moisture have also been reported. Thus Belle (1959) found that the development of eggs of *Bunostomum trigonocephalum* could proceed at a lower relative humidity at low temperatures than at high.

Since the faeces are by no means a homogeneous medium as regards oxygen tension and humidity, it is clear that the range of variation in the time taken to reach the infective stage will be very great. It is probable that in bovine faeces this phenomenon will be even more marked than in the dung of sheep which occurs in rather smaller aggregates although, as Gordon (1967) has pointed out, the faeces of sheep on improved pastures tend to occur as pats

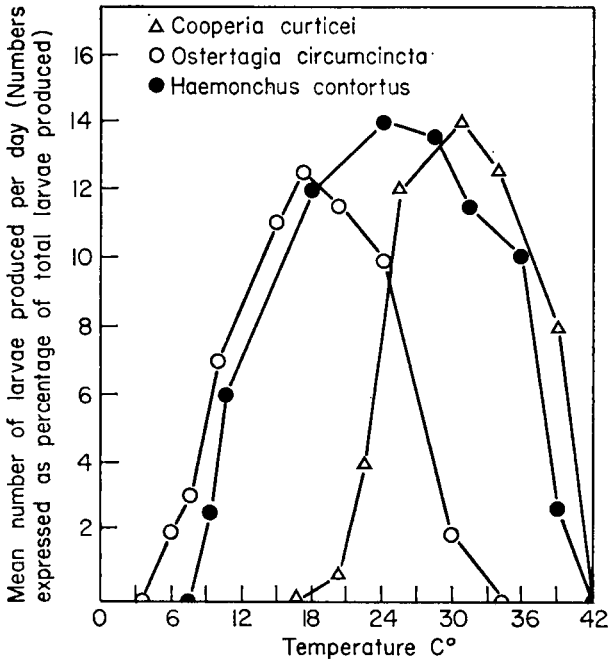


FIG. 10. Showing that the optimum temperatures for the production of infective larvae of *Haemonchus contortus*, *Ostertagia circumcincta* and *Cooperia curticei* are different. (Reproduced with permission from Crofton, 1963.)

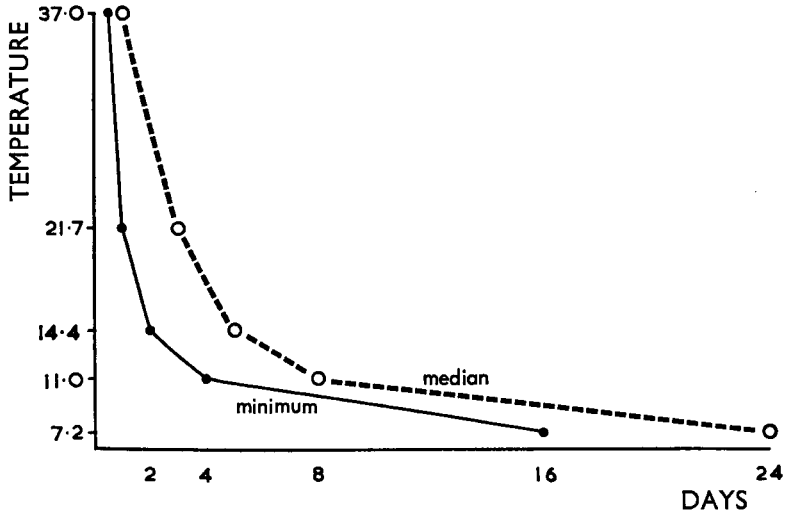


FIG. 11. The time taken by eggs of *Haemonchus contortus* to develop to the pre-hatch stage at different temperatures, showing the difference between the minimum time and the median. (Drawn from data presented by Silverman and Campbell, 1958.)

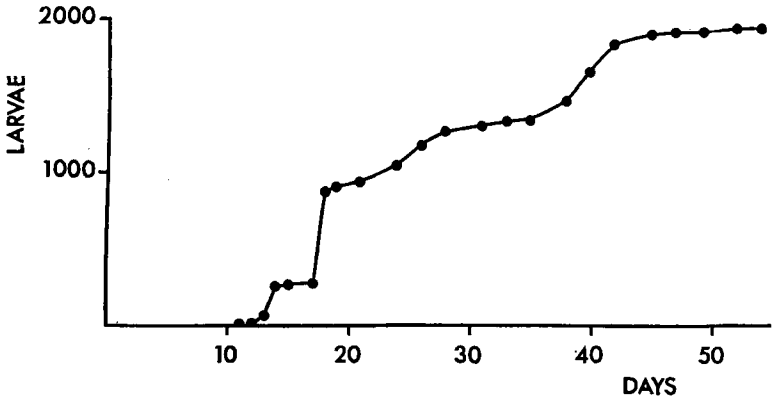


FIG. 12. The cumulative total of larvae of *H. contortus* migrating from a culture of 11 faecal pellets maintained at 11 °C. (Drawn from data presented by Silverman and Campbell, 1958.)

rather than pellets. Silverman and Campbell (1958) have stressed that there will be considerable variation in the time taken to reach the infective stage so that larvae will emerge from the faeces over a long period of time; Fig. 12 shows the cumulative total of infective larvae migrating from a small number of faecal pellets maintained in constant conditions.

### B. MIGRATION OF INFECTIVE LARVAE

Before the infective larva is likely to be ingested by the host, it must leave the faeces and migrate onto the herbage. It is not surprising, therefore, that it was once believed that the larvae perform this migration in a purposeful manner and that the vertical migration up blades of grass was in the nature of a negative geotaxis. Crofton (1954a) has, however, demonstrated that the movements of the larvae could adequately be interpreted as a "random walk", the extent of their movement being limited because it demanded a continuous film of moisture on the herbage. In consequence the larvae were restricted to the lower portions of the herbage unless this was tall and dense, in which case the film of moisture extended correspondingly higher. Activity of the larvae is greater at high temperature than at low (Sturrock, 1965) and, as shown by Rogers (1940), was also affected by light intensity, being greatest when the illumination was rather dull. Both Rogers (1940) and Rees (1950) in experimental systems succeeded, in some measure, in showing that the number of larvae on the herbage was higher in the morning and in the evening than at other times of day. It is now clear that while the larvae may wander fairly actively when young, they soon cease to do so. In consequence there is little or no diurnal fluctuation in the number of larvae on the herbage. Indeed, Crofton (1949) has shown that, assessed per unit weight of herbage, the concentrations of larvae may be greatest in the heat of the day (see Fig. 13). Most workers now recognise that the vertical migrations of the larvae are not of great significance and it is difficult to justify Gordon's (1948) attempt to link the geographical incidence of haemonchiasis and trichostrongyliasis with

Rogers' (1940) finding that the one shows its maximum migratory activity at a higher temperature than the other. The concept of the undecayed fibrous layer at the base of the turf, the so called "mat", acting as a reservoir of larvae whose vertical migration makes them available to the grazing animal, cannot be regarded as useful. (The mat which occurs only in old pastures or acid soils is in any case becoming agriculturally irrelevant.)

A more important process is that which results in the movement of larvae out of the faeces. The time taken for development may be very variable but, especially in dry weather, the emergence of the larvae from the dung may

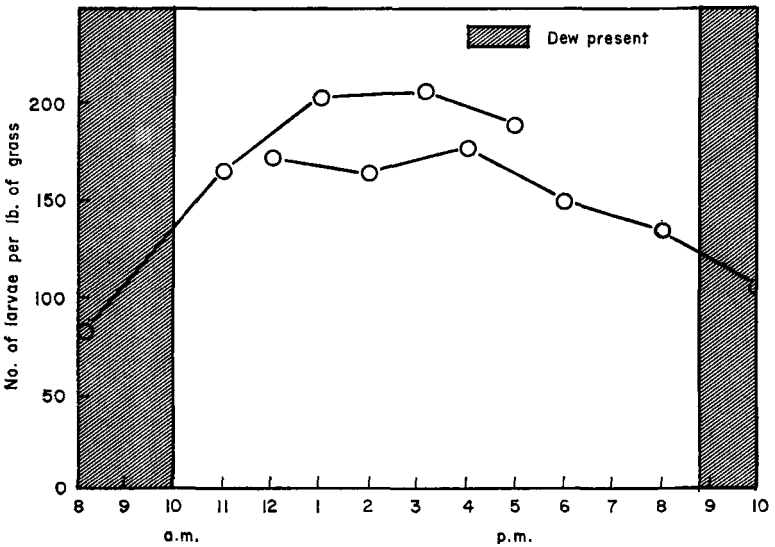


FIG. 13. The number of trichostrongylid larvae per unit weight of pasture herbage at different times of day. (Reproduced with permission from Crofton, 1949.)

continue for a very protracted period. (Figure 14 shows the migration of larvae from a pat of bovine faeces put out on a grass sward at the end of April.) Although all the eggs had developed to infective larvae by the middle of May, they continued to pass from the faeces to the herbage until November.

Trichostrongylid larvae move only a very small distance from the faeces (Furman, 1944b; Dinaburg, 1944; Rose, 1961, 1963a; Sturrock, 1965) and accordingly the part played by the disintegration of faeces has been studied. Christie (1963) pointed out that the disintegration of sheep faeces can take 6–106 days and that even cattle faeces can disappear quickly, as Rose (1960) affirmed. The weather during the first week or so has a marked effect on the persistence of dung pats. They can cease to be recognisable within 4 weeks, but equally they can persist for nearly a year. The faeces, and especially bovine faeces, may act as a reservoir of infective larvae and Rose (1961, 1963b) has shown that larvae of *O. ostertagi* and *C. oncophora* can be recovered from faecal pats for almost as long as these persist. It is probably useful to think of

the faeces as a reservoir of infection from which they are released largely by weathering and only partly by their own activity.

The transference of larvae from the faeces to the herbage by mechanical agencies was considered by Michel and Rose (1954) and Rose and Michel (1957) in connection with *Dictyocaulus viviparus*, the infective larvae of which are extremely inactive. It was argued that if the larvae did not leave the faeces by their own activity, infestations of larvae on the herbage could only arise when the faeces themselves were disseminated over the grass. It was shown

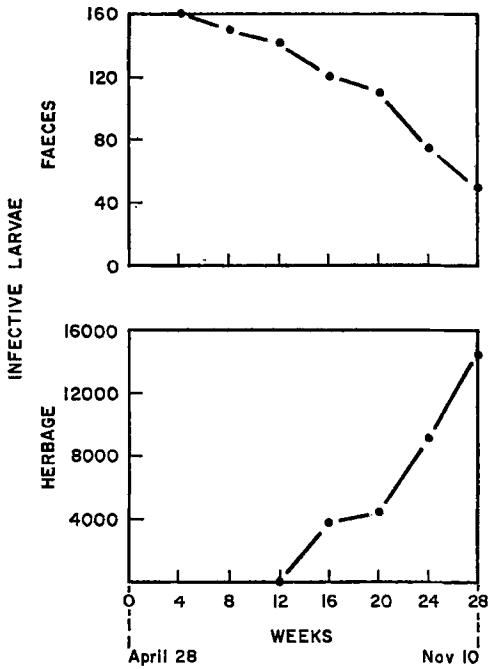


FIG. 14. The migration of larvae of *Ostertagia ostertagi* out of the faeces and onto the herbage. (Reproduced with permission from Rose, 1962.)

that the extent to which the herbage could be contaminated varied with the consistency of the faeces and the effect of the weather in causing spread faeces to disintegrate. Subsequently Robinson (1962) suggested an alternative mechanism. He observed that *Dictyocaulus* larvae climb actively up the sporangiophores of fungi of the genus *Pilobolus* which grow in faeces and shoot their sporangia to considerable distances. The importance of this mechanism in transferring lungworm larvae from faeces to herbage has not been assessed. An observation of a similar kind is that of Jacobs *et al.* (1968), that flies of the genus *Psychoda* which develop in dung may carry larvae of *Oesophagostomum* and *Ostertagia* which have been found clinging to them.

The part played by the activity of the larvae in getting onto the herbage and

thus becoming available to the grazing animal can be over-estimated. It appears that processes in which the larvae are passive may be at least of equal importance.

### C. GRAZING BEHAVIOUR

The transference of larvae from the faeces to the herbage is of obvious relevance to the infection process because the grazing animal eats grass and avoids ingesting faeces. The extent of this coprophobia varies. Cattle actively avoid bovine faeces with such effect that, as shown by Michel (1955b), they pick up a sample of herbage containing only one third as many lungworm larvae as are present in a random sample cut by a human observer. Whether this should be seen as one other adaptation in the process of adjustment between host and parasite is arguable. Crofton (1963) objects to the suggestion that its grazing behaviour may help an animal to avoid infection because he considers that "the evolution of the host–parasite relationship has depended on the increased chances of infection occurring". Support for this view is his finding that flocks of sheep appear to avoid areas of the pasture where high concentrations of fresh faeces occur and to favour areas which were grazed (and contaminated) 5 to 10 days previously (Crofton, 1958a). His suggestion that this may be seen either as an avoidance of fresh faeces or as an attraction to areas where ageing faeces are present and that this has some connection with the fact that a 5–10 day period is also required for most trichostrongylid worms to develop to the infective stage, is probably more complicated than necessary. The grazing pattern of different breeds of sheep is not the same and while some (e.g. Kent or Romney Marsh sheep) tend to spread out over the entire pasture, others (particularly of the Down breeds) do graze in fairly close formation. It is inevitable that these will not return to grazed areas until a fresh growth of herbage is present. It is true that, as pointed out by Taylor (1954), sheep do not avoid grazing close to faecal pellets, but whether their 5–10 day rotation round the pasture increases their uptake of larvae is open to question because few larvae leave the faeces within this period.

The tendency of cattle to avoid grazing near bovine faeces cannot fail to have some effect on their uptake of infection, the more so since the larvae move only a very short distance from the faeces. Observations by Michel, Ollerenshaw and Rose (1956, unpublished), however, indicate that only relatively large aggregates of faeces are avoided and if the faeces are thinly spread over the herbage, either by mechanical means or when diarrhoeic, the contaminated herbage is grazed after a short time.

Doubts are occasionally expressed about the use of assessments of the concentration of larvae in random samples of pasture herbage as an indication of the rate of infection to which grazing animals are subject. The use of such estimates, which at best give relative rather than absolute values, depends on the assumption that there is a fairly constant relationship between the concentration of larvae in the herbage ingested by the grazing animal and in the samples collected for examination. No systematic study has been made but experience seems to vindicate the use of herbage sampling (Michel and Parfitt, 1956; Michel, 1968b).

### D. SURVIVAL OF INFECTIVE LARVAE

Some time may elapse before infective larvae make successful contact with a host and are enabled to resume a parasitic existence. They subsist on their food reserves and may be regarded as in a resting stage. Infective larvae of trichostrongylids are more resistant than the other free living stages to desiccation (Furman, 1944) and extremes of temperature. Infective larvae of *Dictyocaulus viviparus*, on the other hand, are not more resistant than first and second stage larvae although they do seem to survive sub-zero temperatures a little better (Rose, 1956).

Considerable effort has been devoted to the study of the longevity of infective larvae though not always with due regard to the form of the population curve. Taylor (1938a) considered three possible curves (Fig. 15). The first

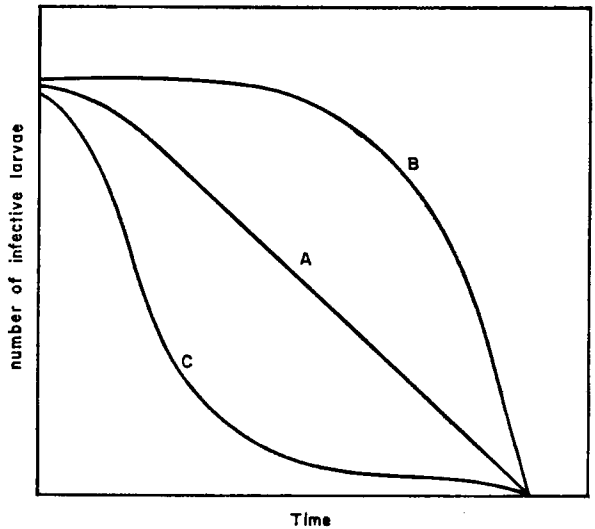


FIG. 15. A and B "showing two alternative theoretical rates of the disappearance of living larvae from a pasture, based on an even death rate among the larvae in the one instance and on even longevity in the other". C "showing the actual rate of disappearance". (Reproduced with permission from Taylor, 1938a.)

implies that a fixed number of larvae died each day and the second that the survival of the larvae depended chiefly on their natural life span. The third curve suggests that the population decreased at a constant rate, the chance that any given individual would perish on any particular day remaining the same. Although the second of these possibilities might not be inappropriate to an organism exhausting limited reserves, Taylor's observations led him to the view that the third pattern was applicable to trichostrongylid larvae. Crofton (1948) agreed that this might be so in hot dry weather but observed that in cool moist conditions the decline in numbers appeared to be approximately linear.

On theoretical grounds this seems improbable but during a period of months both environmental conditions and the inherent mortality rate of the larvae may change.

Studies on the survival of *Dictyocaulus viviparus* larvae indicate that the decrease is approximately logarithmic. Figure 16 shows the decline of a population of larvae maintained in constant conditions. The rate of decrease is influenced by temperature and by other climatic factors, but over a limited period of time populations in the field also decrease according to a curve of similar form. It could not be expected that a population of larvae on the pasture behaves as though homogenous. Some larvae will be in the faeces,

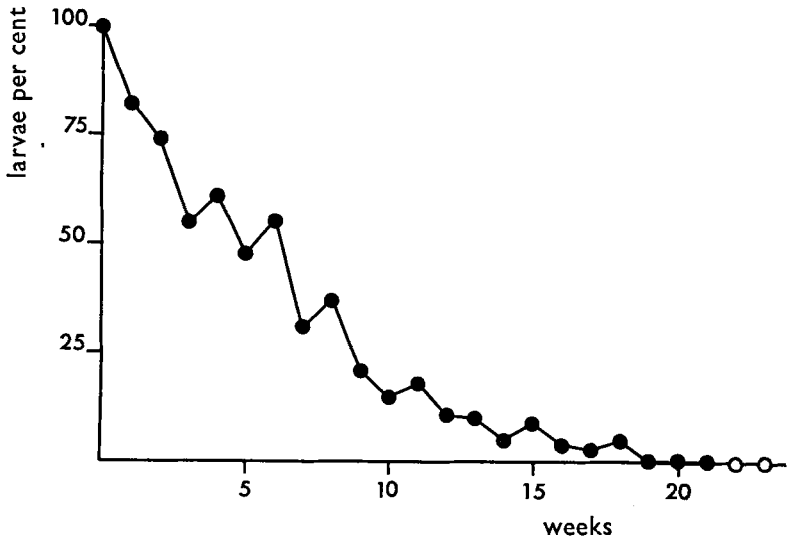


FIG. 16. The decrease of a population of *Dictyocaulus viviparus* in moist faeces maintained at 3–6°C. (Drawn from data presented by Rose, 1956.)

some on the herbage and some more protected from adverse factors than others. Indeed, Michel and Spedding (1955, unpublished) found that a proportion of the population appears to decrease at a slower rate and that as this proportion increases with the passage of time, the rate of decrease of the whole population decreases (see Fig. 17). The natural life span of the larvae does not appear to play a significant part in the decrease of populations. Against this background it is clearly not possible to define maximum longevity unless the initial population and the criteria of presence or absence are specified. Nonetheless, a number of workers have attempted to determine whether or not larvae will survive on pastures through the winter. Of such studies perhaps the best is that of Kates (1950), who infected pastures at Beltsville in May, June and July, and in August, September and October respectively, and periodically tested their infectivity by means of pairs of worm free lambs. He demonstrated that numbers on the pasture were well maintained through the grazing

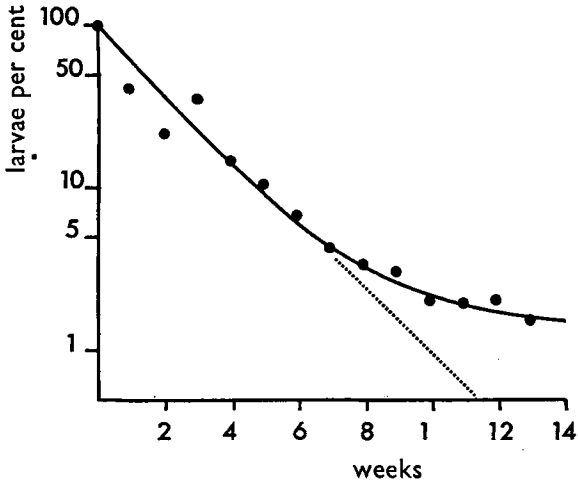


FIG. 17. The course of a population of *Dictyocaulus viviparus* larvae on pasture during the winter. (Drawn from mean values of several series of unpublished observations by Michel and Spedding, 1955.)

season and that small numbers of *Haemonchus*, *Ostertagia*, *Trichostrongylus* and *Cooperia* would survive until the following May if the pasture had been contaminated in August or later. On pastures contaminated in June or July, however, no larvae of these genera were detected in the following spring. A similar investigation by Hawkins *et al.* (1944) in Michigan, in which a pasture was contaminated by ewes and lambs until September and its infectivity tested

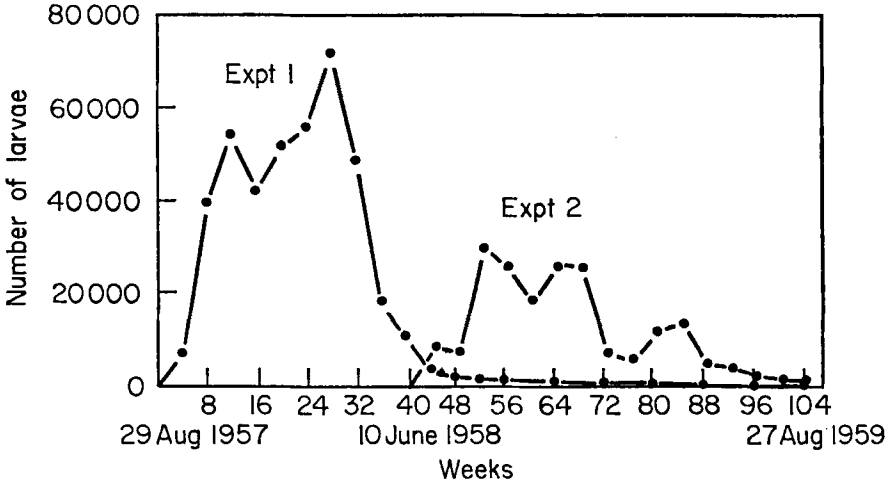


FIG. 18. The number of infective larvae of *Ostertagia ostertagi* on the herbage surrounding two pats of infected faeces exposed in August 1957 and June 1958 respectively. (Reproduced with permission from Rose, 1961.)

with susceptible lambs every month until January, suggested that *Ostertagia, Cooperia* and *Trichostrongylus* survived rather better than *Haemonchus*. An excellent review of the literature is that of Kates (1950). In Britain, Rose (1965) has shown by similar means that a pasture contaminated to the end of October could still transmit *Haemonchus, Ostertagia* and *Trichostrongylus* to lambs grazing it from the following April. This confirmed the findings of Crofton (1952), Large and Spedding (1964) and others. Clearly, most if not all trichostrongyles will survive on the pasture throughout the winter. The residual infestation in the spring, however, is likely to be very small.

In the field, a population of larvae on herbage resulting from the deposition of infected faeces on a single day does not decrease from an early peak as visualised by Taylor (1938a), who like Crofton (1948) had studied infective larvae grown in standard conditions and then pipetted onto experimental plots of grass. As has been shown above, there is a wide variation, between the quickest and the slowest individual, in the time taken for development to the infective stage and migration onto the herbage. The appearance of the larvae on the grass is spread over a long period. Figure 18 shows the number of *O. ostertagi* larvae recovered from the grass surrounding two pats of cattle faeces deposited on 29 August and 10 June respectively. The number of larvae on the herbage increased gradually and for a time the new arrival of larvae and their disappearance seem to have been in balance. It is probable that the mortality rate was very much less in the winter so that a decrease of the expected form did not occur until the spring.

### E. CLIMATE AND THE FREE LIVING STAGES

Both the creation of herbage infestations and their course are influenced by the climate. Donald (1968) observed that climatic factors exert a greater effect on the pre-infective stages than on infective larvae. In consequence it may be impossible for a new infestation of larvae to arise on the pasture although an existing infestation survives well. As observed by Shorb (1943), eggs of *Haemonchus contortus* deposited in winter will not reach the infective stage because at low temperatures their development is greatly retarded and they remain for a long time at a stage susceptible to freezing and other adverse factors. Similarly, in very dry summer conditions contamination of bare pastures will result in only very small infestations on the herbage because the pre-infective stages perish. Shorb (1944) argued that in much of the U.S.A. the winters were too cold and the summer too dry for the transmission of *Haemonchus contortus* although temperatures were sufficiently high from May until September. Working in California with *Ostertagia circumcincta*, Furman (1944) found that herbage infestations did not result from eggs dropped in the summer. Existing infestations, however, survived through the dry months. Temperatures appeared to be adequate throughout the year and on irrigated pasture the transmission of infection was possible at any season.

The idea that temperature and moisture are the chief factors limiting transmission and that the optimum temperatures for the free living development of different species are not the same, led to the use of his so called bioclimatographs by Gordon (1948). By assessing meteorological data against arbitrarily

chosen limits of temperature and rainfall, the probable geographical incidence of a number of species could be worked out. *Haemonchus* and *Oesophagostomum* appear to demand higher temperature than *Ostertagia* and *Trichostrongylus* so that in Australia, haemonchiasis is recognised as occurring in areas of summer rainfall and trichostrongyliasis in areas where the winters are wet. Levine (1963) prefers soil moisture data to rainfall and from this and temperature he calculated periods when development and migration are possible. His term "potential transmission period" may be unfortunate. Larvae which have developed within these periods will persist so that the process of transmission may be completed outside the defined period. Indeed, the larvae may not be released from the faeces and become available until after the end of the period. Further, laying down dates between which development and migration are possible may obscure the important fact that the creation of herbage infestations is not equally effective and does not proceed at the same speed throughout the defined period.

In Britain it is temperature rather than moisture that limits the season during which herbage infestations can be created. Temperatures rise gradually in the spring and therefore trichostrongyle eggs dropped early in the season take much longer before they appear on the herbage as infective larvae than do eggs dropped later. Consequently, eggs dropped over a long period in spring and early summer become available over a rather shorter period. In addition, the optimum temperature for development is reached some time after the minimum and therefore a smaller proportion of eggs dropped early survive to reach the herbage as infective larvae than of those dropped later. Contamination of the pasture during spring and early summer tends to lead to a sharp increase in the herbage infestation in June or July. In later summer and autumn, conditions become increasingly unfavourable for the translation process and the great majority of eggs dropped after September fail to reach the infective stage. The season during which it is possible for herbage infestations to be created extends in England to a little over 5 months but the process can run effectively and rapidly for only half that period.

The free living stages of *Dictyocaulus viviparus* differ from those of trichostrongylid worms in a number of respects. So also do those of *Nematodirus* spp. They will be discussed in later sections.

## IV. Parasitic Gastro-enteritis in Cattle

In Britain, *Ostertagia ostertagi* and to a lesser extent *Cooperia oncophora* are regarded as the most common cause of parasitic gastro-enteritis in cattle. In the course of a survey of post mortem material Rose (1968) has found that *O. ostertagi* occurred more commonly than other species.

In an earlier section some phenomena of host resistance to this species were discussed. Resistance to the establishment of worms develops very slowly and demands an extended experience of infection. In practice this means that calves do not become refractory to reinfection for nearly the whole of their first grazing season. The worm burden, as has been shown, is regulated by a loss of worms related to the number present with the effect that the number of

adult worms depends on the rate at which larvae are ingested, i.e. on the herbage infestation at the time. The length of time for which a calf is exposed to that rate of infection is not of great importance; worm burdens are not built up by the gradual accumulation of worms over a long period. That there is a loss and replacement of worms means that those present at any moment have all developed recently. Their state of development derives from and is indicative of the resistance of the host. Faecal egg output is regulated by a self-contained mechanism which has the effect of limiting the number of eggs produced by the whole population of worms to the same level, irrespective of how many worms are present.

These considerations argue forcibly against the occurrence of a protracted build up of worm populations of the kind formerly visualised. It was thought that as the worm burden increased, the egg output and resulting contamination of the environment would increase likewise. But if it were assumed, as formerly it was, that free living development is rapid and conditions uniformly favourable during a long season, then it would be expected that the infestation on the herbage would reflect the pattern of pasture contamination. As it is known that the worm burdens of calves depend directly on the herbage infestation on the pasture, and that faecal egg counts, and hence the contamination of the pasture, tend to conform to a stereotyped pattern, it follows that worm burdens should also conform to this pattern, rising to an early peak in June and then declining. That they do not follow this pattern is not the only consideration that casts doubt on the assumptions of earlier writers. It is becoming evident from the work of Silverman and Campbell (1958), of Rose (1961, 1962, 1963b), of Donald (1967, 1968) and of Kutzer (1967) that free living development and particularly the migration of the majority of larvae takes far longer than the theoretical minimum and that the season during which conditions favour these processes is not long.

Observations by Michel (1969) on infestations of larvae of *Ostertagia* and *Cooperia* on experimental paddocks grazed by calves showed that these followed a regular seasonal pattern. They fell to low levels in spring so that by the end of April (or exceptionally early May) when new calves were turned out, these acquired only very modest infections. Although the calves began to pass eggs in the second half of May it was not until July or even August that the herbage infestation rose again. This it did rather steeply to a level which was maintained until the following spring (see Fig. 19).

This pattern of herbage infestations was tentatively explained by the suggestion that development and migration of eggs dropped in the spring was slow but that the process became quicker as the season advanced and reached a minimum by the end of June. In consequence the eggs reaching the pasture over a long period would appear on the herbage as infective larvae during a much shorter period. Field observations in which the time interval between the first contamination of pastures and the appearance of larvae on the herbage was studied confirmed this hypothesis. A specimen of such data is shown in Fig. 20. Rose and Wassall (1968, personal communication) have studied the fate of eggs and larvae developing in faeces dropped on experimental pastures by infected calves and confirm this view, but stress that other factors also

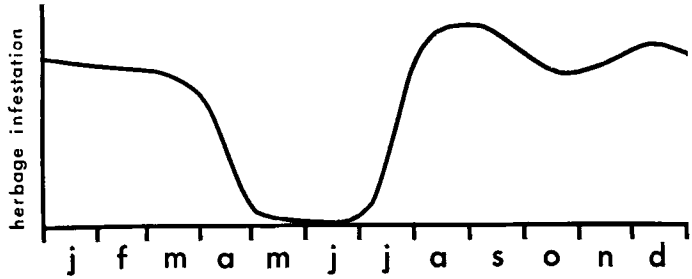


FIG. 19. Typical pattern of the seasonal changes in the infestation of infective larvae of *Ostertagia ostertagi* and *Cooperia oncophora* on the herbage of a pasture grazed by calves. (Reproduced with permission from Michel, 1968b.)

operate, namely that a smaller proportion of the eggs dropped early succeed in completing their development. It may also be relevant that if calves are turned out in late April their egg output does not reach a peak until June, but observations by Michel and Lancaster (1967, unpublished) suggest that even if pastures are heavily contaminated in April, the resulting rise in the herbage infestation does not take place appreciably earlier than if contamination had occurred in June.

From observations conducted over a number of years, Michel (1969) concluded that the level that herbage infestations reached, was influenced more by climatic factors than by the weight of pasture contamination from which they were derived. It was also noticed that some variations in the pattern of the herbage infestation occurred and the same pattern was evident on different pastures in the same year. For example very dry conditions in summer and autumn tended to delay the appearance of large numbers of larvae so that

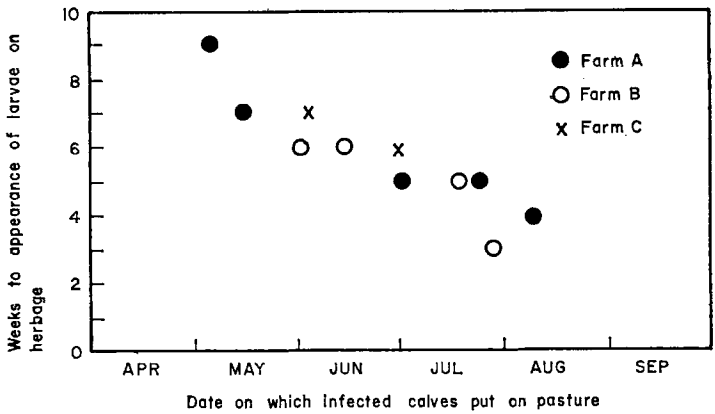


FIG. 20. The time taken for contamination of the pasture by cattle on different dates to result in the appearance of infective trichostrongylid larvae on the herbage. (Data from field observations in Devonshire; reproduced with permission from Michel, 1967d.)

although some increase occurred in July, the level remained fairly low and there was a much greater increase in the autumn. Presumably this was due to the accumulation of larvae unable, during dry conditions, to migrate out of the faeces.

After the herbage infestation has risen in July or August it commonly remains at a high level until the following spring. Michel (1967d) has shown that this is so whether or not the pasture is contaminated after the middle of July, and Rose and Wassall found that after the middle of the season the proportion of eggs which successfully complete their development and migrate onto the herbage decreases. Hardly any eggs deposited after the middle of September complete the process.

It appears that a large proportion of the larvae which persist throughout the winter on the pasture are derived from eggs deposited before the middle of July. To this extent *Ostertagia* and *Cooperia* may be regarded as annuals. Larvae which have overwintered on the pasture are picked up in the spring and it is chiefly their progeny which pass through the following winter on the pasture to take up a parasitic existence in their turn, in the following spring. However, the new generation of worms appears on the herbage in July and it is this, when ingested by the calves, which gives rise to the large infections which are the cause of disease. Commonly, therefore, parasitic gastro-enteritis is the consequences of auto-infestation involving one generation of the parasites. A second generation does occur and a third is just possible but these are not normally of any great importance. Indeed, a large proportion of larvae of the second generation frequently do not appear on the herbage until November or December. An attempt to analyse the generations of *O. ostertagi* in cattle is made in Fig. 21. On a horizontal time scale three winters and two grazing seasons are shown. Autumn born calves of two seasons are depicted as two rectangles *X* and *Y*. The residual infestation on the pasture at the beginning of the first grazing season is regarded as generation **a**. It is picked up by the calf *X* and grows to maturity. Its progeny, generation **b**, appears on the pasture in July and the calf *X* becomes reinfected. This is the potentially disease-producing generation. The eggs produced by these worms may appear on the herbage as larvae in September, generation **c**. They are not of importance in causing or contributing to autumn outbreaks of ostertagiasis but it is possible that they give rise to burdens of inhibited larvae in which form the infection may overwinter in the animal. At the beginning of the second grazing season the residual infestation on the pasture consists partly of generation **b** and partly of **c**. They are picked up by the calf *Y*. Therefore the larvae which appear on the herbage in July of the 2nd year are partly of generation **c** and partly of **d**. Meanwhile the eggs passed by the previous year's calf, *X*, are also of generation **d**. The potentially disease-producing infection contracted by calf *Y* in late summer represents a mixture of generations **c** and **d**.

As the disease-producing generation of larvae appears on the herbage at about the same time each year, control measures can readily be devised. Those now advocated (Michel, 1967c) consist of moving the calves in the middle of July from pastures which they have contaminated, to ground which has not been grazed since the winter, i.e. to aftermaths. At this time of year conditions

for translation are still good and the process is fairly rapid. In consequence there is a danger that if the calves contaminate the clean pastures, a heavy herbage infestation may be built up on these quickly. It is therefore recommended that the calves be given anthelmintic treatment when they are moved in order that this contamination of clean pasture be prevented, or at least postponed. This use of anthelmintics, to prevent pasture contamination rather than to alleviate the pathological effect of the worm burden, is made possible

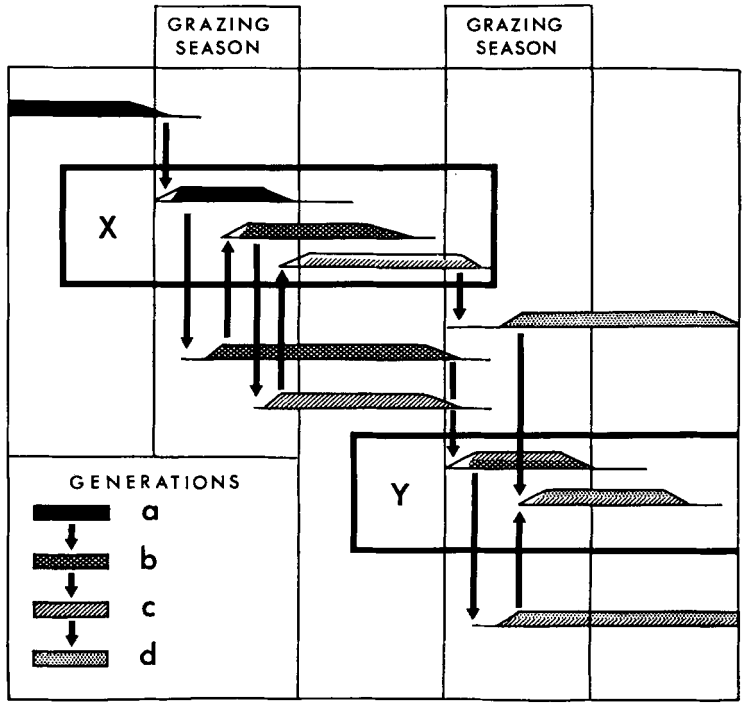


FIG. 21. The generations of *Ostertagia ostertagi* in calves and on the pasture through two grazing seasons.

by the existence of effective anthelmintics. However, for this more exacting purpose the dosage levels usually recommended are not sufficient and larger quantities must be employed, a fact which argues in favour of those materials which have a wide therapeutic index, i.e. which are much less toxic to the host than to the parasite.

The procedure has been and continues to be the subject of experimentation. Trials conducted at Weybridge (Michel, 1968b) have shown that calves turned out on infected pastures and managed in this way maintain an almost linear growth rate throughout the season, and are between 100 and 160 lb heavier than calves which remain on the same ground throughout the season and which suffer a great reduction in growth rate at the beginning of August. Some results from one year of these trials are summarised in Figs 22 and 23. Of

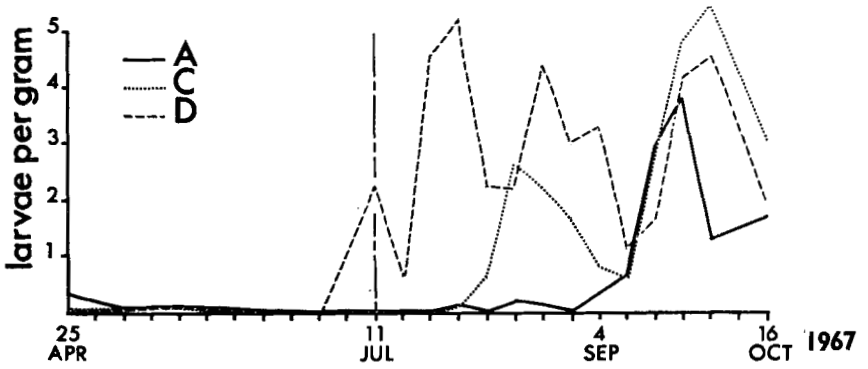


FIG. 22. Trials of measures for the control of parasitic gastro-enteritis in calves. Herbage infestations, expressed as larvae/g of dry matter, on paddocks occupied after mid-July by groups A, C and D. Group A was moved and dosed in mid-July, Group C was moved only and group D remained on the same pasture throughout the seasons. (Reproduced with permission from Michel, 1968b.)

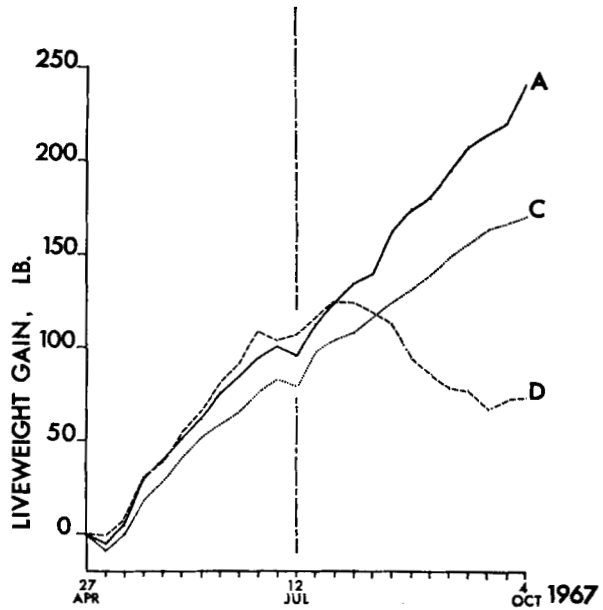


FIG. 23. Trials of measures for the control of parasitic gastro-enteritis in calves. Mean liveweights of groups of calves which were moved and dosed in mid-July (group A), were moved only (group C), and remained on the same pasture throughout the season (group D). (Reproduced with permission from Michel, 1968b.)

these, the first shows the herbage infestation on three paddocks which respectively carried untreated calves throughout the season (group D), was grazed but not contaminated until July and then carried undosed calves (group C), and was grazed but uncontaminated until July and carried calves which were dosed when they were moved onto it (group A). It will be seen that moving and dosing succeeded in postponing access of the calves to heavy herbage infestation. Since the resistance of calves to the effect of such exposure appears to increase with advancing age, the effect of postponing access to heavy infection is reflected in liveweight gains, shown in Fig. 23. During these trials dosing at the time of moving did not consistently show an advantage over moving only.

In practice this approach to the control of parasitic gastro-enteritis is appropriate only to those situations where calves must graze entirely on infected ground. In certain specialist enterprises these measures pose a number of difficulties of management which derive from the necessity of keeping calves, in the second half of the season, off ground which they grazed before July. This area is not likely to be less than one third or, at the very least, one quarter of the total. If older cattle or sheep are available to graze this land in the second half of the season, and they can safely do this, no difficulty arises. Increasingly, however, as enterprises become more specialised, there are no older animals on farms where young cattle are reared. For example, where the semi-intensive production of beef from dairy-bred calves is practised (Baker *et al.*, 1967) there will be no animals in their second grazing year available in July, autumn born animals being sold fat out of the yards after only one grazing season. In these circumstances the measures described would result in an excessive area of grass being conserved, three quarters of the area being cut once for hay or twice for silage before July and the remaining quarter being cut once after July. In such specialist enterprises other measures can be employed, however, if clean pasture is available.

It is to be expected that the size of the initial infestation will have an effect on that of the next generation of larvae on the pasture and hence on the hazard of disease. As has been mentioned, however, there is a tendency for the egg output of large and small populations of worms to be the same and the relationship between pasture contamination and the resulting herbage infestation is very variable and influenced by the climate. For these reasons the number of larvae of the new generation is likely to be less dependant on the residual infestation than might at first sight appear. Nonetheless, if clean calves are put on clean pasture the system will remain sufficiently clean for the whole of the season to make the occurrence of disease extremely unlikely. Some importance must of course be attached to the meaning of "clean" in this context. It has been found that calves that are hand reared under good conditions of housing may be regarded as clean, as may newly sown pasture which has not been grazed before. If such clean calves are grazed on this type of pasture, helminthiasis does not occur and liveweight gains are satisfactory.

This procedure is employed where a semi-intensive beef enterprise on one year leys provides a break between successive crops of cereals. In many situations, however, insufficient newly sown pasture can be provided to maintain

the cattle throughout their first season. If the area of new pasture represents one third of the total, control without resort to anthelmintic treatment should still be possible. If the calves are put first onto the new grass and remain there until the end of June while the infected pasture is conserved, they and the pasture on which they graze will remain clean. If they are moved to the aftermaths in late June they will not begin to pass worm eggs until the middle of July, and since the infestation on the aftermaths will by this time be very low indeed, the numbers of eggs that they pass will be too small to produce a harmful herbage infestation.

No reference has been made, in the foregoing account of parasitic gastroenteritis, to the inhibition of development or its epidemiological consequences. *Ostertagia ostertagi* is more liable to be inhibited in its early parasitic development than is *Cooperia oncophora* or the other nematodes of cattle and very large numbers can accumulate in young cattle exposed to infection. It seems that the phenomenon cannot be ascribed to host resistance. Michel (1967, unpublished) found that in calves infected daily a small but constant proportion of the worms ingested, accumulated as early fourth stage larvae. The proportion so inhibited did not change as the host's experience of infection increased; it was not affected by the infection rate, nor by treatment of the host with an immunosuppressant drug. The number of inhibited larvae established in experimental infections is never very great (Anderson *et al.*, 1967; Michel, 1963) in comparison with the very large burdens commonly found in naturally affected cattle at the end of their first grazing season. Michel (1967, unpublished) found outwintered yearlings to carry burdens of inhibited larvae ranging from 30 000 to 1 700 000, the highest values being reached in February. Anderson *et al.* (1965a, b) have shown that after a certain point of time in the autumn, a large proportion of larvae ingested even by entirely susceptible calves is inhibited. Subsequently they produced evidence (Armour *et al.*, 1967) suggesting that some strains of *Ostertagia* are more liable to be inhibited than others.

Normally the very large burdens of inhibited fourth stage larvae are associated with quite small burdens of adults. An analysis of the worm burdens of naturally infected young cattle has shown a much closer correlation between the number of late fourth stage (actively developing) worms and the number of adults than between the number of early fourth stage (inhibited) larvae and the number of late fourth stage larvae. It may be concluded that a turnover of worms was taking place, the number of adults being regulated in the normal way and depending on the rate at which inhibited larvae were resuming their development. This, in turn, did not depend on the number of inhibited forms present but there were grounds for believing that it was in some measure influenced by the number of adults worms present. It could not be said, however, that anything approaching a complete understanding exists either of the causation of inhibition or of the resumption of development.

The phenomenon is of significance partly because the large burdens of immature worms which are capable of developing represent one means whereby the infection is carried on from year to year, and partly because the numbers which resume their development are not always restrained and a

massive development can occur to give rise to outbreaks of disease in winter or early spring in animals which, in many cases, have not been exposed to infection for a period of months (Martin *et al.*, 1957). Anderson *et al.* (1965b) have rejected the commonly used term "winter ostertagiasis" for outbreaks of this kind and instead classify ostertagiasis into three types, simple ostertagiasis as seen in calves in late summer and autumn being designated Type I and winter ostertagiasis being called Type II. Animals carrying large burdens of inhibited forms and therefore at risk as far as winter ostertagiasis is concerned are, according to this classification, regarded as cases of "pre-type II ostertagiasis".

Concerning the factors which cause the inhibited larvae to resume their development in very large numbers instead of at the normal closely regulated rate, ignorance is still profound. Treatment of winter ostertagiasis is particularly difficult even with the aid of modern anthelmintics. Three methods of prevention are theoretically possible: (1) The inhibited forms could be removed at the end of the grazing season by anthelmintic treatment if a suitable anthelmintic existed. However, the inhibited forms, presumably because their metabolism is extremely slow, are highly resistant to all known anthelmintics. (2) The situations and factors which stimulate a massive development of worms could be avoided were they known. (3) The only course open at present is to avoid accumulation of large burdens of inhibited forms by limiting the access of calves to infection especially in autumn and winter. This point has been stressed by Anderson *et al.* (1965b), who recommend that calves be transferred to clean pasture in September, a suggestion that would raise considerable practical difficulties. The control measures advocated by Michel (1967b) reduce the uptake of infective larvae in the autumn but they can on occasion permit the accumulation of moderate burdens of inhibited larvae, and the possibility cannot be excluded that they do not remove all hazard of winter ostertagiasis.

## V. Parasitic Gastro-enteritis in Sheep

Of the 21 species of nematodes which occur in sheep in Britain, four or five are at present of importance. These are *Ostertagia circumcincta*, *Trichostrongylus vitrinus*, and to a lesser extent *T. axei*, *Nematodirus fillicollis* and *N. battus*. Nematodiriasis will be dealt with in a later section; it is proposed to consider here ostertagiasis and trichostrongyliasis with particular reference to lambs.

It is inevitable that an annual periodicity must be evident in worm burdens. In addition to climatic changes which are highly relevant both to free living development of the parasites and the physiology of the host, there is also, every spring, a new entry of susceptible lambs into the flock. Only certain aspects of the seasonal pattern have been studied in detail. Among these, the egg output of ewes and the increase in worm burden of lambs are of particular interest to the present discussion.

Seasonal fluctuations in the worm burdens of hill sheep in Scotland were studied in the late forties and early fifties by D. O. Morgan and his collaborators

(Morgan and Sloan, 1947; Morgan *et al.*, 1950, 1951; Wilson *et al.*, 1953). Hill sheep were chosen partly for their intrinsic interest and partly because they could be regarded as representing a natural rather than an artificial system. However, they presented certain practical problems because they could not be collected together and handled at frequent intervals.

In its early stages this work was based entirely on faecal egg counts, which in the case of ewes showed a very marked seasonal pattern; they began to rise in March, reached a peak in May and then declined to low levels (Fig. 24).

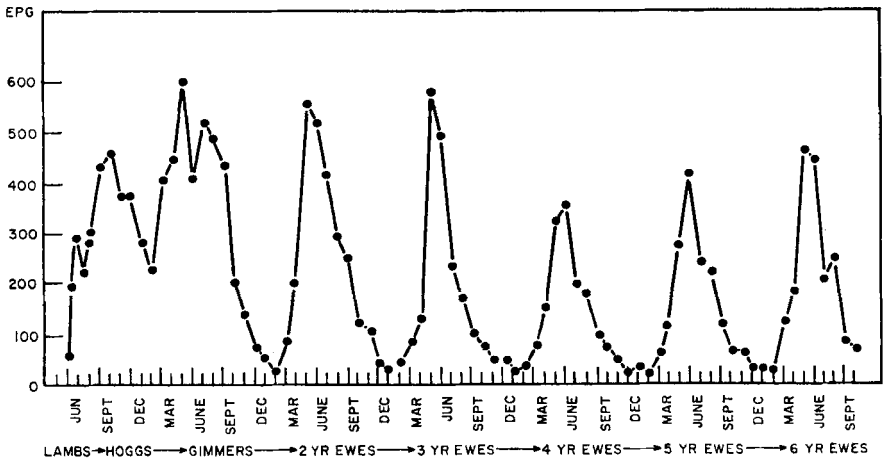


FIG. 24. Worm egg counts of strongyles of an "average" Scottish hill sheep throughout life; based on worm egg counts from numerous sheep on many farms from several districts of Scotland. (Reproduced with permission from Morgan *et al.*, 1951.)

Morgan *et al.* (1950) interpreted the increase in worm burden as being due to an increase in worm numbers which, they suggested, was attributable to worms acquired during the late winter. Since the hill grazings in question were at that time frequently deep in snow, this suggestion did not meet a ready acceptance, the more so since Taylor (1935) had observed a similar phenomenon in ewes that had been housed throughout the winter. Naerland (1952), Spedding and Brown (1956) and Field *et al.* (1960) have reported similar findings. Taylor's suggestion that the rise was due to increased fecundity of a constant number of worms was for some reason entirely neglected and it was generally held that it must be due to the development of formerly inhibited worms. Morgan *et al.* (1951), in an extensive series of post mortem examinations, demonstrated that the spring rise was indeed associated with an increase in worm numbers, but they failed to find appreciable numbers of inhibited larvae during the winter (Fig. 25). While admitting that the number of larvae picked up during the winter might not be large, they suggested that the susceptibility of the sheep might be greatly increased in the late winter and early spring and that a very much larger proportion than usual of those larvae that were ingested, became established. This was consistent with the findings of Paver *et al.* (1955) that the

rise was more marked after a severe winter. White and Cushnie (1952), on the other hand, had found that the phenomenon was not affected by supplementary feeding, and Parnell *et al.* (1954) observed that the spring rise occurred earlier in those ewes that lambed early in the season.

An important advance was made by Crofton (1954b), who showed that the spring rise was associated with parturition or lactation. According to Crofton's results the increase in worm egg counts of individual ewes was of short duration and its timing was closely correlated with the time of lambing, occurring after 6–8 weeks (Fig. 26). Other workers, e.g. Parnell *et al.* (1954),

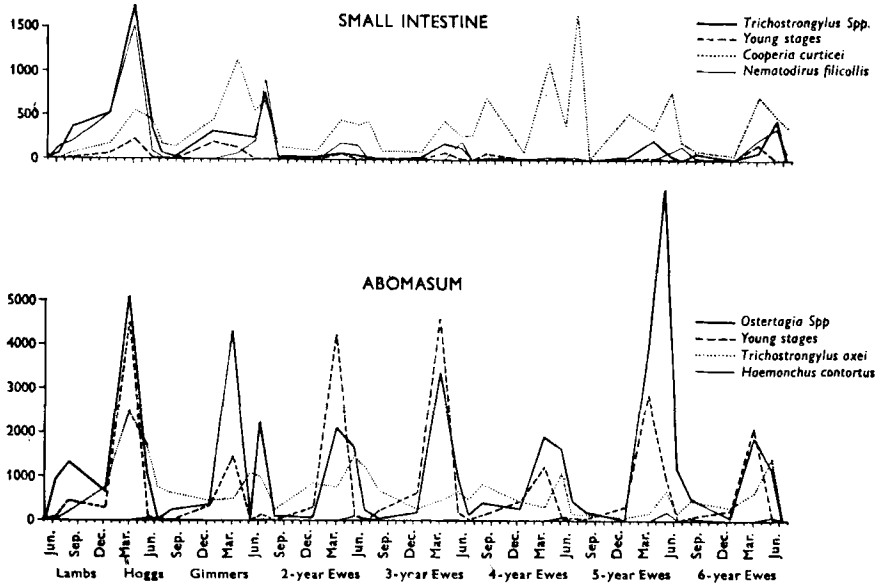


FIG. 25. The seasonal variations in worm burdens of hill sheep. (Reproduced with permission from Michel and Ollerenshaw, 1963, based on data presented by Morgan *et al.*, 1951.)

Spedding and Brown (1956) and Herweijer (1965), were not able to demonstrate such a close relationship, but that the spring rise and lambing are connected is no longer challenged. Crofton (1958b) showed that in flocks lambing in the autumn the post-parturient rise, as it is now called, also occurs in the autumn. Crofton, in attempting to explain the phenomenon, suggested that it was due to a reduction in the animals' resistance arising from the strain of lambing and that this caused an increased establishment of the worms being ingested and also prompted the development of inhibited forms. Although it is widely accepted that the resumed development of inhibited worms plays an important part in the spring rise phenomenon, it is only very recently that their presence in the ewes during the winter has been demonstrated. Soulsby (1965) made a passing reference to finding inhibited forms of trichostrongylid worms but did not make it clear what species were involved. James and

Johnstone (1967) have shown that numbers of inhibited forms of *Ostertagia* spp. rise to a peak in the winter and decrease in the spring, and Connan (1968) has demonstrated that formerly inhibited *Ostertagia* and *Haemonchus* resume their development to contribute to the post-parturient rise. He has also shown that the fecundity of the females is increased. If the efficiency of his techniques for recovering immature worms from post mortem material is not less than that for recovering adult worms, then he has also demonstrated that some of the worms contributing to the spring rise are newly acquired.

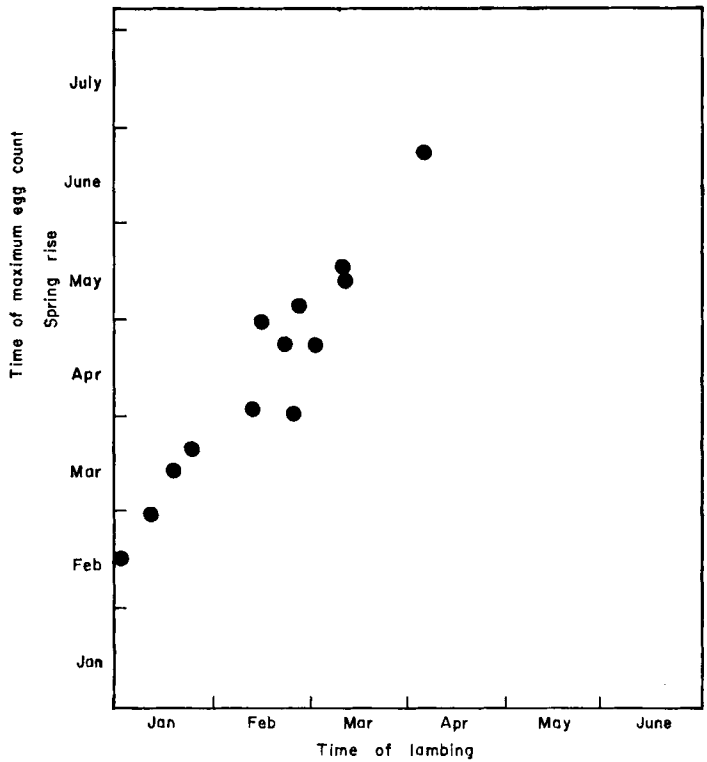


FIG. 26. The time of maximum egg count of individual ewes in spring plotted against the time of lambing. (Reproduced with permission from Crofton, 1954b.)

Explanations involving a reduction in resistance due to poor nutrition or the strain of lambing are less attractive than those entailing endocrine mechanisms. The importance of these has been forcibly argued by Dunsmore (1965). An intimate connection of the phenomenon with lactation has been demonstrated by Connan (1968) and by Jansen (1968), who found that if the lambs were prematurely weaned the expected increase in the faecal egg count of the ewes did not occur (Fig. 27). Although this represents substantial progress a complete understanding of the spring rise phenomenon has not been achieved and

the common finding that wethers and barren ewes show a modest but nonetheless real increase in egg count at the same time, remains to be explained. The decline in worm burdens and faecal egg counts by which the spring rise is terminated has been regarded as due to self-cure (Soulsby, 1960), but it should not be necessary to invoke this phenomenon. If the burden of adult worms is not being further augmented either because the sheep is again refractory to reinfection or because no further inhibited forms are developing, then the burden would decline in just the manner that appears to be reflected by the observed faecal egg counts.

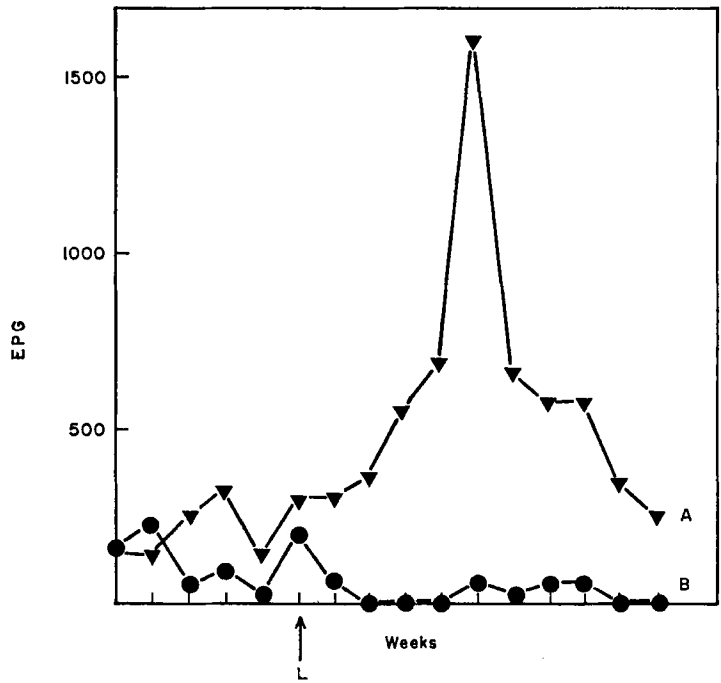


FIG. 27. The effect on the faecal egg counts of ewes, of weaning their lambs prematurely. Group A ewes suckling their lambs, group B ewes from which the lambs were removed at birth. L, lambing time. (Reproduced with permission from Connan, 1968.)

That the worm egg output of the ewes is greatly increased at a time when the lambs are beginning to graze has been seen by many as a device calculated to ensure the infection of the lambs. The relative importance of the residual infestation on the pasture in the spring and the contamination of pasture by the ewes is not easy to assess. The infective larvae of all species will survive through the British winter and meaningful numbers of *Ostertagia* spp. and *Trichostrongylus* spp. are likely to be present on the herbage in the spring. The initial infection available to the lambs is generally held to derive partly from larvae that have overwintered on the pasture and partly from new contamination of the pasture by the ewes. If it is assumed that the initial infection is

likely to be small, the manner in which populations in lambs increase to the high levels present when outbreaks of disease occur from July onwards, must be considered.

From a study of worm egg counts of lambs Crofton (1955) deduced that the population increased logarithmically. He interpreted this as reflecting a process of auto-infestation involving numerous generations. The logarithmic increase of worm burdens is referred to as the "Crofton line" by Whitlock (1963), who appears to regard it as an immutable feature of *Haemonchus* infections.

The theme was developed further by Crofton (1957) when he considered the effect of fecundity and generation interval of different species on the rate of population increase, in an effort to explain the fact that different species appeared to be most abundant in the lambs in a constant sequence through the year. If the increase is logarithmic, as Crofton assumed, the characteristics of the population increase depend on the increase in each generation and the interval between generations. Crofton calculated the generation interval for each species by adding the minimum time required for free living development to the prepatent period. To this minimum generation interval he added either a week or a fortnight to give estimates of an actual generation interval and assumed that these figures would apply from the time that the lambs first become infected until the worms become most abundant (in some cases from April until December). The intrinsic rate of increase of each species was calculated according to their relative fecundities from a figure for the increase per generation of *Haemonchus contortus*, derived by trial and error from a variety of data, some from Australia. By these means curves were constructed which showed that populations of the different species would reach certain arbitrary levels in the expected sequence. Only *Nematodirus* spp. did not fit, in that they would require nearly a year to achieve the burdens which occur very early in the lamb's life. Crofton claims the right to make an exception for this genus on the grounds that it is known to complete only one generation per annum. It appears to the present writer, however, that this is not justified. If the method of calculating the intrinsic increase per generation is sound in the case of the other genera, it should apply to *Nematodirus* also. Now according to Crofton's figures, *Nematodirus* in the course of one generation increases by an increment of $\frac{1}{3}$, i.e. from 1 to $1\frac{1}{3}$. Since each successive generation of *Nematodirus* occurs in a different crop of lambs this means that each generation would only be one third as numerous as the last. Obviously the calculated value of the intrinsic increase is far too low and this must cast doubt on the figures calculated for other species. These do indeed appear very low. For example, a sixfold increase per generation for *H. contortus* seems unlikely when females of this species lay up to 10 000 eggs per day, 250 000 in the space of one minimum generation interval, and when, according to Donald (1968), up to 20% of those eggs may actually become available on the herbage as infective larvae.

Now if the values for intrinsic increase used by Crofton were too small and the calculated worm burdens in late summer and autumn were nonetheless of the right order, then it follows that the generation interval must also have been

too small. This must emerge also from the work of Silverman and Campbell (1958) and of others which, as discussed in an earlier section, indicates that free living development and migration of larvae onto the herbage takes far longer than the minimum period. Indeed, it is open to question whether more than one or at most two generations of the parasite are involved. This in turn raises the question of how a succession of dominant species comes about, if it occurs at all. The data of Parnell (1954) and of Tetley (1941) suggest that burdens of most species increase to a peak simultaneously, and Tetley (1949) considered that the succession of species was primarily a matter of the time when eggs ceased to appear in the faeces. More recently, Brunsdon (1963) has shown that the reported sequence *Ostertagia–Haemonchus* and *T. axei–T. vitrinus, T. colubriformis* and *C. curticei* is reflected also in the number of larvae available on the herbage.

It must also be questioned, if the increase in worm populations of lambs is logarithmic as Crofton (1955) claimed, whether or not this can be due to a succession of generations. This interpretation would fit only if both the rate of development of the larvae and the proportion which completed their development remained constant throughout the season. These conditions are not met. The time taken for eggs in faeces to appear on the herbage as infective larvae is far longer at the beginning of the season than at its height, and a larger proportion of the eggs successfully complete the process in summer than do so in spring or autumn. Furthermore, the residual pasture infestation and the infection contributed by the ewes must be taken into account. The curves presented by Crofton (1957) assumed that the lambs acquired a minimal infection on 1st April and that thereafter their intake of larvae depended entirely on the infestation which they themselves had created. If the residual level on the pasture were greater this would make a considerable difference to the rate of population increase, and since the extent to which larvae of different species overwinter is different, it is not justifiable to assume a minimal infestation (presumably two worms) on 1st April for all species. Furthermore, if the spring rise is of importance in providing the initial infection and these eggs appear some 6–8 weeks after lambing, not only would they markedly distort the curve at that time but, since a number of species participate in this phenomenon, this distortion would be greater in the case of those species alleged to increase slowly, than in the case of those said to increase more rapidly.

If the interpretation that is offered for the logarithmic increase of worm burdens is suspect, then it is appropriate to question whether indeed the increase really is of logarithmic form. The evidence for this, presented by Crofton (1955), consists of ten sets of mean egg counts derived from three flocks in 3 years, and while four of these certainly show a resemblance to a logarithmic curve, the other six are much less convincing. To this extent the apparently excellent fit calculated by Crofton may be misleading. The observed curve might resemble a logarithmic curve by chance or might conform to a similar curve of rather different characteristics. The fact that development and migration of the larvae become faster during the relevant period and that the proportion of the larvae which successfully complete the process increases, are both factors which would tend to make the herbage infestation increase

according to this pattern if contamination were at a constant rate. This, however, it is not. The mean egg output of a flock of ewes after lambing may also increase according to a pattern resembling a logarithmic curve. A good example is published by Connan (1968), and another (Fig. 28) is drawn from

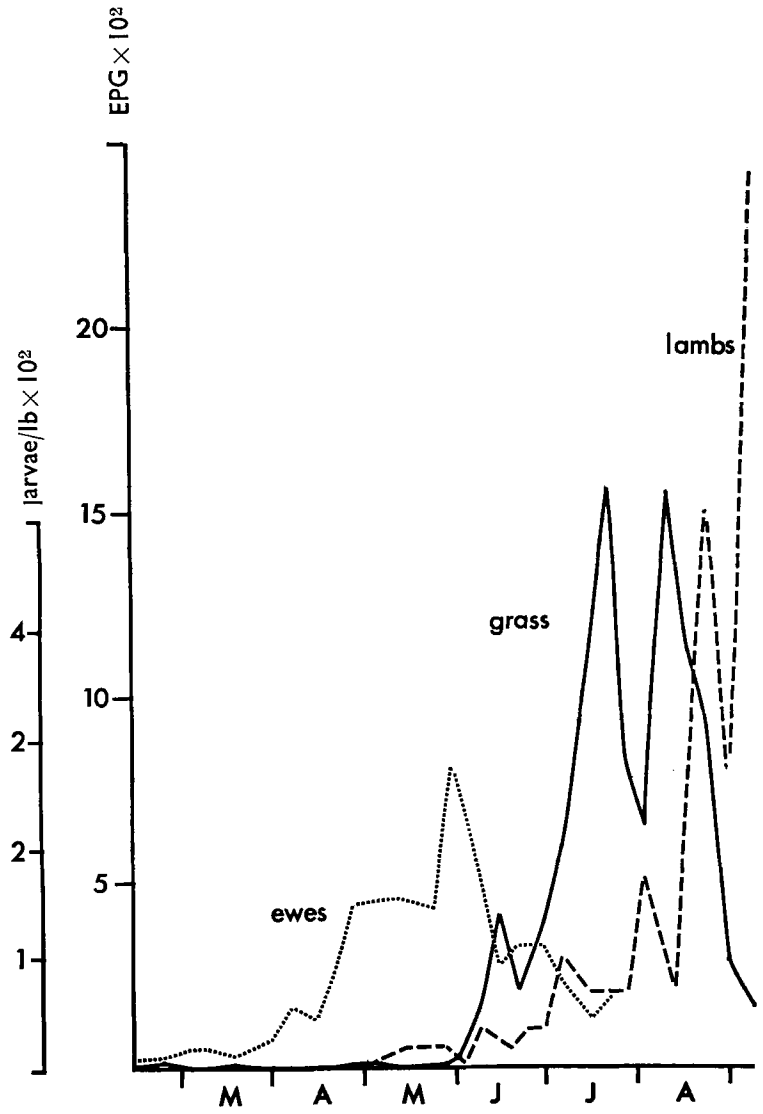


FIG. 28. The faecal egg counts of ewes and their lambs and the herbage infestations (chiefly *Ostertagia* spp. and *Trichostrongylus* spp.) on the pasture which they grazed. (From data made available by G. B. S. Heath.)

data kindly made available by G. B. S. Heath of Newcastle, who studied the egg count of ewes and lambs in a flock in Cumberland as well as the infestation on the pasture on which they grazed. It will be seen that all three conform to the same pattern and it is difficult to avoid the conclusion that the herbage infestation was a reflection of, and almost entirely due to, eggs deposited by the ewes while the egg output of the lambs reflected, and was a direct consequence of, the herbage infestation. Clearly the pattern of increase of the egg count of ewes cannot be attributed to a repeated process of auto-infestation but is due, more probably, to variation in the date of lambing, in the time at which worms resume their development, in the rate of development of worms and in the time they take to reach their maximum egg output. The increase in herbage count is a little steeper than this, presumably because of the increasing rate of free living development and migration and the increasing proportion of eggs developing successfully. The egg count of lambs appears to increase rather less steeply and it seems probable from post mortem data that this is due to the fact that worms in small populations lay relatively more eggs than those in large populations. It appears from these results that the infection had been transmitted from the ewes to the lambs. The eggs passed by the lambs can have made little contribution up to the end of June, especially when it is considered that the ewes passed a considerably greater weight of faeces than the lambs. It is of interest that when the lambs began to pass large numbers of worm eggs the herbage infestation was no longer increasing and indeed decreased sharply. Auto-infestation did not occur to any significant extent.

By preventing the post parturient rise in the egg ouput of ewes by anthelmintic treatment, Nunns *et al.* (1965) and Thomas and Boag (1968) have shown that if ewes and lambs are subsequently maintained on a clean pasture, worm burdens acquired by the lambs will be very much reduced.

It is probable that if the ewes produced no eggs the residual infestation on the pasture studied by Heath might have produced enough infection in the lambs to give a second generation of worms sufficient to produce disease, and that this generation would have appeared on the herbage as soon as the larvae derived from eggs passed by the ewes. That sufficient larvae of *Haemonchus*, *Ostertagia* and *Trichostrongylus* survive on the pasture through the winter has been shown by Rose (1965) in Britain and by Kates (1950) in the U.S.A.

The normal pattern of infestations on the herbage has not been extensively studied. From the data presented by Gibson and Everett (1967) it might be deduced that herbage infestation on a pasture contaminated at a constant rate would begin to rise steeply in late June and remain at a high level until the following March or April. This pattern is revealed in the data presented by Large and Spedding (1964). The same increase in late June or July was also evident in the work of Spedding and Large (1959) and of Heath, although in these cases counts did not remain at a high level for as long. The herbage counts of Gibson and Everett (1968) suggest that some increase in level may occur in early June, but dangerous numbers did not appear until August. Crofton (1949) published herbage counts on a hill pasture which increased gradually from May to reach a peak in September. Subsequently Crofton (1952) presented series of counts from lowland pastures. These also increased

from May to peaks which occurred variously from June until September. Details of when these pastures were contaminated are not given and peak levels of infestation are not high. However, it is clear that larvae of the trichostrongylid nematodes of sheep begin to appear on the herbage rather earlier than those of cattle. Gibson and Everett (1967) also showed that contamination of the pasture before April and after the middle of September will not result in more than negligible infestations on the herbage. The season during which translation is possible is short. The assumption that levels of infestation on the herbage will be low in the spring and will not rise to dangerous levels until late June, may prove to be a suitable basis for the control of parasitic gastro-enteritis in lambs on infected pastures. If the ewes and lambs can be kept on newly sown, clean pastures the infection will be derived entirely from the ewes and these should be treated to prevent pasture contamination. It is not then likely that meaningful infestations will occur in the lambs and no further precautions need be taken, at any rate until the late autumn. If ewes and lambs have to graze on pastures contaminated in the previous year, treatment of the ewes may not be justified, but when the herbage infestation begins to rise towards a dangerous level which is likely to be in late June, the lambs should be dosed and moved to pasture not contaminated since the winter. In practice this is best arranged by weaning the lambs at this time.

Such a recommendation would depend on the assumption that infection at a low rate does not adversely affect the growth of lambs. Spedding (1956a, b) showed that moderate worm burdens had an adverse effect on the growth of lambs, but the deduction that there is a continuous relationship between worm numbers and damage to the growth of the host was not supported by the subsequent work of Spedding and Brown (1957) and has not in general been vindicated. Parasitic gastro-enteritis is not generally a problem in fat lamb production if the lambs can be got ready for slaughter before the beginning of July. The poor thriving which becomes evident in that month is frequently but by no means always attributable to uncomplicated helminthiasis. The condition known as "July disease", which is probably similar to the "hoggett ill thrift" discussed by Clarke and Filmer (1956), is likely to be due to the interaction of a number of factors. Of these a moderate worm infestation may be one, and the circumstance that herbage infestations appear less suddenly than is the case with cattle may be of relevance and may mean that the measures suggested, while probably effective in preventing the occurrence of large worm burdens, may not in all circumstances avoid unsatisfactory growth.

## VI. Parasitic Bronchitis of Cattle

The epidemiology of infections of *Dictyocaulus viviparus* is dominated by the relative ease and rapidity with which the host acquires a resistance to the establishment of worms and by the speed at which the free living phase of the life history can be completed. The critical temperature for the development of the free living stages is low, and some development can proceed almost throughout the year (Daubney, 1920) although it is considerably slower in winter than in summer (Rose, 1956). All the free living stages from the first

stage larva passed in the faeces to the infective third stage are susceptible to desiccation, and while the infective larva does withstand freezing a little better (Rose, 1956) this species cannot be said to have a resistant stage comparable with the embryonated egg or the infective larva of trichostrongylid nematodes. From the moment the faeces are passed there is a heavy mortality of larvae. This is more rapid at high temperatures and in conditions of drought than when it is cool and moist.

The infective larvae are quite inactive yet they can be present on the herbage in considerable numbers so quickly after contamination of the pasture that transfer from faeces to herbage must take place almost immediately development is complete or even sooner. Michel and Rose (1954) and Rose and Michel (1957) stressed the part played by the consistency of the faeces and its physical dissemination over the herbage, and this seems to be important. Subsequently Robinson (1962) discovered, however, that the dissemination of the larvae may also be assisted by fungal "gunnery". The relative importance of different means by which the larvae get from the faeces onto the herbage has not been established, but the process can be extremely rapid in favourable conditions. At the same time the faeces on the pasture can act as a reservoir of infective larvae which can subsequently be made available.

The creation of infestations on the herbage appears to be fostered by those conditions that favour the rapid growth of the herbage (Michel and Parfitt, 1956). Conditions favouring the survival of larvae on the pasture are not the same as favour the creation of herbage infestations. In general, while a new herbage infestation can only be created with ease in autumn and in spring, an established infestation survives well in the autumn and winter. Consequently, heavy though short lived infestations may occur in spring and early summer and rather longer lasting infestations will occur in autumn and may survive into the early winter. Heavy infestations are less likely to occur in the height of summer unless abnormal conditions of microclimate obtain. It is almost impossible to create a new herbage infestation in the winter. As development is completed rapidly during much of the grazing season, favourable conditions of climate or of microclimate need prevail for only a relatively short time for sharp increases in herbage infestation to occur. The system shows the instability referred to on p. 212, in which a small change in environmental pressure can produce an unduly large increase in population. As has been indicated, the larvae die off rapidly. In summer a population of infective larvae on the herbage can decrease to one hundredth in 23 days. In the winter, however, the same decrease could take 96 days. In the spring the mortality rate would increase again. The rate of decrease is not constant but itself tends to decrease. A population of larvae on the pasture is heterogeneous, a part decreasing in number more slowly than the rest, probably because these larvae occupy a more protected situation. This protected minority comes to represent an increasing proportion of the population.

A surprising measure of attention has been devoted to the question of whether lungworm larvae survive on the pasture through the winter. In view of the form of the population curve an answer to this question should not be

sought in absolute terms. It might be proper to ask what proportion of a population of larvae might be expected to persist from October until April. Alternatively, the circumstances and the criteria of presence or absence would have to be specified.

In the spring residual levels of pasture infestation are likely to be very low indeed, although they may be a little higher in S.W. Scotland than in S.E. England (Jarrett *et al.*, 1955). Whether the infection survives throughout the winter on the pasture depends on the size of the infestation in the autumn and on how early in the spring the pasture is grazed. In S.E. England the infestation present in late April is normally so small that it could most readily be defined in terms of the number of animals that must graze the pasture together to give a good chance that both a male and a female worm will become established in one of them. In a situation in which a pasture is grazed by a new bunch of calves every year, the over-winter survival of the infection may depend in the spring on only two worms in a single calf.

A number of writers have therefore stressed the importance of older animals as a source of infection. Experimentally infected animals cease to pass larvae in their faeces after about 70 days but they are likely to retain very small numbers of worms, usually immature worms, for long periods. This residue of worms is larger in animals that have been infected several times or in naturally infected animals. Jarrett *et al.* (1955), in a survey of animals brought to a Scottish knackery, found adult lungworms in 4·8% of cows and 30·7% of yearling cattle during April. The intake of a knackery does not represent a random sample of cattle and contains a disproportionate number of sick and debilitated animals, and these figures may therefore be rather high, but it is clear that adult animals can and do represent a source of pasture contamination.

It has been indicated in an earlier section that in infections of *Dictyocaulus viviparus* a resistance to the establishment of new worms develops quickly and as a result of only a moderate experience of infection. It might be expected, therefore, that even if the generation interval is short, auto-infestation is unlikely to occur because the animals will be refractory by the time a second generation of larvae has become available on the pasture.

Michel and Parfitt (1956) exposed calves to natural infection on an experimental paddock, adding new calves at regular intervals, and observed that the fate of each calf in terms of how long it survived the effects of the lungworm infection, was related to the herbage infestation during the first nine days of exposure (Fig. 29). From this they concluded that auto-infestation did not occur and that outbreaks of husk were always the consequence of exposing animals to a herbage infestation which had been created by some other group of animals. This conclusion, though supported by the results of Michel and Mackenzie (1956), was erroneous and further analysis showed that, in the experimental situations on which it was based, when herbage counts were very low the potential rate of increase was also low. Subsequent work (Michel and Parfitt, unpublished) has made it clear that if the infection to which calves are initially exposed is very low they will acquire a negligible resistance to reinfection. However, such calves will pass only very small numbers of larvae and

would not normally be in a position to create herbage infestations sufficiently heavy to cause disease. Moreover the second generation of the parasite would be likely to render the calves immune. For auto-infestation to end in disease, the increase in population from one generation to the next must be large; a small number of larvae in faeces must rapidly produce a heavy infestation of infective larvae on the pasture. This will demand conditions in which the normal heavy losses do not occur, and as the process of translation can be very rapid, these favourable conditions need obtain for only a short time. The outcome of auto-infestation depends on the initial level of the pasture infestation and on the effectiveness of the translation process. This is most readily considered in terms of a number of hypothetical cases.

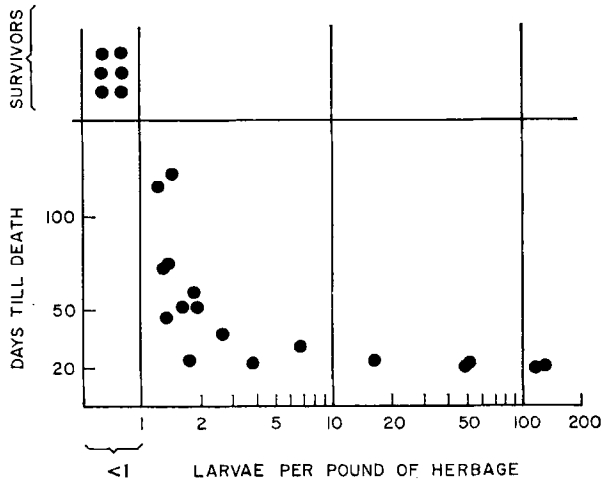


FIG. 29. The relationship between the mean number of *Dictyocaulus viviparus* larvae per pound of pasture herbage during the first nine days that calves were exposed to infection, and the subsequent fate of the calves. (Reproduced with permission from Michel and Parfitt, 1956.)

(1) If the initial infestation is extremely low no immunity will result from it. If conditions now do not favour translation, the second generation will still be too few to engender effective resistance, and whether a third generation causes disease will depend on how numerous it is. However, the immunising effect of small infections is cumulative and it is extremely unlikely that a fourth generation could become established in the host. If conditions for the free living existence of the second generation are more favourable it will be sufficiently large to engender resistance. If conditions are very favourable indeed the second generation could produce disease.

(2) If the initial infestation is rather greater but still not sufficient to confer on the calves more than a negligible resistance, the chances that the second generation will be sufficiently great to produce disease will be somewhat

increased. It is, however, extremely unlikely that the calves will remain susceptible to a third generation.

(3) If the initial infection is greater, the calves, while showing only the mildest of symptoms, will become resistant to larvae of the second generation.

(4) If the initial infestation is still greater the first generation of worms will produce disease.

The third and fourth cases are illustrated in Fig. 30, which depicts the course of the herbage infestation on an experimental paddock and the faecal larval counts of three calves put on it at different times through the season.

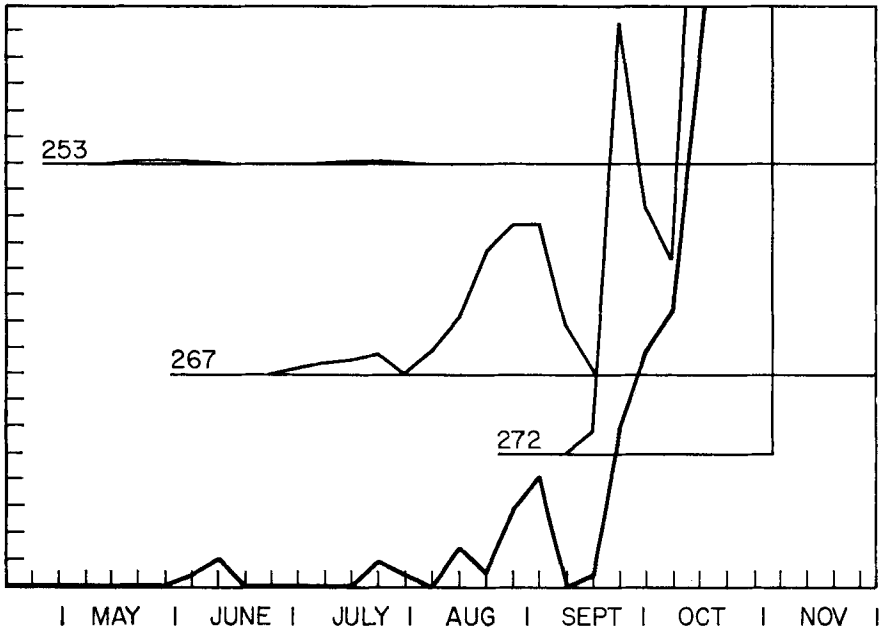


FIG. 30. The infestation of *Dictyocaulus viviparus* larvae (shown by the heavy black line) on an experimental paddock and the faecal larval counts of three calves turned out on it at different times through the season. (Reproduced with permission from Michel, 1957.)

The first calf (No. 253) was put on the paddock in late April when the herbage infestation was very low. It passed only very small numbers of larvae and remained unaffected even when the herbage infestation rose to high levels later in the season. The small numbers of larvae which this calf passed produced a moderate herbage infestation which was present when a second calf (No. 267) was added at the end of May. This animal became rather more heavily infected, passed rather more larvae and gave rise to a very much greater herbage infestation. The third calf (No. 272) succumbed to this heavy infestation. Outbreaks of disease of this type due to the exposure of one group of cattle to a heavy herbage infestation derived from contamination of the

pasture by another group of cattle, do occasionally occur. Not infrequently one lot of calves is severely affected while on the same pasture another group which has been there for a longer time, remains unaffected. Such outbreaks would be avoided if all calves which were to run together were turned out for the first time in the same week and if calves were not allowed to graze pastures which had been occupied by other cattle in the same season.

The occurrence of lungworm disease conforms to one of two patterns. On some farms the infection is present at a subclinical level but outbreaks occur only very rarely and the disease may occur in stock of any age. On other farms disease occurs every year in calves but is never seen in adult animals. It is in herds of the second type, where outbreaks are generally due to auto-infestation, that the disease is appropriately controlled by vaccination.

A vaccine which is now widely used depends on the fact that infective larvae which are attenuated by irradiation fail to reach the lungs of a calf to which they are administered, in sufficient numbers to cause disease (Jarrett *et al.*, 1958, 1959, 1960; Poynter *et al.*, 1960). The immunity which it produces is not as strong as that derived from infection with normal larvae (Michel and Mackenzie *et al.*, 1965) and it is questionable whether a vaccinated animal will withstand sudden exposure to a heavy uptake of larvae. Nearly all cases of husk in vaccinated calves appear to be attributable to infection from pastures contaminated by other animals and not to auto-infestation. As has been shown, many outbreaks of husk are the result of auto-infestation and occur when the population increase from one generation to the next is greater than the resistance of the host will accommodate. It is the effect of vaccination to insert one, or more precisely two, generations of the parasite, insofar as their effects on resistance are concerned, without at the same time contributing to population increase. Occasionally indeed, vaccinated animals become a source of infection (Cornwell and Berry, 1960) but not apparently on a sufficient scale to produce infestations harmful to the vaccinated calves. They may, however, be a hazard to entirely susceptible calves, and vaccinated and unvaccinated calves should not graze together. In practice the vaccine has proved generally effective in preventing outbreaks due to auto-infestation.

The ready development of a protective immunity and the presence of the parasite on almost every farm where cattle are kept might lead to the expectation that parasitic bronchitis is invariably a disease of cattle in their first grazing season. As has been intimated above, however, outbreaks in adult cattle do occur, especially on those farms where the level of infection in most years is very low.

A resistance to the establishment of *Dictyocaulus* decreases in intensity and is lost if the cattle are withheld from contact with infection for some time. If thereafter they are again infected, worms may become established in their lungs. The subject has been extensively studied by Michel and Mackenzie *et al.* (1965), who showed that if calves were infected and then received no further infection for a period of months, at the end of which time they were challenged by the administration of large numbers of larvae, they were no longer refractory. Initially they behaved as though entirely susceptible. Those effects of resistance which affect the worms during the first 10 or 11 days of

their parasitic life and which may presumably be ascribed to a residual immunity, had disappeared after nine months' freedom from contact with infection. Those effects of resistance which operate after the 11th day and may in this case be associated with an anamnestic response continued to be fully effective even after the animal had been withheld from infection for 27 months. In consequence, the worms of a challenge infection, given 9 months or more after the immunising infection, became established and developed normally for 10 or 11 days. Thereupon the development of some of the worms was arrested and that of the remainder retarded. A large proportion of the worms was lost from the host before the 30th day and a negligible number of larvae appeared in the faeces. Meanwhile, and starting about the 11th day, pathological changes in the lung which were suggestive of a hypersensitive reaction gave rise to clinical signs more acute, though not as long lasting, as those seen in previously worm free animals. Some years previously, Michel (1954) had speculated that the oedema and cellular infiltration seen when lungworms invaded a sensitised lung might be associated with the expulsion of worms in much the same way as these phenomena appeared to be connected in the self-cure of infections of *Haemonchus contortus* in sheep (Stewart, 1953).

The results of Michel and Mackenzie *et al.* (1965) helped to illuminate some puzzling features of husk in adult cattle. Michel and Shand (1955) had investigated numerous outbreaks of the disease, which at that time was frequently misdiagnosed, and had concluded that it occurred in animals which, while they had had some contact with infection in their 1st or 2nd year, had subsequently been withheld from infection, that violent symptoms appeared suddenly about 12 days after exposure to reinfection, and that the worms frequently failed to grow to maturity. Subsequently Michel and Coates (1958) produced an experimental outbreak of husk in previously infected animals by withholding them from infection for some months before re-exposure. They showed that previously infected animals were affected earlier and more severely than previously uninfected animals and that their infections failed to become patent.

## VII. Nematodiriasis

### A. Nematodiriasis in sheep

Two species of *Nematodirus* are of importance in Britain as parasites of sheep. They are *N. filicollis* and *N. battus*, which was identified as a new and distinct species by Crofton and Thomas (1951). The life history of these parasites differs from that of other trichostrongylids in that free living development to the third stage proceeds entirely within the egg shell. Hatching, especially of *N. battus*, does not occur spontaneously. Gibson (1958) has shown that although lambs can become infected by ingesting eggs containing third stage larvae, the hatched larvae are far more infective. Moreover, eggs are likely to be far less accessible to the grazing animal than larvae which have migrated onto the herbage, and the hatching of the egg is an important factor in the epidemiology of nematodiriasis. The attention devoted to the phenomenon is therefore justified.

Thomas and Stevens (1960) working in N.E. England found that development of *N. battus* was even slower than that of *N. filicollis*, eggs deposited in faeces in May taking until September and July respectively before the majority of larvae had reached the third stage. However, little or no hatching occurred until the following March. As a result of laboratory experiments they advanced the hypothesis that after exposure to cold the eggs hatched when the temperature rose to 50°F. A slightly different theory proposed by Christie (1962) also entailed a cold process followed by a warm process. Other circumstances can lead to hatching, however, and various forms of

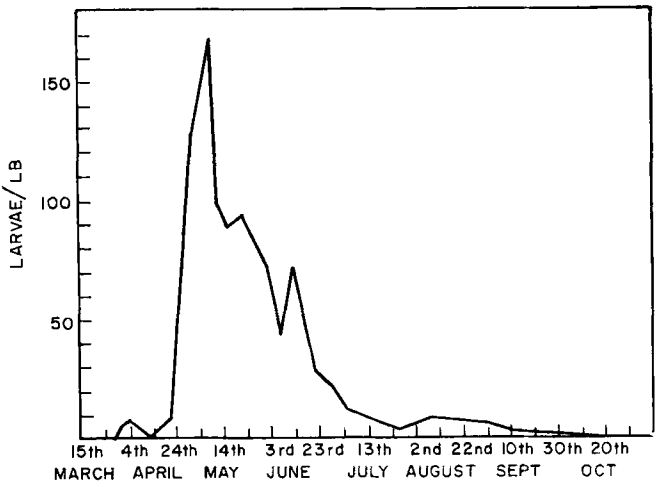


FIG. 31. The seasonal pattern of infestations on the herbage of infective larvae of *Nematodirus battus* and *N. filicollis*. (Reproduced with permission from Thomas and Stevens, 1956.)

mechanical interference, squeezing and shaking, are used by experimenters to this end. It is thus reasonable to expect that while a mass hatching may be the result of conditioning by low temperature and stimulation by warmer conditions, some spontaneous hatching will occur at other times.

Outbreaks of nematodiriasis occur in lambs in the spring and Thomas and Stevens (1956) showed that this was preceded by a sharp increase in the number of larvae on the herbage (Fig. 31). Thomas and Stevens (1960) contaminated an area of pasture with eggs of *N. filicollis* and *N. battus* by allowing infected lambs to graze on it in May and June. They then tested its infectivity by means of worm free lambs and demonstrated that such test lambs did not become infected in summer and autumn but did pick up both species of *Nematodirus* in the following spring and summer. This evidence lent considerable support to the view first advanced by Tetley (1941) that the infection was transmitted from one year's lambs to the next year's lambs. Gibson (1959) found that in southern England *N. filicollis* larvae on the pasture showed a slightly different pattern. Appreciable numbers were present in Autumn as well as in Spring. Evidently some hatching occurred without the experience of

winter cold. Baxter (1958) also reported an autumnal peak of herbage infestation, as did Brunsdon (1960) and Tetley (1959) in New Zealand.

It appears, however, that the lambs are no longer susceptible when these larvae derived from eggs which they have deposited appear on the herbage in the autumn. Exposure to this second generation of worms does not result in clinical effects. Baxter (1967), claimed, however, that some of the worms do become established, deducing this from egg count data of Baxter (1958) and Brunsdon (1960) and from the experimental infections of Dineen *et al.* (1965). That exposure to infection with *Nematodirus* results in a fairly prompt and effective immunity to reinfection is plain not only from field evidence but also from an experiment carried out by Gibson and Everett (1963). A form of age resistance has also been described, but while Brunsdon (1960) maintains that it results in a lengthened prepatent period, reduced egg production per female and a greatly reduced worm burden, Gibson (1959) reported that its chief effect was to cause the development of the worms to be inhibited.

The epidemiology of nematodiriasis, the manner in which it is transmitted from one year's lambs to the next year's lambs, thus depends both on the acquisition of an effective immunity before auto-infestation can occur, as visualised by Tetley (1935), and on the special requirements for the hatching of larvae which ensure that a heavy infestation is available on the herbage when the next group of susceptible animals begins to graze.

As suggested by Thomas (1959), the control of nematodiriasis in Britain is readily achieved by preventing lambs from grazing pastures which were occupied by lambs in May and June of the previous year. Gibson (1963a) showed that on ground grazed every year by lambs, infestations of *N. battus* and *N. filicollis* were built up in two years to levels resulting in outbreaks of severe disease. Keeping the pasture free of lambs for a year reduced the infestation to a harmless level from which, however, it could again be built up by annual grazing with lambs.

Although the recommended control measures are not difficult to apply, attempts have been made to devise methods of prophylactic anthelmintic treatment. The use of a number of regular doses of Bephenium embonate during May and June was shown to be effective (Scarnell and Rawes, 1959), and Gibson (1963b) in confirming these results demonstrated that the first of the doses he gave was alone as effective as all five in his complete programme. This, however, was attributable to the fact that this first dose was given a few days after the peak of herbage infestation and when this was already declining rapidly. The peak of herbage infestation occurs at a slightly different time in different years. Moreover, there is variation in the form of the larval count curve, which tends to rise rapidly to a high peak where spring is late and to climb more gradually to a rather lower plateau where warm weather occurs early in the year.

When it becomes possible to predict the date of appearance and course of pasture infestations on the basis of meteorological data, the control of nematodiriasis as advocated by Thomas or by anthelmintic treatment will be easier. Methods of forecasting are at an immature stage of development but some success has been achieved in predicting the incidence and severity of the

disease. Ollerenshaw and Smith (1966) have shown a good correlation between incidence and two indices based respectively on the extent to which mean temperatures are below average in winter and particularly in March and on the date when the one foot soil temperature is likely to reach 43°F.

### B. NEMATODIRIASIS IN CATTLE

Although four species of *Nematodirus* occur in British cattle only one, *N. helvetianus*—the last species to be reported in this country (Morgan and Soulsby 1956)—is of any consequence. Some observations on the free living stages were reported by Rose (1966), who found that development proceeded at a rate similar to that of *N. filicollis*. Hatching, however, occurred shortly after development was complete and did not appear to require the special conditions demanded by *N. filicollis* and *N. battus*. Nonetheless, the season during which larvae hatch and appear on the herbage is very short (Fig. 2). Rose also studied the longevity of infective larvae on the pasture and it is evident from his results that a small residual infection will be present in early spring on pastures contaminated during the previous summer.

A consideration of the course of infections in calves turned out in April, shows that they will become lightly infected and will, in turn, contaminate the pasture in late May and particularly during June and July. Most of these eggs will hatch during August and a sharp increase in the herbage infestation will occur in that month. It is what remains of this infestation that constitutes the residual infestation in the following spring. The rather small number of eggs passed by the calves in the autumn will overwinter as eggs and will augment the residual infestation to a limited extent during May.

By the time the second generation of larvae appears on the herbage in August, and it is a numerous and potentially dangerous infestation, the calves which gave rise to it will be resistant to reinfection. Auto-infestation is therefore prevented and in these circumstances disease will not occur. It may be expected, therefore, that outbreaks of nematodiriasis in calves are either the consequence of simple transmission when a group of susceptible calves is put to graze on pastures contaminated in early summer by other calves, or are due to a failure of resistance and occur as a secondary complication to some other helminthiasis or disease.

## VIII. HUSBANDRY AND THE CONTROL OF NEMATODE INFECTIONS

In an important book M. C. Hall (1936) discussed the control of parasitic diseases in purely military terms. The style of this work may have been dismissed by most of its readers as whimsical or ludicrous but it expressed a philosophy which was widely accepted. This was that in a campaign using all available means of reducing parasite populations, ultimate victory was possible. Demoralised, the vanquished foe would fold up his tents and steal away, never again to return. This outlook owes much to a preoccupation with the presence or absence of the causative organism which had been inherited from classical bacteriology. It could be argued that the doctrine of spontaneous

generation might offer a more useful approach. A situation in which a parasitic life-cycle can readily be completed represents an empty ecological niche which will quickly be filled. The eradication of parasites, which is frequently presented as an attainable goal, should be seen against this background. A parasite may be regarded as eradicated if no precautions or special measures need be taken beyond those aimed at preventing its re-entry to the herd or farm. To be eradicable, a disease must either produce obvious symptoms rapidly in every infected animal or its rate of spread through the herd must be slow and every infected animal identifiable by some test. Helminthiases do not satisfy these requirements. The rate of spread through the herd is rapid yet the infection can remain at a subclinical level for many years. No test can adequately establish that an animal is worm free or that a pasture is absolutely clean. Infection can be introduced mechanically and remain capable of infecting animals for long periods. The worm free unit, described by Spedding *et al.* (1965), to permit grazing experiments uncomplicated by the presence of parasitic worms, may be regarded as a prototype of a worm free farm in a wormy world. It is, in effect, a fortress and the most rigid precautions are taken to exclude infection. Yet this unit does not remain worm free in the sense that all precautions can be relaxed within it. Populations of worms do remain at a very low level for long enough to allow experiments to be completed but pastures do not remain worm free indefinitely and a rotation of crops must be practised so that lambs reared worm free may be put on new clean pasture.

That total eradication cannot be regarded as a realistic aim does not mean that it may not be profitable to rear a particular group of animals free from worms. The procedure suggested by Spedding (1956a) for rearing fat lambs by close folding over clean ground is an example of this, although its object of achieving a rate of growth unhampered by worm infestation would probably not demand complete freedom from worms and could be achieved by simpler means.

Worms must be regarded as potentially ubiquitous; their presence on the farm may be assumed and control methods must aim at preventing the exposure of susceptible animals to excessive infection.

It is often assumed that good husbandry tends to diminish the hazard of helminthiasis and it is implied that good husbandry in this context denotes a system of practices that can be defined and is generally known and understood. This assumption is dangerous because it suggests that such a set of universally applicable precepts could be devised and that any means of reducing worm numbers in the host or on the pasture must necessarily contribute to their control (see, for instance, Gordon, 1967).

A relatively ineffective pressure on every available part of the life cycle is frequently mistaken for cutting the life cycle. Cutting a life cycle entails very positive action to prevent transmission. In the present context it is achieved by the practice of soiling, now known as zero-grazing.

A number of factors and practices are widely discussed as affecting the size of nematode infections. Among these the density of stocking, alternate stocking and rotational grazing merit some discussion. It has for long been generally accepted that maintaining many animals on a limited area is an important

element in the causation of clinical helminthiasis. Taylor (1930, 1938b), making a number of assumptions, sought to show that worm burdens would vary as the square of the stocking density. It is possible that a simple relationship between stocking density and worm burdens may exist under conditions of ranching where not all the herbage is utilised and where a decrease in density even further extends the already long average period for which infective forms must persist on the pasture before being picked up. Under agricultural conditions, however, it is not possible to alter stocking density without at the same time altering other factors, and it becomes difficult to determine whether the density of stocking, of itself, plays any part. Thus, if all the herbage grown is to be utilised, maintaining more animals on a given area must mean that the quantity of herbage grown must be increased. If a given number of infective organisms is to be suspended in a certain bulk of herbage, it matters little whether that bulk of herbage is grown on a large or a small area. However, the microclimate among dense, rapidly growing herbage will differ from that among sparse slow growing vegetation and the faeces of animals grazing such different pasture will also differ. Yet other factors come into play. Overcrowded animals may have to graze close to the ground and the concentration of larvae on the lower portion of the herbage tends to be greater. On the other hand the microclimate among a short sward will be less favourable to the larvae than that in a long sward. The plane of nutrition of the stock will also be affected. Where the pasture is stocked beyond its capacity the animals will be undernourished. On the other hand the digestibility of an intensively grazed sward will be better than that of an undergrazed pasture. The effects of stocking density, of which these are only a few, are highly complex and some are likely to affect worm burdens in one directon and some in the other. The assertion that there is a simple relationship between stocking density and worm burden cannot be justified.

Equally complex in their effects are mixed stocking and rotational grazing. It is often claimed that resistant animals or animals of another species, either when grazing alternately or together with susceptible stock, have an effect in cleaning the pastures. They have been compared with vacuum cleaners. It is obvious, however, that such animals do not selectively remove larvae from the herbage. If it were assumed that the larvae were uniformly distributed throughout the herbage, then removing a part of the herbage would not influence the concentration of larvae in the herbage remaining, and it is the concentration that is of relevance to the grazing animal. In fact, however, the distribution is not uniform, a rather greater concentration being found on the lower parts of the herbage. It is possible, therefore, by removing the upper portion, actually to increase the concentration per unit weight.

However, the practice of mixed stocking will, in effect, reduce the density of susceptible animals without introducing some of the incidental effects discussed above and may in some circumstances reduce the hazard of helminthiasis. If the interval between grazing by different host species is sufficiently long, as in the system practised in parts of N.E. England where ground is grazed in alternate years by sheep and cattle, the effect on worm burdens is, of course, very marked.

In discussing the effect of grazing resistant adult animals with susceptible juveniles, Taylor (1957) constructed balance sheets of the gain and loss of larvae to the pasture which purported to show that the larvae removed from the pasture by ewes outweighed the contamination which they contributed. This may well be true during certain parts of the year which are, however, unlikely to be of relevance to the causation of disease. Nearly all the infection producing disease in the lambs may be attributed to the ewes, and if the pasture on which ewes and lambs graze is initially clean then any contamination due to the ewes will be of importance.

Rotational grazing has been the subject both of discussion and of experiment. It is based on the belief that all the eggs in faeces develop quickly to the infective stage and that thereupon they die off quickly. It has been shown on theoretical grounds by Silverman and Campbell (1958) that even in the case of *Haemonchus contortus*, the infective larvae of which survive less well than those of other trichostrongylid worms, this is not likely to be true. Gibson and Everett (1967) have found that *Trichostrongylus colubriformis* will persist on the pasture for a prolonged period, and Michel (1967a) showed that the number of *O. ostertagi* and *C. oncophora* on pastures contaminated in early summer will remain high from July until the following March. It is evident that any practicable system of rotational grazing can have no relevance to the control of trichostrongylid worms. The disease producing generation of larvae is likely to appear on all paddocks about the same time. Before this time all paddocks will be equally harmless, after it all will be equally dangerous and will remain so for a prolonged period. After some years of experimentation Levine (1959) concluded that no system of rotational grazing could be devised to protect lambs against parasitic gastro-enteritis. Roe *et al.* (1959), Levine and Clark (1961) and Gibson and Everett (1968) made similar observations. In Australia, however, Ross *et al.* (1937) found a rotational system in which sheep were on each plot for a month and off for 3 months, and with lesser extent a second system in which the sheep were on for 1 week and off for 3 weeks, to result in smaller worm burdens and better liveweight gains than set stocking. *Dictyocaulus viviparus*, which develops faster and persists less well on the pasture, is more likely to be influenced by the practice of rotational grazing. Even here, however, the issues are complex. They were discussed by Michel and MacKenzie (1956) in the following terms:

> The comparison of rotational grazing with set stocking is likewise influenced by two antagonistic factors. In both set stocking and rotational grazing, provided that the larval output of the calves remains constant, the same total number of larvae is deposited on the entire area during the period that the rotationally grazing animals complete one circuit of all the strips. In the case of rotational grazing, however, each strip receives its full quota of larvae over a short period, so that all these larvae may contribute to the resulting herbage infestation. Under conditions of set stocking on the other hand, each portion of the field receives its quota of larvae, not at once but over a period of weeks. Since the larvae begin to die off immediately, the herbage infestation which is established in these circumstances can never be so high as that under conditions of rotational grazing.

> On the other hand, while the animals are immediately exposed to the lower herbage infestation that is achieved on the set stocking, they are not exposed to the higher herbage infestation which results on the rotationally grazed strips until some time later, by which time that higher herbage infestation has fallen. The relative merits of the two systems of grazing depend therefore on the translation of faecal larvae into herbage larvae and on their survival. Thus, if conditions for translation are particularly good, the animals grazing under conditions of set stocking may be exposed to a high herbage infestation. On the other hand, particularly favourable conditions for survival will lead the rotationally grazing animals to return to herbage infestations that are still high.

This rather variable effect of rotational grazing on lungworm infection is seen in the contrast between the observations of Michel and Shand (1955), who described a number of outbreaks of husk in adult cattle which appeared to be associated with strict rotational grazing, and those of Gregoire *et al.* (1958) and of Pouplard (1964), who are convinced that in Belgium this practice is valuable in preventing parasitic bronchitis in calves.

Two modifications or refinements of rotational grazing have been developed and are widely advocated with particular reference to sheep. In the first, known as forwards creep grazing, the lambs are given access to ground ahead of the ewes. Thus the lambs return to contaminated ground more quickly than the ewes but it is argued that in the course of their more lenient grazing the lambs will not become heavily infected. This procedure is similar to the so called Ruakura system, according to which calves graze ahead of dairy cows in a scheme of strict rotational grazing. In the second variant, known as sideways creep grazing, the lambs have access to an adjacent area which is never grazed by the ewes and it is assumed that the lambs will graze almost entirely on this area. They will, however, become infected and are then likely to contaminate the adjacent area more heavily than the ewes are contaminating the main area.

The effect of these practices on worm burden is ambiguous or small and it is open to question whether they can play a useful part in the control of helminthic disease. They could only be justified if, indeed, worm burdens increased by that continuous process of reciprocal increase on the pasture and in the animal involving many generations each greater than the last. It has been shown, however, that this is not the pattern of events. To be effective control measures must be directed with greater force and precision to protecting the stock from the disease producing generation of worms.

The same criticism may be directed against the manner in which prophylactic anthelmintic treatment is at present carried out. Hall (1932), considering primarily *Haemonchus* infections of sheep in America, advocated regular anthelmintic treatment at intervals of 3 or 4 weeks or fortnightly where conditions were more severe.

Monthly dosing is still advised for both sheep and cattle, although a number of workers have questioned whether it is economically justified (Banks, 1958; Heath, 1961) and whether fewer treatments are not equally effective (Brunsdon, 1965, 1966). The theoretical basis for monthly treatment has also been challenged, Crofton (1958c) objecting on the following grounds: "The choice

of monthly treatment has been made in the past because the majority of the more pathogenic forms complete their life history in approximately this period. The only condition under which this choice could be justified would be that in which the anthelmintic killed all stages of the parasite within the host".

Anthelmintic treatment may be employed with two distinct aims. It may be intended to reduce the worm burden in the animal and thereby alleviate its harmful effects, i.e. it may have a clinical purpose. Alternatively it may serve to reduce or prevent contamination of the pasture, i.e. have a hygienic purpose. While it was believed that worm burdens in the animal accumulated steadily until harmful levels were reached, and that the pattern of egg output would reflect its increase, these two purposes of anthelmintic treatment were almost indistinguishable and regular dosing seemed logical. This is no longer the case. In the case of gastro-intestinal worms of cattle, for example, little purpose is served by dosing calves during the early part of the season when the uptake of larvae and consequently worm burdens are small. If an anthelmintic is used which is effective only against adult worms, monthly anthelmintic treatment would interrupt the output of worm eggs for only a few days each month. Moreover, since the worms are in a state of constant turnover, and those present at any time represent what is picked up over a relatively short time, worm burdens will return rapidly to their former level after anthelmintic treatment. Further, since egg output per female in small infections is greater than in large, the effect of anthelmintic treatment on the contamination of the pasture is likely to be even less than its effect on worm burdens.

The most important contribution to thinking on the use of anthelmintics is that of Crofton (1958c), who observed that in lambs dosed at regular monthly intervals, the increase in egg counts after each dose was the same as the increase before the first dose. He argued that if the proportional decrease in worm burden was the same at each dosing and the subsequent increase at the same rate, then the effect of treatment would merely be to delay the increase and it would not matter how a given number of treatments was distributed in time. As there was no adequate reason for regular monthly dosing, treatment could be given when convenient or when the lambs were young and the costs less. This theory is probably unsound. It is based on the belief that the apparently logarithmic increase in worm egg output is due to the development of a number of generations of the parasites in a uniform environment. Even if this were so the population curve during the first two or three generations should be seen not as representing a continuous process but rather as consisting of a number of separate events with intervening areas of confusion. It is doubtful whether an increase occupying a shorter time than one generation interval may be regarded as having the same characteristics as the entire curve. The conclusion that the increase is the same, and has only been delayed, is therefore suspect. As has been shown in an earlier section, however, a very much smaller number of generations is involved than visualised by Crofton and it is clear that the timing of anthelmintic treatment is of considerable importance.

The very much greater efficiency of modern anthelmintics makes treatment with a hygienic purpose more readily possible. For example, where ewes and

lambs are turned out onto entirely clean pasture, treatment of the ewes is successful in preventing helminthiasis in the lambs (Nunns *et al.*, 1965; Thomas and Boag, 1968). The treatment of calves when they are moved to clean pasture in mid-July (Michel, 1967d) is a further example of treatment for purposes of pasture hygiene.

Since worm burdens are not built up by a protracted process of accumulation but are in a state of constant turnover so that those present at any moment have been acquired during a short space of time, even efficient anthelmintic treatment will only remove worms which in any case have only a short expectation of life. Treatment intended primarily to reduce the worm burden can only be justified, therefore, to remove a disease producing or potentially disease producing worm burden. However, if the worms that are removed are very rapidly replaced where animals remain on a heavily infested pasture or where, as in winter ostertagiasis, replacement is by the development of formerly inhibited forms, the value of such clinical treatment is limited. It is only if the rate of replacement is greatly reduced, either by removing the stock from the source of infection or, as in *Dictyocaulus* infection of cattle, because the host becomes rapidly refractory to reinfection, that the efficient drugs now available are used to full advantage.

The possible effect of anthelmintic treatment on the development of host resistance has been considered by Soulsby (1962) and others. Some workers have shown in experimental systems that the premature removal of worms will reduce the resulting immunity (Roberts and Keith, 1959; Ross, 1963; Kendall, 1965). The only evidence obtained under field conditions is provided by Crofton (1958c), who showed that in lambs which had been regularly dosed during the summer, faecal egg counts remained high in autumn while those of undosed lambs fell to a low level. He also suggested a connection between winter trichostrongyliasis in hoggs and the intensive anthelmintic treatment they had received as lambs.

It is occasionally suggested that the control of all helminthiases may ultimately be achieved by vaccination. This is open to question. Live vaccines consisting of attenuated larvae like that used against lungworms and the experimental vaccines against *Uncinaria* (Dow *et al.*, 1961) and *Ancylostoma caninum* (Miller, 1965) demand that the parasite should satisfy a number of requirements. First, limited experience of infection must rapidly engender in the host an adequate resistance to the establishment of worms. Second, this resistance must be evoked by developmental stages of the parasite earlier than those which cause disease. Third, it must be technically possible so to attenuate the organism that it will develop sufficiently far to elicit resistance but not far enough to cause disease. The number of nematodes which satisfy these requirements is small. The possibility should not be neglected, however, that dead vaccines could be developed by the perfection of methods of *in vitro* culture which, by suitable formulation and the use of adjuvants, might overcome some of these difficulties.

Vaccination as a method of control can only be effective when worm populations are normally regulated by the protective immunity of the host. Thus it is appropriate in the case of *Dictyocaulus viviparus* where the number of

generations of the parasite is limited by the rapid acquisition of a resistance to the establishment of worms, and inappropriate in the case of *Ostertagia ostertagi* where the number of generations is limited by the time occupied by free living existence and the disease producing infection appears fairly suddenly. Vaccination may also prove to be an unsuitable method of control where disease is due to the development of inhibited forms, as in winter ostertagiasis of cattle and probably winter trichostrongyliasis of sheep. It may be impracticable where disease is caused by the infection of animals that have lost their resistance, such as husk in adult cattle and haemonchiasis of Australian ewes. Vaccination is likely to be ineffective where disease results from the simple transmission of infection from one group to another, a situation which, as noted above, can in most cases be avoided.

## IX. Conclusions

Disease due to the nematode infections considered here, is the consequence of exposing insufficiently resistant animals to grazing that is too heavily infected so that they pick up infective larvae at an excessive rate. The disease producing infection is acquired over a relatively short space of time, in some cases because the animals quickly become refractory to infection, in others because worm numbers are regulated in the host to maintain a level proportional to the rate at which new infection is acquired.

Susceptible animals can encounter heavy infections either when they graze a pasture contaminated by another group of animals (simple transmission) or when they themselves build up an infestation on the pasture without, in so doing, becoming resistant (auto-infestation). Sometimes the disease producing infection is derived from both sources. The number of generations of the parasites involved in the process of auto-infestation is always small. This is due either to the rapid development by the host of a resistance to reinfection or to the long time taken for the free living phase of the life cycle to be completed. In some cases both factors operate.

Disease due to the simple transmission of infections can be avoided by withholding groups of susceptible animals from pasture contaminated by other groups. The choice of control measures where disease is due to auto-infestation depends on how the number of generations involved is normally limited. Where this is due to a lengthy free living phase, the appearance of the disease producing generation of larvae can generally be predicted and measures taken to remove susceptible stock from exposure to it. Where the rapid development of host resistance restricts the number of generations of the parasite, the artificial enhancement of resistance by vaccination is a promising approach to control.

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# Meteorological Factors and Forecasts of Helminthic Disease

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## I. Introduction

In recent years much research has been carried out on the epidemiology of helminthic diseases of farm livestock. In many published reports mention is made of the important effect of climate* on the development or availability of some stage of the life cycle, possibly even on the distribution of disease, yet very few reports enable the variations in populations of helminths and the resulting incidence of disease to be determined or predicted from an analysis of climate. There are several reasons for this. Perhaps climate does not play a sufficiently important part to allow such relationships to be developed, although there is little evidence that this is so. Individual workers have often been concerned primarily with some other aspect of epidemiology and their studies on the effect of climate on the helminth life cycle have had a limited objective. Even in more broadly based investigations problems have arisen.

* In this review, some difficulty is encountered in the use of the terms "weather" and "climate". "Weather" is a description of the meteorological conditions at a given time and place. In English usage "climate" is generally taken to refer to the weather over a long series of years expressed in terms of averages or frequencies. In French "climât" refers to the accumulation of weather over a series of days or months in quite short periods of consecutive time. Wherever possible herein, the French sense of the term has been indicated by use of the qualifying adjectives "current" or "ambient" to distinguish it from the long term "climate".

The environment of the parasite is never that in which standard meteorological measurements are made. The interaction between the macroclimate, the soil, and a variable herbage cover providing a micro-scale environment in which the parasite passes from fresh but inconsistent faeces onto the herbage, defies precise analysis. With some helminthic diseases, e.g. parasitic gastro-enteritis in sheep, several different species of nematodes may be involved. These are unlikely to show identical responses to changes in weather or host. Even where single species are involved, different stages in the life cycle may respond differently to changes in weather and, for instance, the nematode egg is usually more resistant to desiccation than the larva. With many helminthic diseases the intensity of disease is not simply related to the level of the population of the infective stage on the pasture. The final host develops varying degrees of resistance and this is an important factor in determining whether or not disease occurs. With trematode parasites, an added complication is that an intermediate snail host is necessary for completion of the life cycle so that the response of the intermediate host to ambient weather also must be taken into account. Faced with the task of solving these problems it is not surprising that a variety of methods of investigation have been employed.

## II. Laboratory Observations

The simplest approach is typified by the collection of helminth eggs, separation from faeces and incubation at constant temperatures. The time taken for eggs to hatch at different temperatures is noted. In due course it is possible to plot a time-temperature graph. An example of this simple approach into the effect of temperature on the development and hatching of the egg of *Fasciola hepatica* is given by Rowcliffe and Ollerenshaw (1960). Where the hatching process is more complex, as with *Nematodirus battus*, there is a need for a more sophisticated approach, as described by Thomas and Stevens (1960), where eggs were developed in one temperature until the third larval stage was completed, then maintained in a number of low temperatures for varying periods before being returned to a higher temperature for hatching. Christie (1962) used even more elaborate techniques for his laboratory studies on the same species. In the interpretation of such research it must be remembered that in the field the temperature is varying almost continuously.

Whether or not studies of this type are valuable in helping to assess the role of temperature in the epidemiology of a particular disease depends greatly on the results. Where development shows a direct relationship with temperature or where it takes a relatively long time for a particular biological process to be completed or where the critical temperature below which development does not take place is high relative to those occurring under natural conditions, then such studies can be of great value. For example, there is no development of the egg of *F. hepatica* below 10°C whilst in the range 10–20°C development to hatching may take several weeks. Clearly these findings are of great importance in assessing the rate of development of this parasite in cool temperate climates where temperatures may be below 10°C for several months in each year. Moreover, since development in the remaining months

is likely to be slow, deviations from normal temperatures are likely to cancel each other out. With the final host showing little resistance to *F. hepatica* there is likely to be a continuous output of fluke eggs onto the pastures unless infected animals are treated to remove adult flukes. In these circumstances, temperature is likely to play an important role in determining the seasonal incidence of disease, and it is possible to make a reasonable speculation concerning events in the field from simple laboratory observations in constant temperatures.

In many instances it is more difficult to make any valid comment on the life cycle of a parasite in the field as a result of limited laboratory observations. Development may be rapid and measured in days in temperatures similar to those obtaining under natural conditions, as occurs with *Haemonchus contortus* according to Dinaburg (1944), or the critical temperature may be so low so that some development is possible all the year round, as with *Dictyocaulus viviparus* (see Michel and Rose, 1954). Further difficulties of interpretation arise where development of eggs in water may not be the same as in faeces, or where other climatic factors such as oxygen tension exert an important effect on the rate of development. Daily variations in temperature may be important where hatching takes place in two or three days. Resistance mechanisms may develop quickly, so that the supply of eggs to pastures is variable and discontinuous. In these circumstances, it may be unprofitable to speculate on the seasonal distribution of disease from laboratory observations at constant temperatures.

In a similar way it is possible to study the response of eggs, larvae and metacercariae to the effect of moisture by establishing chambers at varying humidities in a variety of constant temperatures. The parasites may be studied either in faeces or individually on microscope slides. Modifications to this approach may involve simulating dew or alternating periods of wetness and dryness, as described by Taylor (1934). The results of these experiments in the laboratory are even more difficult to interpret in terms of natural conditions than those involving temperature, because it is extremely difficult to make a valid assessment of the variable microclimate within the faeces–herbage complex.

A number of workers have attempted to overcome the difficulty of interpreting results obtained in constant temperatures in terms of fluctuating temperatures by placing cultures outdoors. Often this type of experiment is carried out alongside those in constant temperatures (see Rowcliffe and Ollerenshaw, 1960) so that a more reliable assessment of events in the field is possible. Even this type of experiment can be criticised in that the eggs are usually maintained in a very artificial environment.

## III. Plot Observations

Most workers regard results obtained in the artificial environments obtainable in the laboratory as a guide to help formulate experiments on the bionomics of the free living stages of parasites under conditions more closely approximating to those on pasture. These involve the use of small plots.

Some workers used normal pasture divided into units by metal dividers (Gibson, 1958). Others used metal boxes containing soil and turf where some degree of moisture control was possible by leaving the boxes covered or exposed to rain, or kept moist artificially (Rose, 1961, 1962). A number of workers used even smaller units, for example plant pots containing soil previously sown with grass seed (Thomas and Stevens, 1960). Dinaburg (1944) used plant pots sunk into the ground in a variety of situations, such as direct sunlight and shade. Purists might argue that such small plots bear little resemblance to natural pasture, and that even sinking metal dividers into permanent pasture is likely to interfere with the microclimate in the herbage. These objections can be set aside provided that the limitations of these methods are recognised by all concerned.

The plots are then infected with the appropriate stage of the parasite usually at regular intervals over a period of time. Faeces containing eggs have been made diarrhoeic artificially and spread on the turf or they have been left as pellets or made into large pats. Representative samples of faeces and herbage are examined at intervals in time and space in order to study rates of development, viability, movements of larvae from faeces onto herbage, and vertical distributions of larvae within the herbage. At the same time climatological records are kept, mainly of temperature and rainfall. Thus, much valuable information on the specific characteristics of eggs and larvae has been obtained. Michel and Rose (1954) and Rose and Michel (1957) showed that the larvae of *Dictyocaulus viviparus* did not succeed in moving out of faeces onto herbage, and that the consistency of faeces had a great bearing on the subsequent intensity of the herbage infection. If the faeces were diarrhoeic it was possible for a high population to develop, though at the same time the larvae were much more exposed to the possibility of desiccation. From such results it is possible to speculate on the type of weather which facilitates translation of larvae from faeces into herbage. It is rarely possible, however, to quantify these results in terms of the incidence of disease.

Another difficulty with the use of the experimental plot is that of simulating the grazing animal. The relationship between grazing stock and growing grass is not static; there is a continual and selective turnover of herbage which must influence any parasite population, and this is difficult to reproduce on an experimental plot. A further drawback with this type of experiment is that it relates events in the free living stages of the parasite's life cycle to a particular set of weather conditions. It rarely provides sufficient information as to what would happen to the parasite under a different set of weather conditions. Some of the most interesting results derived from experimental plots have been obtained when they were carried out under unusual weather conditions. Thus Gibson and Everett (1967) noted that *Trichostrongylus colubriformis* survived much better in winter when there was a ground cover of snow over a long period, an observation made possible solely by S.E. England experiencing one of its snowiest winters on record in 1963. Similarly Rose (1961, 1962) showed that *Ostertagia ostertagi* survived a long drought in the summer of 1959 and subsequently developed in the autumn rains to produce an unusual and late peak infection on the herbage.

The main advantage of the experimental plot is that with limited resources it reinforces laboratory observations particularly in respect of the effect of temperature. From an analysis of the results it is usually possible to show that temperature determines the seasonal availability of the infective stage of the parasite on the herbage, particularly in cool temperate climates where temperatures tend to be near or below the critical temperature in winter. Temperature determines the seasons when it is possible for the herbage to be infected, but the magnitude and duration of the infection usually depends on other factors.

It does not always follow that disease is an inevitable consequence of a high population on the herbage of the infective stage of the parasite; the grazing animal may or may not be susceptible or even available at the crucial period.

## IV. The Experimental Paddock

Because of the difficulty of relating herbage infections to disease in grazing stock a number of researchers have utilised experimental paddocks as a means of following the epidemiology of helminthic disease. A worm free pasture is obtained and infected sheep or cattle introduced to set up an initial infection. First attempts usually involve single species, but recently more elaborate observations involving more than one species have been undertaken. Subsequently, worm free animals are introduced at varying intervals and both faeces and herbage samples are examined regularly in order to ascertain the course of events. By dosing and/or moving stock at selected times, the most advantageous techniques for controlling disease can be devised. Some skill in husbandry is required in the manipulation of this technique, because experimental paddocks cannot be large in area. It is not easy to select suitable pastures which may be divided into uniform paddocks or to adjust the stocking rate so that the quantity of grass is in reasonable balance with the grazing of stock. Similarly there may be a need to maintain some balance between the parasite and its host; it may be important that not all the parasites or hosts should die. As there is often a need to continue observations for more than 1 year, there may be difficulty in keeping stock free from other parasitic infections or diseases.

Where it is not possible to set up a parasitic infection on normal pasture, as with fascioliasis, then a suitable natural snail habitat is selected, fenced off and used as the experimental area. At times experimental paddocks are used for more limited objectives. Stock may graze them for set periods when they are removed and killed in order to determine when the level of infection is at its peak (Ross, 1967). This technique had been used earlier in America by Goldberg and Rubin (1956), who placed worm free calves at intervals on a natural pasture which had been contaminated with a mixed infection of nematodes in order to determine the survival times of these parasites under natural conditions.

Some workers, such as Michel (1966, 1968), have carried out observations on experimental paddocks and continued their researches on natural populations to determine whether their conclusions were valid under different

conditions of management and climate. Crofton (1949, 1952) also studied the development and survival of sheep strongyles under varying natural conditions such as hill and lowland farms. In one series of observations he noted that the peak herbage infection occurred at different times in different fields on the same farm, and suggested that other factors besides the macroclimate were of great importance in the epidemiology of helminthic disease.

The results of all these various approaches to understanding the epidemiology of helminthic disease and its control have been variable. On the one hand research by Thomas and Stevens (1960) and Gibson (1963) on *Nematodirus battus* showed that the infection passed from one lamb crop to the next with the adult ewe playing a minor part in the contamination of pasture with eggs. Under these conditions they were able to prescribe suitable husbandry practices, namely, lambing in fields not grazed by the previous season's lambs, which prevented the possibility of infection. In a similar way Michel (1966, 1968) has recommended controlling parasitic gastro-enteritis in cattle in Britain by dosing and moving young stock to new pastures in mid-July, normally to aftermaths following hay or silage.

Neither of these two recommendations has been formulated as a result of detailed knowledge of all aspects of the epidemiology of these parasites. The recommendations for controlling nematodiriasis stem mainly from the resistance of the ewe, whilst those for controlling parasitic gastro-enteritis in cattle depend on the fact that high herbage infections are not normally encountered until after mid-summer. With both these diseases control does not depend on assessing the intensity of infection, which can vary from year to year; it offers a system of management which can be undertaken each year to control any disease that might arise.

Another result of these studies is to be found in the use of the bioclimatograph introduced by Gordon (1948) in Australia. This was based largely on the observations of Dinaburg (1944) on *Haemonchus contortus* in America. He reported that monthly rainfall of at least 2 in when mean maximum temperatures were above 65°F was required for the eggs of *H. contortus* to hatch and produce infective larvae on the herbage. Gordon (1948) plotted the monthly means of maximum temperature and rainfall for a number of meteorological stations in Australia and by comparing these values with the selected parameters of Dinaburg he was able to determine where and when the parasite was likely to occur. Other workers, notably Roberts *et al.* (1952), Forsyth (1953) and Pullar (1953), used the same approach in Australia, Cameron (1956) in Canada and Levine (1959) in America. A number of different criteria were used both for *H. contortus* and for *Trichostrongylus* spp. but, as reported by Levine (1963), no investigator has been fully satisfied with the results obtained with bioclimatographs. This was to be expected when mean monthly figures were used and when the simple rainfall statistic was employed as an estimate of soil moisture in different countries experiencing varying conditions of transpiration, temperature, sunshine and soil type.

In spite of its defects, the use of the bioclimatograph to explain the distribution in space and time of pasture nematodes must not be condemned out

of hand, because it represented the first rational attempt to utilise climatic data to explain important features of the epidemiology of helminthic disease. The attempt did not succeed mainly because the basic data on the response of the parasite to climate and the analysis of that climate were much too crude to allow the detailed correlations which were attempted.

Apart from these achievements there is a wealth of knowledge showing how individual parasites respond to their environment in a particular situation, either controlled or under natural conditions, but it remains extremely difficult to use this information as a basis for determining the response of the parasite (possibly as a mixed population) to a different and varying sequence of weather conditions. Levine (1963) provided a succinct summary of our knowledge in this respect. When describing the preferred climates of different species of nematodes he had to resort to descriptions such as cool moist, cool dry or warm moist.

In trying to evaluate our present knowledge on this subject one is struck by its increasing complexity. The free living stages tend to live longer and be more resistant to adverse factors than expected. Resistance mechanisms by the final host become more involved. Parasite populations are not static, for there is continued loss and replacement. Different species involved in one disease entity such as parasitic gastro-enteritis behave differently. Even the same species in different countries responds differently, cf. response of *H. contortus* to temperature according to Dinaburg (1944) in America, and Silverman and Campbell (1959) in Scotland. The most recent views are that some disease syndromes are caused by the interaction between a variety of pathogens, the nutrition and stress of the host and the external environment. If this is so, experimentation and control will become increasingly complex. In this situation seemingly the only constant factor in epidemiological studies on helminthic disease is that the assessment of current climate is still measured in terms of the effects of temperature and rainfall.

Certainly one feature of all recent work has been to shatter many of the preconceived ideas on control. It is no longer accepted that parasites need to complete several generations in a grazing season in order to produce dangerous levels of infection on herbage. Control by simple rotational grazing or by mixed stocking has been shown to be untenable. Apart from a few notable exceptions there has been little to replace these old ideas. There are now a number of valuable drugs available for treatment, but we are not able to make the best use of them. We still persist in the view that the free living stages of helminths are weather dependant, yet in spite of a great deal of painstaking research very few attempts have been made to relate the level of a parasite population to weather or to predict what might happen to the parasite population from knowledge of climate.

## V. Climate and Incidence of Disease

It is perhaps an opportune time to consider a different approach to this problem. One of the major difficulties of research into the role of climate in biological processes is that there is no way of controlling the climate. With

any of the approaches discussed earlier, investigations cannot be carried out under all possible variations of weather. There is, however, sufficient evidence both from past epizootics and from isolated epidemiological investigations (see p. 286) that it is under extremes of weather that unusual and extreme variations occur in helminth populations. Clearly it is not practical for most workers to repeat a series of observations for more than 3 years, yet this period is unlikely to encompass all possible variations of weather. Faced with this situation and recognising that the basic relationships between ambient climate and helminthic disease will be more easily identified under extremes of weather and incidence, it would seem both reasonable and profitable to attempt to relate the two, directly using all available information but without the necessity of having to understand in detail all the processes involved.

The approach initially necessitates the collection of data on the overall incidence of disease and in particular information about years of extreme incidence. Depending on the reliability of disease surveys such records serve several purposes. They demonstrate which diseases are the more important economically and hence where research effort should be concentrated. Examination of such records in relation to changes in farming patterns may reveal disease problems which are on the increase or in decline. Variations in the geographical distribution of disease may reveal unexpected features. These benefits are not confined to helminthic diseases.

Where a disease entity is caused by several different species, as with parasitic gastro-enteritis in sheep, the most important species in any area should be revealed, and again research effort can be concentrated on those species doing most damage. If weather factors do in fact have a major role in the epidemiology of any helminthic disease, a comparison between the weather in high and low incidence years may in the light of existing knowledge reveal suitable parameters for establishing a relationship between climate and disease.

Where unusual changes in the incidence of disease arise over a short period of time these may be identified as being due to a particular weather sequence. There is often much uninformed speculation about particular diseases being on the increase or decrease, or whether disease pathogens are changing in virulence or becoming resistant to drugs. Whilst disease pathogens do change, an awareness of the importance of meteorology in disease processes often reveals that the pathogen is responding normally to an abnormal sequence of weather at a critical time or place. The severe epizootic of foot and mouth disease in Cheshire in 1967/1968 serves as a good example.

There are of course a number of difficulties in this approach to epidemiological research. The objective assessment of disease incidence may be a formidable task. There is, in fact, very little information and that which does exist is highly subjective. The scarcity of information on incidence is in marked contrast with that available on weather, of which there is an embarrassing wealth of data. In these circumstances there is a need to handle the information with caution if reliable relationships are to be established. It is important to recognise that there may be some reluctance to accept what might be considered an empirical approach which may be valid only over a limited area.

In spite of these difficulties there are a number of advantages which accrue from this approach. It makes the most of existing knowledge, progress can often be made with limited resources, and it is a constructive process in that relationships which are established can be put to practical advantage either in respect of forecasting disease incidence, or identifying weather patterns favouring particular diseases, or in pinpointing critical aspects of disease processes which demand further experimental work. For instance, if it is shown that dry weather in a particular series of months appears to be an important factor in giving rise to a particular disease, it should be profitable to devote research effort to that particular problem should the necessary sequence of weather occur. This method of approach is admirably suited to studying the complex disease syndromes which seem to be on the increase, because it concentrates attention on the occurrence of disease in relation to the total environment.

This approach is part of the essential review of any disease problem which must be carried out before progress can be planned. It may be useful to consider in some detail some of the approaches which have been made with a number of helminthic diseases particularly in Britain.

### A. FASCIOLIASIS

Fascioliasis is a disease which involves an intermediate snail host in addition to free living stages, and might therefore be expected to show varied responses to climate. In this instance, however, there is much evidence that epizootics are associated with wet summers, and indeed this has long been part of traditional knowledge. Documentation of past epizootics of this disease in Britain is perhaps second only to that of potato blight, no doubt because of the serious economic consequences which followed epizootics. Prominent among those who have contributed to our knowledge of epizootics are Dun (1881), Thomas (1881), Walton (1917, 1922), Montgomerie (1926), Thompson (1929), Peters (1938) and Jones (1964), as well as a number of anonymous reports in Ministry of Agriculture Journals and the Farming Press. There are in fact reports of epizootics extending back to the early part of the eighteenth century, a period when the distribution of sheep was much different from that today and when there were few meteorological records. Thanks to the work of Nicholas and Glasspoole (1931) it is possible to express monthly rainfall over England and Wales as far back as 1727 as a percentage of the monthly average from 1881–1915. In the early part of this period these figures were based on the records of very few meteorological stations. Although more meaningful rainfall parameters are now available this parameter has been used throughout Table I in order to facilitate comparison of the weather over the entire period. Serious outbreaks of disease were reported in the winters following 1735, 1738, 1745, 1747, 1766, 1768, 1783, 1789, 1792, 1794, 1797, 1808, 1809, 1816, 1817, 1823, 1824, 1828, 1829, 1830, 1831, 1852, 1853, 1860, 1879, 1880, 1881, 1912, 1913, 1920, 1924, 1931, 1936, 1946, 1954 and 1958. The majority of the reports concerning the eighteenth and early nineteenth centuries stem from Baker quoted by Jones (1964), and should not be

TABLE I

*Monthly rainfall over England and Wales expressed as a percentage of the monthly average from* 1881–1915. *A high incidence of disease was recorded in the winters of the years marked with an asterisk*

| Year | Jan. | Feb. | Mar. | Apl. | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1734 | 56 | 167 | 107 | 56 | 230 | 137 | 81 | 141 | 81 | 96 | 63 | 200 |
| 1735* | 103 | 93 | 115 | 97 | 98 | 119 | 139 | 97 | 103 | 62 | 117 | 68 |
| 1737 | 48 | 132 | 138 | 85 | 63 | 66 | 35 | 276 | 185 | 64 | 33 | 81 |
| 1738* | 96 | 40 | 64 | 76 | 108 | 148 | 37 | 69 | 115 | 73 | 39 | 54 |
| 1744 | 52 | 66 | 94 | 182 | 65 | 165 | 45 | 55 | 191 | 136 | 108 | 43 |
| 1745* | 75 | 38 | 171 | 162 | 80 | 176 | 43 | 56 | 52 | 106 | 104 | 73 |
| 1746 | 111 | 112 | 116 | 75 | 56 | 160 | 102 | 24 | 113 | 77 | 91 | 6 |
| 1747* | 163 | 101 | 69 | 115 | 139 | 122 | 77 | 11 | 110 | 34 | 141 | 165 |
| 1765 | 34 | 55 | 161 | 165 | 32 | 73 | 23 | 120 | 81 | 152 | 70 | 40 |
| 1766* | 9 | 96 | 30 | 118 | 179 | 163 | 93 | 53 | 112 | 72 | 69 | 50 |
| 1767 | 96 | 179 | 105 | 57 | 117 | 45 | 201 | 82 | 100 | 85 | 92 | 29 |
| 1768* | 132 | 199 | 27 | 137 | 61 | 208 | 169 | 107 | 212 | 109 | 170 | 95 |
| 1782 | 130 | 47 | 124 | 200 | 213 | 70 | 151 | 154 | 174 | 96 | 54 | 38 |
| 1783* | 110 | 140 | 70 | 29 | 155 | 149 | 78 | 101 | 191 | 52 | 78 | 33 |
| 1788 | 61 | 100 | 58 | 54 | 47 | 81 | 118 | 93 | 141 | 29 | 34 | 14 |
| 1789* | 116 | 145 | 58 | 97 | 138 | 202 | 158 | 38 | 149 | 128 | 84 | 105 |
| 1791 | 175 | 124 | 50 | 123 | 71 | 51 | 139 | 95 | 59 | 103 | 159 | 100 |
| 1792* | 111 | 66 | 130 | 181 | 144 | 115 | 128 | 123 | 213 | 94 | 53 | 117 |
| 1793 | 96 | 123 | 101 | 85 | 45 | 69 | 56 | 110 | 124 | 62 | 90 | 88 |
| 1794* | 51 | 132 | 84 | 129 | 85 | 32 | 83 | 88 | 192 | 128 | 136 | 70 |
| 1796 | 139 | 93 | 25 | 60 | 171 | 80 | 135 | 37 | 106 | 79 | 71 | 64 |
| 1797* | 80 | 20 | 50 | 117 | 169 | 175 | 97 | 136 | 219 | 71 | 84 | 119 |
| 1807 | 54 | 101 | 44 | 63 | 193 | 87 | 76 | 73 | 136 | 64 | 138 | 51 |
| 1808* | 67 | 51 | 18 | 145 | 106 | 73 | 130 | 91 | 135 | 121 | 95 | 65 |
| 1809* | 148 | 118 | 25 | 125 | 94 | 96 | 91 | 144 | 166 | 17 | 66 | 93 |
| 1815 | 41 | 86 | 155 | 91 | 128 | 110 | 77 | 92 | 114 | 103 | 74 | 97 |
| 1816* | 100 | 77 | 85 | 113 | 107 | 110 | 173 | 77 | 130 | 96 | 94 | 113 |
| 1817* | 112 | 110 | 94 | 16 | 154 | 139 | 144 | 162 | 39 | 41 | 37 | 131 |
| 1822 | 43 | 98 | 107 | 127 | 83 | 57 | 202 | 71 | 57 | 124 | 154 | 49 |
| 1823* | 109 | 170 | 73 | 99 | 111 | 91 | 141 | 135 | 113 | 127 | 75 | 119 |

TABLE I (*continued*)

| Year | Jan. | Feb. | Mar. | Apl. | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1824* | 47 | 90 | 123 | 118 | 103 | 155 | 60 | 84 | 175 | 137 | 164 | 149 |
| 1827 | 82 | 55 | 162 | 70 | 134 | 71 | 68 | 90 | 147 | 139 | 76 | 143 |
| 1828* | 71 | 82 | 53 | 182 | 101 | 103 | 243 | 112 | 126 | 60 | 76 | 102 |
| 1829* | 38 | 61 | 35 | 219 | 32 | 142 | 177 | 183 | 186 | 66 | 63 | 26 |
| 1830* | 65 | 93 | 35 | 163 | 153 | 181 | 104 | 113 | 187 | 37 | 131 | 64 |
| 1831* | 76 | 138 | 122 | 114 | 80 | 97 | 127 | 96 | 148 | 141 | 111 | 112 |
| 1851 | 154 | 53 | 155 | 92 | 60 | 110 | 128 | 81 | 54 | 85 | 47 | 37 |
| 1852* | 209 | 102 | 25 | 44 | 107 | 219 | 97 | 167 | 182 | 131 | 233 | 133 |
| 1853* | 131 | 68 | 65 | 127 | 69 | 155 | 161 | 93 | 103 | 108 | 76 | 32 |
| 1859 | 72 | 87 | 97 | 150 | 62 | 105 | 91 | 108 | 175 | 98 | 98 | 86 |
| 1860* | 158 | 69 | 118 | 71 | 150 | 243 | 87 | 159 | 123 | 85 | 96 | 101 |
| 1878 | 94 | 79 | 58 | 126 | 205 | 130 | 53 | 172 | 112 | 102 | 132 | 69 |
| 1879* | 98 | 167 | 59 | 128 | 126 | 213 | 156 | 178 | 144 | 36 | 43 | 41 |
| 1880* | 21 | 128 | 73 | 100 | 66 | 126 | 190 | 46 | 177 | 154 | 107 | 122 |
| 1911 | 58 | 95 | 88 | 88 | 67 | 124 | 23 | 66 | 96 | 93 | 136 | 174 |
| 1912* | 152 | 104 | 180 | 15 | 92 | 195 | 131 | 216 | 73 | 100 | 91 | 113 |
| 1913* | 169 | 54 | 163 | 184 | 114 | 70 | 49 | 51 | 117 | 103 | 103 | 55 |
| 1919 | 161 | 119 | 185 | 120 | 45 | 59 | 83 | 96 | 87 | 70 | 85 | 149 |
| 1920* | 148 | 74 | 135 | 221 | 131 | 103 | 187 | 60 | 108 | 71 | 52 | 94 |
| 1923 | 83 | 235 | 77 | 126 | 119 | 34 | 102 | 113 | 130 | 136 | 108 | 105 |
| 1924* | 123 | 42 | 57 | 139 | 217 | 102 | 153 | 113 | 172 | 129 | 83 | 125 |
| 1930 | 170 | 44 | 99 | 134 | 103 | 59 | 145 | 132 | 180 | 92 | 148 | 102 |
| 1931* | 108 | 128 | 33 | 175 | 153 | 149 | 148 | 143 | 120 | 34 | 150 | 47 |
| 1935 | 68 | 157 | 37 | 181 | 62 | 154 | 29 | 76 | 208 | 147 | 170 | 96 |
| 1936* | 175 | 123 | 89 | 94 | 54 | 154 | 187 | 40 | 138 | 61 | 121 | 93 |
| 1945 | 115 | 121 | 49 | 83 | 141 | 134 | 94 | 84 | 99 | 105 | 23 | 105 |
| 1946* | 115 | 135 | 58 | 88 | 132 | 138 | 108 | 163 | 193 | 39 | 178 | 101 |
| 1953 | 46 | 81 | 41 | 139 | 109 | 98 | 134 | 98 | 125 | 72 | 80 | 36 |
| 1954* | 87 | 131 | 117 | 29 | 131 | 142 | 124 | 139 | 139 | 122 | 189 | 89 |
| 1955* | 116 | 104 | 83 | 65 | 178 | 143 | 37 | 35 | 81 | 77 | 71 | 108 |
| 1957 | 97 | 152 | 102 | 19 | 84 | 79 | 146 | 122 | 196 | 75 | 72 | 84 |
| 1958* | 97 | 185 | 83 | 50 | 124 | 206 | 119 | 120 | 157 | 89 | 58 | 126 |

relied on implicitly. In all probability a number of these reports refer to events in Baker's own locality, though at times he refers to losses as far afield as Norfolk, Hereford and Lancashire. As far as can be judged, the years 1830, 1860 and 1879 were the worst years in the nineteenth century, whilst 1924, 1946, 1954 and 1958 were the worst in the present century. Perhaps the most serious epizootic ever reported was that occurring over the period 1878–1880, when some estimates put the loss of sheep at 6 000 000. It was at the instigation of the Royal Agricultural Society following this epizootic that Thomas began the researches which led to the discovery of the life cycle of *F. hepatica.*

Although some of these outbreaks were limited in their occurrence, that in 1920 being confined mainly to N. Wales, a feature of many outbreaks was their simultaneous occurrence in England, Wales and Ireland. Generally, there are few reports of catastrophic outbreaks in Scotland. On this evidence any relationship between climate and disease need not necessarily apply to a localised area. Except on one occasion (1794) a high incidence of disease always followed a wet summer. The usual weather pattern associated with epizootics was where every summer month from May to September had above average rainfall. Some latitude in this sequence was possible and there were occasions when May, July, August, or September, but not June (with the exception of 1808), had well below average rainfall without preventing a high incidence of disease. In 1860, 1879 and 1958, probably even the worst fluke years ever recorded, rainfall in June was more than twice the long term average. The importance of June weather in the production of disease is confirmed by the absence of a high incidence of disease following wet summers in which June had below average rainfall. Such years occurred in 1799, 1877, 1889 and 1908.

There is a suggestion in the records that a further weather pattern may produce a high incidence of disease. The important sequence in this case is a wet late summer followed the next year by a wet early summer and a dry late summer. This sequence was seen in 1737/1738, 1912/1913 and 1954/1955. Additional information regarding the timing of losses in 1955 is available from the records of the Veterinary Investigation Centre in Cardiff which covers S. Wales, where this particular outbreak was most serious. In 1955 losses from acute disease began much earlier than usual—in late July—a peak being reached in September and losses declining to a low level by November. This pattern contrasts with the normal sequence as in 1958, when losses in the same area reached a peak in December/January.

A further feature about the correlation between incidence and weather was that a high incidence could arise following a single wet summer. For instance in 1919 there was below average rainfall in each summer month prior to the outbreak in late 1920. There appeared no necessity to have a sequence of wet summers as a necessary prerequisite to an epizootic, though when such a sequence did arise as in 1877/1878/1879 or 1953/1954 or 1957/1958 the resulting incidence was correspondingly greater.

Apart from summer, the weather in the remainder of the year appeared to have little effect on the course of the life cycle and the incidence of disease. For instance, one could postulate that a dry autumn and early winter might

facilitate the access of stock to the wetter areas of pasture where the snail intermediate host is found. This period was dry in 1879 and 1958 but in 1954 it was very wet. Similarly dry springs seem to have little effect on subsequent incidence, because the springs of 1936 and 1958 were dry.

These observations indicate the great dependance of *Fasciola hepatica* on weather factors, irrespective of the wide variations in husbandry practices and methods of control over a period of two centuries. They show that in some way both the parasite and its snail host can respond rapidly to any relaxation in the environmental pressure with consequent rapid changes in the incidence of disease. Further development of this approach would have resulted in a simple reliable relationship between weather and the incidence of disease without any real knowledge of the course of events during a wet summer. Information from experimental studies on the free living stages of the parasite and the snail host was becoming available so that a more soundly based approach to the problem appeared possible.

Studies on the bionomics of the fluke egg (Rowcliffe and Ollerenshaw, 1960) confirmed previous work that there was no development and hatching of eggs below 10°C. By observing the development and hatching of eggs under outdoor conditions in S.E. England they were able to show that with few exceptions hatching occurred in the period May to October—the months when soil temperatures exceeded 10°C. Eggs hatching in May were in cultures placed outside in the previous autumn. Under field conditions few of these eggs were likely to survive and infect snails in May.

During the months from June to September development to hatching could be completed in about 3 weeks, but in May and October the rate of development was about half that in mid-summer (Fig. 1). Kendall and McCullough (1951) demonstrated that there was no development of the parasite or liberation of cercariae from the snail host *Lymnaea truncatula* below 10°C. By infecting snails from May to October, and maintaining them under optimum conditions outdoors, it was found that snails infected up to early August produced cercariae before the end of October. In snails infected after mid-August the parasite was unable to develop sufficiently to emerge from the snail before winter temperatures restricted development (Fig. 1). The parasite overwintered in the snail and emerged to infect herbage in the following early summer (Ollerenshaw, 1959, and in press). Under the temperature regime of S.E. England, herbage could become infected from two sources in each year.

On the basis of these limited observations some comment on the geographical distribution and course of the life cycle of *F. hepatica* is possible. In the extreme north of Scotland, where temperatures are above the critical 10°C only from June to September and where summer temperatures are lower than in S.E. England, completion of the life cycle in one summer is difficult. In these areas the overwintering infection is likely to be the more important. In Iceland and northern Norway where summer temperatures barely exceed the critical 10°C even at the height of summer, one would not expect the life cycle to be completed even in two seasons. Information from these countries (Einarsson and Helle, personal communications) shows that there is no disease

in these areas in spite of the presence of the intermediate snail host and the occasional introduction of infected sheep.

Observations on the viability of metacercariae on herbage in S.E. England (Ollerenshaw, 1959, and in press) showed that they survived for several months during autumn and winter, and for several weeks during the height of summer. Experimental infections of sheep with large single doses of metacercariae produced deaths from acute disease from about 6 weeks after infection (Roberts, 1968). In the field, where equally high doses are likely to be acquired more slowly, deaths seem unlikely to occur before eight weeks. From

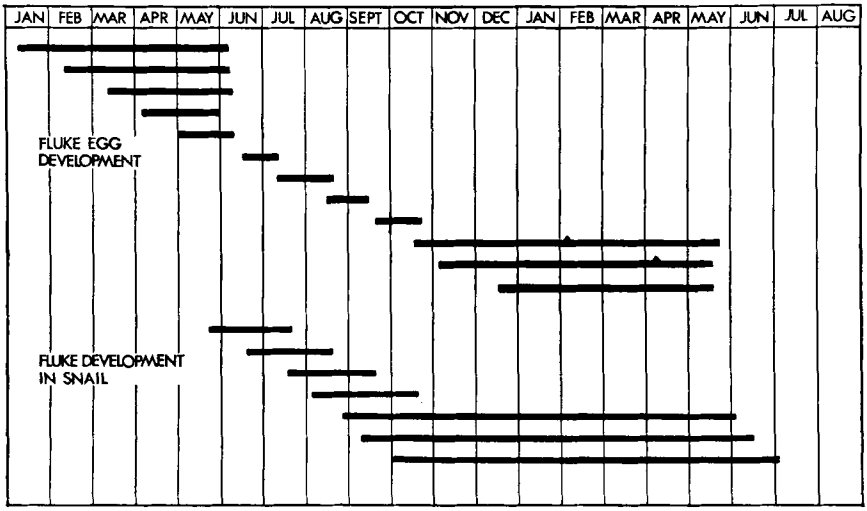


FIG. 1. The effect of temperature on the rate of development of fluke eggs to hatching and the development of the parasite in the snail to emergence of cercariae in outdoor temperatures at Weybridge, S.E. England. A summary of the results obtained during 1959–1961. (Reproduced with permission from Ollerenshaw, in press.)

these observations it was possible to establish a time-table showing the seasonal distribution of eggs on pasture, infected snails, infected herbage and disease in sheep (Ollerenshaw, 1959). This was based solely on the response of the parasite to seasonal variations in temperature. (See Fig. 2).

The extent to which these stages occur in any year depends on moisture conditions in snail habitats during the summer period, when temperatures do not limit development. The fluke egg is not resistant to desiccation and it dies if the surface film of moisture is lost. Although faeces help to protect the egg against the effects of the ambient macroclimate, there is no development whilst the egg remains in identifiable faecal material. The effect of dry conditions is to prevent any hatching of eggs. As the final host shows little or no resistance to the parasite, and as stock graze pastures throughout the year, there is invariably a continuous supply of viable fluke eggs ready to take advantage of any occurrence of wet ground conditions.

There has been much speculation about the role of the intermediate snail host in the production of disease and whether it is an important limiting factor. Much evidence shows that the snail is able to respond almost as quickly as the parasite to favourable moisture conditions. It must be recognised that *L. truncatula* has exploited an ecological niche where there are few competitors or predators, but the mode of life is not without its disadvantages, because

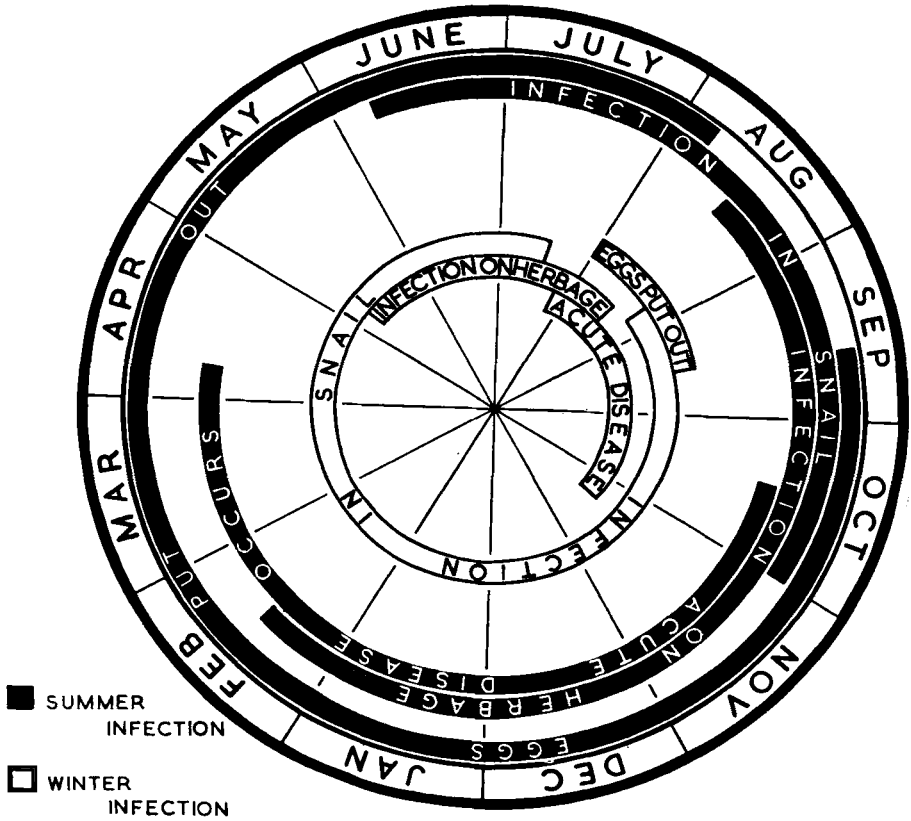


FIG. 2. A time-table showing the seasonal distribution of the various stages of the life cycle of *F. hepatica* as a result of seasonal temperature changes in Britain, assuming optimum moisture conditions are maintained throughout the year. (Reproduced with permission from Ollerenshaw, 1959.)

variations in moisture conditions present the greatest problems. Basically, *L. truncatula* is an aquatic snail in a habitat that is not completely aquatic. Habitats are found on pastures which are badly drained but not too acid, in the wet areas around springs or seepages, or in shallow drainage ditches. In winter these areas are invariably wet, but in spring and summer when the snail is most active, moisture conditions vary depending on the prevailing weather. Kendall (1949) showed that the snail is able to survive these adverse conditions

for a considerable time, but eventually it dies. With a return of wet conditions the snail becomes active and having high reproductive potential it is able quickly to repopulate its habitat. The recovery of snail populations in Wales by the autumn of 1960 following the very dry summer of 1959, and that of early summer in 1960 as reported by Ollerenshaw (in press), shows the speed of this repopulation and provides some evidence that the snail is not a major limiting factor in the production of disease. A feature of many snail collections is that whenever a sequence of weather occurs which is likely to have resulted in some hatching of eggs, it is unusual not to be able to find any snails.

The maintenance of wet conditions in habitats enables existing snails to feed and grow and, as reported by Kendall and Ollerenshaw (1963), the better the state of nutrition of the snail host, the larger the parasite population it can support. A number of other consequences follow from the maintenance of wet ground conditions in summer. Habitats may expand in area, and they certainly carry an increased population of growing snails. Fluke eggs are able to hatch and give rise to a high rate of infection in snails; furthermore, large snails produce more cercariae. All these factors combine to produce a high intensity of infection on the herbage. As this infection is relatively long lived on the herbage and the final host shows no marked resistance to infection, the incidence of disease is directly related to the intensity of infection on the herbage.

The existence of snail habitats depends on the interaction of a number of physical factors in the environment. Some geological formations are impervious to water, others not; clay soils are more water retentive than sandy soils. The topography of the land may hinder or facilitate natural drainage, and field drainage systems may be comprehensive or non-existent. All these factors are relatively constant from one year to another and they determine whether or not snail habitats can occur in any given area. The extent to which habitats actually arise depends on the climate. The all-important factor is the balance between rainfall and evapotranspiration during the summer.

With this knowledge it is possible to explain many features associated with epizootics of fascioliasis. Wet weather throughout the summer period facilitates the development of the free living stages of the parasite and those in the intermediate snail host, and it results in a heavy infection of herbage in late summer and autumn. As this infection persists during the winter it gives rise to losses commencing in late autumn and extending throughout winter into the spring. Wet weather in late summer, autumn and the following early summer facilitates infection of herbage in early summer from the overwintering infection, and this gives rise to stock losses in late summer extending into autumn. A dry late summer in such years probably encourages stock to graze flukey areas by limiting the growth of herbage in the better drained areas. This factor does not arise in winter because grass growth is limited by temperature, so that irrespective of the wetness of winter all available herbage is generally eaten before the spring.

The overriding importance of June weather in the causation of epizootics follows from the fact that the weather in this month is the key to the all-important first stage of the life cycle, namely, the development and hatching of

the fluke egg and infection of the snail. Apart from ensuring a high level of the overwintering infection, wet weather in late summer cannot itself produce a high level of herbage infection unless infection of snails has occurred earlier in that summer.

In establishing a suitable numerical relationship, the two most important climatic parameters concerned with soil moisture were rainfall and evapotranspiration. The latter was represented as potential transpiration since these values were readily available from the Meteorological Office (see Technical Bulletin No. 16). Rain-days were included since this provides a measure of the distribution of rain during a month.

$$\text{The formula } M_t = (\mathrm{R} - \mathrm{P} + 5)\mathrm{n}$$

where $R$ = rainfall (in)

$P$ = potential transpiration (in)

$n$ = number of rain-days

was suggested as a useful monthly expression of soil moisture in snail habitats. Observations showed that when the value $M_t$ reached 100, habitats tended to be wet for the whole month, hence values exceeding 100 were assessed as 100. As temperatures permitted less development in May and October than in the remaining summer months the values for May and October were halved. In this way the index $M_t$ represents a measure of the amount of fluke development in each month and may be summated to indicate the expected intensity of disease. Values from May to October each year provided a measure of the expected incidence in stock in the following winter. Values for August, September and October of one year and May and June of the following year provided a measure of the intensity of the overwintering infection in the snail.

The first comparison between the incidence of disease and climate assessed on this basis was carried out by Ollerenshaw and Rowlands (1959) for the county of Anglesey over the period 1948–1957 (see Fig. 3). This showed that losses from acute disease did not commence until the $M_t$ value reached 300, losses were above average beyond 400, and the epizootic level was placed at 475. The correlation seemed sufficiently good to allow forecasts of incidence to be issued to farmers, and these commenced in 1958. An assessment of the accuracy of these forecasts in England and Wales over the period 1958–1962 together with some modifications in approach was given by Ollerenshaw (1966). Recently, Ollerenshaw (in press) has given a more comprehensive review of the situation in Wales from 1958–1966. As well as comparing forecasts with actual incidence in both sheep and cattle, information on the varying population of snails, the degree of infection, and the development of the parasite in the snail is given. Throughout the period, apart from some minor discrepancies, there was a good correlation between the incidence of disease and current climate, whilst data from snail collections enabled the course of the life cycle to be followed each year.

One of the practical difficulties in producing a forecast has been the necessity to issue it in early August, so that in years with an expectation of above average or high incidence a warning of the need to use alternative

grazing or apply molluscicides can be given before a high intensity of infection has occurred on the herbage. The results reported by Ollerenshaw (1966, and in press) show that a forecast can be based on an analysis of climate up to the end of July. That this is possible again shows the importance of the weather in early summer and in particular in June. Indeed, there is some evidence that analysis of the weather up to the end of July provides an even more reliable basis for estimating incidence than of that in the whole period from May to October. But for this automatic weighting of the importance of early summer

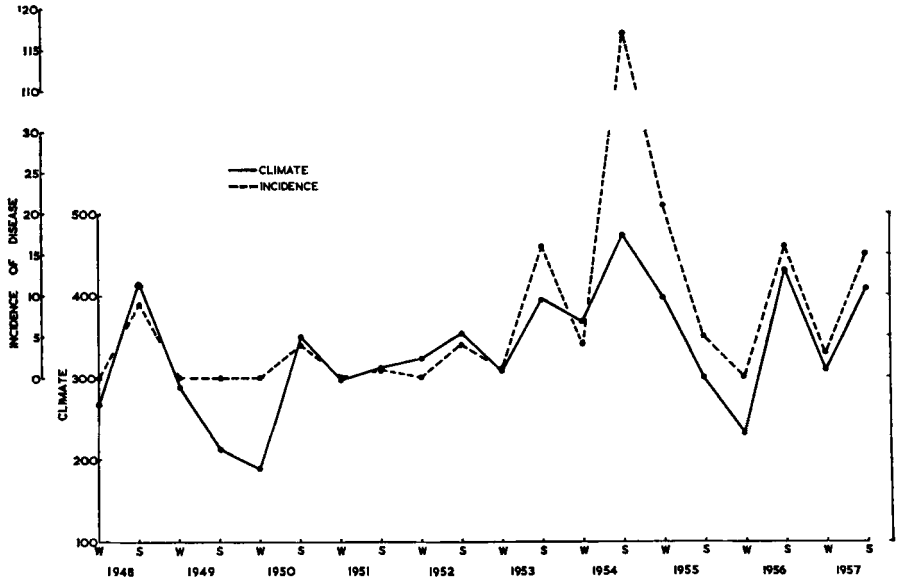


FIG. 3. A comparison between climate ($M_t$ value from May to October inclusive) and the number of incidents of acute fluke in Anglesey reported to the Veterinary Investigation Centre in Bangor 1948–1957. (Reproduced with permission from Ollerenshaw and Rowlands, 1959.)

weather in forecasting disease incidence there would have been a need to modify the original approach, where incidence was assessed on the weather throughout the summer period.

The results reported by Sloan (in press) provide a most valuable and independent comment on this relationship between current climate and incidence. His records of incidence based on the examination of a large but not random selection of cattle faeces samples sent in by veterinary practitioners, extended over the period 1947–1966. A comparison of the percentage of samples containing fluke eggs in the month of peak incidence each year plotted against the mean $M_t$ value up to the end of July for the ten stations in England and Wales recording the highest $M_t$ values, were in close agreement. Throughout this period the $M_t$ value reached or exceeded 200 on five occasions—in 1947,

1954, 1958, 1965 and 1966. In the appropriate months following these years but in no other did 40% or more of the faeces samples contain fluke eggs.

Analysis of the climate in this way can be used also to explain the geographical distribution of fascioliasis in Britain. In its simplest terms the analysis depends on a comparison between rainfall and potential transpiration. When rainfall exceeds potential transpiration, development of the parasite is possible. Maintenance of these conditions for at least three of the 6 summer months allows the life cycle from deposition of the fluke's egg in faeces to infection of the herbage to be completed.

Analysis of the climate over Britain in this respect reveals three broad patterns. Over much of E. and S.E. England it is rare for rainfall to exceed potential transpiration in more than 3 of the summer months, and it is in these areas that little disease occurs. Over most hill land, particularly in the west and north, rainfall almost invariably exceeds potential transpiration in all 6 months. Contrary to expectation this does not give rise to a serious perpetual fluke problem. These climatic conditions tend to produce a waterlogged acid soil, and if drainage is at all impeded, peat is formed by the anaerobic decomposition of plant material. The acidity of peat is inimical to the snail, so that extensive snail habitats rarely occur on hill farms. The snail is restricted to wet areas of inbye land and to flushes on the hillsides caused by springs of non-acidic water. Occasionally where wet hill land is improved by lime and fertiliser applications but with no drainage improvements, fluke disease may become a serious problem. In general, however, fluke disease is a constant but minor problem on most hill farms.

The situation on lowland farms in the west presents the greatest variation; rainfall sometimes exceeds potential transpiration in all the summer months, and occasionally the reverse is true. Usually in at least three and sometimes four months rainfall exceeds potential transpiration. It is in these areas that the disease is endemic but, depending on the weather in summer, all possible variations in incidence occur. It follows that it is in these areas that a forecast of incidence can be of greatest value to farmers who have to farm land which is inherently prone to fluke disease.

There are areas in Britain—for instance parts of S.E. England—where disease occurs under climatic conditions which would normally prove unfavourable for completion of the life cycle. In these areas farmers deliberately maintain a high water table for other reasons, such as the enclosure of stock by drainage ditches or easy access of stock to water. It is the high water table which enables the life cycle to be completed.

A similar situation also occurs in Holland where disease occurs under climatic conditions which are not particularly conducive to the development of the parasite. Nevertheless, it is of interest to note from the work of Honer and Vink (1963a,b, and in press) and Donker (in press) that the broad trends of incidence in Holland are similar to those in England. In both countries 1958 was a bad year, whilst 1959, 1960, 1961 and 1962 were average to good years. Then followed a series of wet summers and above average incidence resulted in 1963 and 1964, rising almost to the high incidence category in 1965 and 1966. In 1967 the situation improved following a mainly dry summer.

It would appear that the approach to forecasting the incidence of fascioliasis outlined above is applicable (with suitable modifications) in other parts of Europe. An almost identical approach now in operation in Russia has been reported by Mereminskii (1967).

Recognition of the importance of climate in the life cycle of *F. hepatica* can be of value in countries where the course of events in the field is not known in great detail. For instance in Portugal fascioliasis is a problem in many areas and particularly in the north. Analysis of the climate shows that temperature is much less limiting than in northern European countries. Although summers are usually dry, winters are wet and there is every opportunity in the endemic areas for fluke eggs to hatch and infect snails following autumn rains but before the temperature falls below 10°C. Resumption of development in the snail occurs early the next year so that infection of herbage is most likely in spring before the summer drought sets in. The main season of development is likely to be in autumn, winter and spring, and not in summer as occurs in Britain. The problem may of course be further complicated in areas experiencing a summer drought, because irrigation to maintain grass growth is becoming increasingly important. In Australia, for instance, the increasing use of irrigation is recognised as a major factor in increasing the distribution of the disease. In this context it may be considered that analysis of climate can play no part in the control of fascioliasis. This is not so, inasmuch as an awareness of the importance of climate in relation to soil moisture may help in devising suitable irrigation techniques which will allow grass growth but not the development of the parasite.

### B. NEMATODIRIASIS

Nematodiriasis in lambs is in many ways an unusual disease. It was not recognised as a specific disease entity until the early 1950s, when the principal parasite involved was identified as a new species of nematode—*Nematodirus battus*. At that time the disease proved a very serious problem, no drugs were available to kill this worm and many farmers lost many lambs, particularly in the north of England. As a result of the work of Thomas (1959), Thomas and Stevens (1956, 1960), Gibson (1958, 1959a,b, 1963) and Christie (1962), a good deal is known about the epidemiology of this disease and its control by husbandry practices.

Infected lambs pass worm eggs on to pastures during late spring and early summer. The eggs develop slowly and by September they usually contain third stage infective larvae. The egg overwinters and hatches sometime in late winter and spring of the following year, when the succeeding lamb crop is available to eat the infected herbage. Animals quickly become resistant to the parasite so that adult sheep play little part in the life cycle of the parasite.

Very early in these investigations it was recognised that the hatching mechanism of the egg is the key to an understanding of the variations in incidence of this disease. Though temperature was clearly involved it proved impossible to demonstrate a quantitative relationship between climate and the incidence of disease. A general observation noted by most of these investigators and also

by Black (1959) was that incidence was high following cold winters and late springs, and low following mild winters and early springs. It was suggested that the hatching of the egg in cold winters and late springs was delayed and coincided with the period when lambs were eating herbage. In contrast, following mild winters and early springs hatching occurred early before lambs were eating herbage, and in such seasons there was little or no disease. Ollerenshaw and Smith (1966) made use of these observations in suggesting an empirical approach to forecasting the incidence of this disease (see Fig. 4).

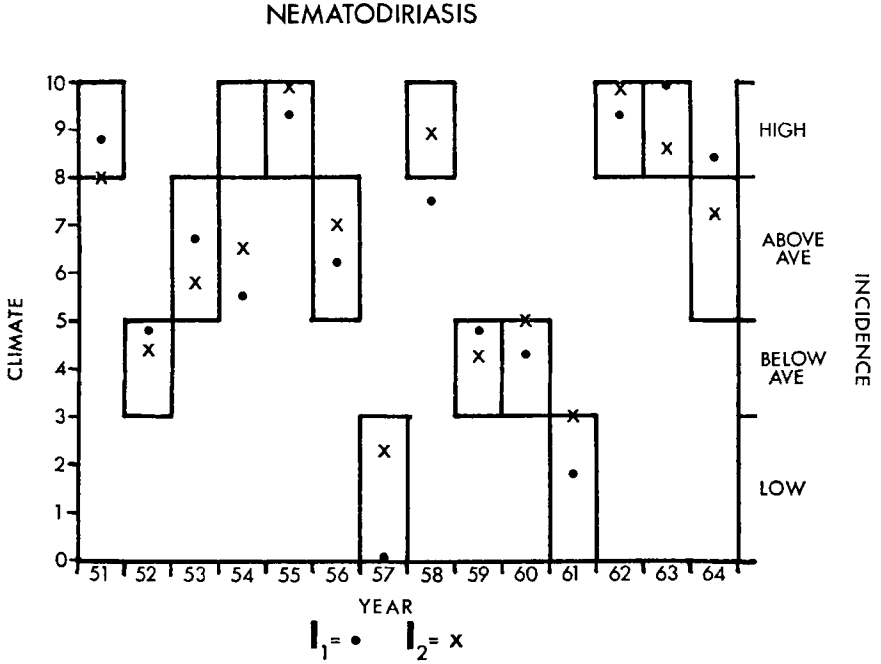


FIG. 4. A comparison between the incidence of nematodiriasis and two assessments of climate ($I_1$ and $I_2$). (Reproduced with permission from Ollerenshaw and Smith, 1966.)

These workers were fortunate in having some record of the incidence of disease over the period 1951–1964 in England and Wales, based on the reports of the Veterinary Investigations Officers of the M.A.F.F.

Two types of meteorological data were examined to provide information about the coldness of winter and the earliness of spring. An analysis was made of the deviations from the monthly mean air temperatures over England and Wales in the period December to March, and an examination was made of the 1 ft earth temperatures at Oxford interpolated linearly to find when this temperature reached 43°F to give an accepted date of spring. The relationships were further developed so as to enable a direct comparison between the two assessments of climate and incidence.

The regression equation

$$I_1 = 5{\cdot}3 - 0{\cdot}33u - 0{\cdot}58v$$

where $u$ = the sum of the temperature deviations of December, January and February and $v$ = the deviation from average of March temperature, gave values of an index ($I_1$) which was approximately 10 for a high incidence of disease and zero for a low incidence.

The 1 ft earth temperatures in March at two meterological stations, Oxford (o) and Cockle Park (c.p.), were used and the equation

$$I_2 = \tfrac{1}{2}\,(94 - T_{\text{o}} - T_{\text{c.p.}})$$

gave values of an index $I_2$ which enabled a direct comparison with the values of $I_1$, using the same index criteria to assess incidence. These criteria were

| | |
|---|---|
| Above 8 | high incidence. |
| 5–8 | above average incidence. |
| 3–5 | below average incidence. |
| Below 3 | low incidence. |

A further equation developed since 1966, namely

$$I_3 = 52{\cdot}5 - T_{\text{o}} - \frac{T_{\text{c.p.}}}{10}$$

would appear to provide a more accurate assessment of incidence than $I_2$.

As shown in Table II, only in 1954 was there a real discrepancy between these assessments of climate and actual incidence. This was a year with a particularly dry April, and it was suggested that this may have delayed the onset of the infection of herbage and resulted in a higher incidence of disease than was expected. As the correlation between weather and incidence is based on temperatures up to the end of March it is possible to issue a forecast of incidence in April long before disease arises in May, June and July. Forecasts of incidence have in fact been issued since 1964.

Experience with fascioliasis suggested that the introduction of forecasts stimulated an interest in the disease and provided more comments on the actual incidence of disease. Unfortunately, with nematodiriasis this has not proved to be the case. Two other influences have been noted: nematodiriasis is now so well recognised and expected that relatively few farmers request help from their veterinary surgeons or the Veterinary Investigation Centres, and secondly, the introduction and use of new drugs capable of giving good control of the disease means that the disease is much less of a problem than when it first made its appearance. In spite of these factors it would seem that there has been no major discrepancy between the forecasts and actual incidence in the period 1964–1968, though in this period no extremes of incidence have occurred.

Much more work remains to be accomplished before this approach to forecasting can make an important contribution to the control of this disease. At the moment it simply makes some comment on the expected overall

TABLE II

*Comparison of climate and the incidence of nematodiriasis*, 1951–1968

| | | | | March 1 ft earth temperature | | | | |
|---|---|---|---|---|---|---|---|---|
| Year | u°F | v°F | $I_1$ | Oxford | Cockle Park | $I_2$ | $I_3$ | Incidence |
| 1951 | −7·0 | −2·0 | 8·8 | 40·6 | 37·3 | 8·0 | 8·2 | High |
| 1952 | −1·9 | +1·9 | 4·8 | 44·2 | 41·0 | 4·4 | 4·2 | Below average |
| 1953 | −3·4 | −0·4 | 6·7 | 41·2 | 41·1 | 5·8 | 7·2 | Above average |
| 1954 | −0·4 | −0·1 | 5·5 | 42·2 | 38·7 | 6·5 | 6·4 | High |
| 1955 | −3·5 | −4·9 | 9·3 | 37·3 | 35·7 | 10·5 | 11·6 | High |
| 1956 | −6·3 | +0·3 | 6·2 | 40·8 | 39·2 | 7·0 | 7·8 | Above average |
| 1957 | +6·6 | +5·6 | 0·0 | 46·6 | 42·8 | 2·3 | 1·6 | Low |
| 1958 | +0·4 | −4·0 | 7·5 | 40·0 | 36·2 | 8·9 | 8·9 | High |
| 1959 | −2·8 | +2·4 | 4·8 | 44·1 | 41·5 | 4·2 | 4·3 | Below average |
| 1960 | +2·3 | +0·5 | 4·3 | 44·0 | 40·0 | 5·0 | 4·5 | Below average |
| 1961 | +3·3 | +4·1 | 1·8 | 45·3 | 42·6 | 3·0 | 2·9 | Low |
| 1962 | −2·3 | −5·6 | 9·3 | 38·1 | 36·0 | 10·0 | 10·8 | High |
| 1963 | −21·9 | 0·0 | 12·5 | 38·8 | 37·9 | 8·6 | 9·9 | High |
| 1964 | −3·9 | −3·1 | 8·4 | 40·8 | 38·8 | 7·2 | 7·8 | Above average |
| 1965* | −3·6 | −1·4 | 7·1 | 40·8 | 37·6 | 7·8 | 7·9 | Above average |
| 1966* | +1·4 | +1·4 | 4·0 | 43·7 | 40·3 | 5·0 | 4·8 | Below average |
| 1967* | +5·0 | +2·3 | 3·2 | 44·2 | 41·2 | 4·3 | 4·2 | Below average |
| 1968* | −2·5 | −1·1 | 5·1 | 41·7 | 39·4 | 6·4 | 6·9 | Above average |

* Forecasts issued in these years proved reasonably accurate.

incidence of disease. It is known, however, that the nematodirus season may be short or long, early or late, and this influences the most opportune times for and the required frequency of dosing. Where farmers use husbandry practices to control the disease, they would undoubtedly appreciate information as to when lambs should be moved to clean pastures, as there is no doubt that the onset of infection on the herbage may vary in different years. These problems are likely to be resolved as more information becomes available on the basic relationship between ambient climate and the hatching mechanism of the

nematodirus egg. Even then a factor in disease incidence which will remain important is the age of the lambs when the herbage becomes infected.

### C. PARASITIC GASTRO-ENTERITIS IN CATTLE

Two species of nematodes usually predominate in outbreaks of parasitic gastro-enteritis in cattle, *Ostertagia ostertagi* and *Cooperia oncophora*, the former the more important. Anderson *et al.* (1965) reported that clinical disease in S. Scotland may be classified into two forms, Types I and II. Type I disease, also called summer ostertagiasis, occurs in young stock during their first season at grass from late July until the end of autumn. Subsequently Reid *et al.* (1968) indicated that Type I disease could in fact occur from June onwards. Type II disease, or winter ostertagiasis, which was first described by Martin *et al.* (1957), occurs mainly in late winter and early spring, and results from the maturation of larvae which have been inhibited in their development in the final host when ingested during the previous autumn.

Type I ostertagiasis, the more common of these two syndromes, has been studied in some detail in different parts of England by Michel (1966, 1968), who observed the course of the infection mainly in calves turned out on to experimental paddocks over the period 1959–1966, and to a lesser extent in natural populations. He found that the pattern of the herbage infection on these paddocks remained remarkably constant in different years. Infection was low in spring when the calves were first turned out. The calves became lightly infected and produced a further infection on the herbage from July which increased during August to a level which was well maintained through the following winter, when it declined rapidly during March and April. There was some evidence that in dry seasons the increase in the herbage infection starting in July was more gradual and continued into autumn. In 1960 and 1965 the decline in the herbage infection at the end of winter was possibly more gradual than in the other years. It was the second generation of larvae appearing on the herbage from mid-July which produced disease in grazing stock.

Winter ostertagiasis has been studied in detail by parasitologists in Glasgow. The mechanism underlying the inhibition of larvae acquired during grazing in autumn and their subsequent development at a varying interval later is not thoroughly understood. Jennings *et al.* (1967) suggested two factors necessary for the production of inhibited *O. ostertagi*, namely, a strain of larvae susceptible to inhibition, and the environmental circumstances of late autumn which appear to produce the optimal changes in the larvae for their subsequent inhibition when ingested by the host. Larvae ingested from late September onwards may become inhibited and, as shown by Anderson *et al.* (1965), resumption of development and the occurrence of clinical disease may start in early December and extend to June. The last case studied occurred within a week of the animals being turned out for their second summer.

Martin *et al.* (1957) considered that the cases which were investigated in the winter of 1955/1956 may have been related to the abnormally dry summer of 1955. Subsequent observations reported by Reid *et al.* (1967) noted the

prevalence of Type II disease in winters following dry summers such as occurred in 1955 and 1959. Ross (1965) in Northern Ireland considered that dry summers may aggravate the situation, but he noted that winter ostertagiasis also occurs in winters following wet summers. He considered that other factors such as nutrition were important in the production of disease. In Australia, Hotson (1967) noted also the occurrence of Type II ostertagiasis following prolonged dry weather.

The ecology of the free living stages of *O. ostertagi* has been studied by Rose (1961, 1962) and more recently by Kutzer (1967). The observations of Rose fortunately extended over the dry summer of 1959, and he was able to show that larvae survived in the dung pat during the dry summer and emerged in large quantities on to the herbage following the onset of autumn rains. He also noted that wet weather during the summer facilitated the translation of larvae from faeces on to the herbage.

These facts were utilised by Smith and Ollerenshaw (1967) in a brief report on the possibility of forecasting the incidence of parasitic gastro-enteritis in cattle. Assessments of the incidence of disease over the period 1953–1965 were obtained from the reports of the Veterinary Investigation Officers in England and Wales. Although these assessments of incidence were based on very limited information there was good evidence that not all losses were in accord with existing accounts of the epidemiology of the disease. Parasitic gastro-enteritis was commonly reported in late summer and autumn, mainly in the months August to October. Sporadic losses in housed cattle were reported during the winter. In the years of highest incidence in 1956, 1960 and to a lesser extent 1965, the most serious losses were reported in May, June and July. Most losses occurred after stock was turned out and outbreaks were recorded in young stock. Generally *O. ostertagi* was the most common worm involved though other species besides *C. oncophora* were found. Ross and Woodley (1968) in Northern Ireland have noted outbreaks in spring in addition to those in late summer and winter. These were termed acute outbreaks and were due to a combined infection of *Trichostrongylus axei* and *O. ostertagi*, with the former species predominating.

In discussing the possibility of forecasting parasitic gastro-enteritis in calves in spring, Ross and Woodley (1968) stressed the importance of warm spells in early spring and suggested that a rise in the one ft earth temperature above 42°F may be critical. On this basis there should be an inverse relationship between the incidence of parasitic gastro-enteritis in cattle and nematodiriasis in lambs, but no such relationship has been noted in England and Wales in recent years. The springs of 1957 and 1961 were exceptionally early, implying an early accumulation of day-degrees over 42°F, yet in neither season were losses from parasitic gastro-enteritis in cattle unduly high. Conversely the springs of 1956, 1960 and 1965 were not particularly early, yet incidence was high in the early summer of these years. Although rainfall was below average in the period March to May in 1956 and 1960, that in 1965 was above average in these months. The main weather feature which was common to these years was that they all followed years in which late summer and early autumn were particularly dry.

Clearly there is a need to carry out further work to determine the origin of disease which occurs in stock in early summer. There is the possibility that herbage remains infected longer in springs following dry summers (Michel, 1966); the work of Ross and Woodley (1968) has shown that *T. axei* may be important, whilst the resumption of development of inhibited larvae acquired during the previous autumn may be a factor in outbreaks in older cattle.

On present information the same weather sequence, namely, a dry late summer and autumn, is responsible for a high incidence of both Type II ostertagiasis (though according to V.I.O. reports this syndrome appears sporadic in its distribution apart from 1956, 1960 and 1965) and for disease which occurs in early summer.

On the basis of very limited information, Smith and Ollerenshaw (1967) suggested that incidence in early summer could be related to rainfall in the months August, September and October in the previous year, the lower the rainfall meaning the higher the incidence. Incidence in late summer and autumn was related to the saturation deficit in the soil up to the end of July, the lower the saturation deficit, which implies a high, well distributed rainfall, the higher the incidence. It was clear, however, that the weather in August continued to influence incidence, and knowledge of this helped to resolve some of the borderline cases as judged by the analysis of current climate up to the end of July. It was apparent that incidence also depended on the previous level of disease in stock, and this parameter was included in the formulae suggested as worthy of trial. The limited information on incidence did not permit assessments for different parts of the country, so that the analysis of climate was based on data from two meteorological stations.

The suggested formula for estimating early summer incidence was

$$\text{Disease Index } I_1 = 13 - R - 0{\cdot}5\,D$$

where $R$ = the mean of the August to October rainfall in inches at Sutton Bonington and Cirencester in the previous year, and $D$ = total period disease rating in the previous year. Only one year in the period 1953–1965 was seriously in error (see Table III), this being 1961 when the April soil temperatures were extremely high.

For late summer incidence the suggested formula was

$$\text{Disease Index } I_2 = 8{\cdot}0 - 1{\cdot}7J + 0{\cdot}6\,D_1$$

where $J$ = Soil moisture deficit at the end of July, and $D_1$ = early summer disease rating.

The results of these analyses are given in Tables III and IV.

It is perhaps a little early to suggest how this approach to forecasting the incidence of parasitic gastro-enteritis in cattle can make a practical contribution to controlling disease. Michel (1966, 1968) recommended a system of control by dosing and moving stock to clean pastures in mid-July. This move is recommended before it is possible to provide a forecast of incidence in early August. If the intake of larvae in the last fortnight of July is critical then a forecast of incidence in early August is of limited practical value. With little disease occurring until well into August a forecast in early August in years of

TABLE III

*A comparison between the incidence of parasitic gastro-enteritis in cattle in early summer, and climate*, 1953–1965

| Year | $R$ | $D$ | $I_1$ | Observed rating | Error | Forecast |
|---|---|---|---|---|---|---|
| 1953 | 7·3 | 2 | 4·7 | 5 | 0·3 | Average |
| 1954 | 7·8 | 7 | 1·7 | 1 | 0·7 | Low |
| 1955 | 10·2 | 6 | 0·2 | 1 | 1·2 | Low |
| 1956 | 3·1 | 1 | 9·4 | 9 | 0·4 | High |
| 1957 | 8·5 | 9 | 0·0 | 1 | 1·0 | Low |
| 1958 | 9·4 | 3 | 2·1 | 1 | 1·1 | Low |
| 1959 | 8·4 | 5 | 2·1 | 1 | 1·1 | Low |
| 1960 | 3·5 | 1 | 9·0 | 10 | 1·0 | High |
| 1961 | 12·2 | 10 | −4·2 | 7 | 11·2 | Low |
| 1962 | 6·8 | 7 | 2·7 | 3 | 0·3 | Below average |
| 1963 | 8·7 | 3 | 2·8 | 4 | 1·2 | Below average |
| 1964 | 6·6 | 7 | 2·9 | 3 | 0·1 | Below average |
| 1965 | 3·0 | 3 | 8·5 | 8 | 0·5 | High |

The "forecasts" were based on $I_1$ using criteria at 2·5, 5·0 and 7·5

high incidence could be a timely reminder of the need for action by the many farmers whose husbandry practices are not in accord with the most up-to-date techniques or who have limited opportunity for moving stock to clean fields.

In order to avoid winter ostertagiasis, Ross and Woodley (1968) have suggested that farmers would need to be advised in early September of the likely prevalence of disease in winter and of the need to move stock to clean pastures or alternatively to house them. Similar control measures have also been recommended by Reid *et al.* (1968). Whether farmers regard these recommendations as practical or whether one can in fact predict the incidence of winter ostertagiasis by the end of August remains to be seen.

If incidence in early summer is due mainly to the overwintering infection on the herbage (see Kutzer, 1967) and not to other factors, then a forecast of incidence could be available in good time to allow farmers to delay turning out young stock in spring, a procedure already suggested by Michel (1966).

TABLE IV

*A comparison between the incidence of parasitic gastro-enteritis in cattle in late summer, and climate*, 1953–1964

| Year | $J$ | $D_1$ | $I_2$ | Observed rating | Error | Forecast |
|---|---|---|---|---|---|---|
| 1953 | 2·7 | 5 | 6·4 | 7 | 0·6 | Above average |
| 1954 | 1·7 | 1 | 5·7 | 6 | 0·3 | Above average |
| 1955 | 3·0 | 1 | 3·5 | 1 | 2·5 | Below average |
| 1956 | 3·6 | 9 | 7·3 | 8 | 0·7 | Above average |
| 1957 | 4·3 | 1 | 1·3 | 1 | 0·3 | Low |
| 1958 | 1·3 | 1 | 6·4 | 6 | 0·4 | Above average |
| 1959 | 4·3 | 1 | 1·3 | 1 | 0·3 | Low |
| 1960 | 2·1 | 10 | 10·4 | 10 | 0·4 | High |
| 1961 | 4·0 | 7 | 5·4 | 7 | 1·6 | Average |
| 1962 | 4·2 | 3 | 2·7 | 3 | 0·3 | Below average |
| 1963 | 2·6 | 4 | 6·0 | 6 | 0·0 | Above average |
| 1964 | 2·8 | 3 | 5·0 | 3 | 2·0 | Average |

The "forecasts" were based on $I_2$ using criteria at 2·5, 5·0 and 7·5. The August mean rainfalls at Sutton Bonington and Cirencester in 1955, 1956, 1961 and 1964 (the years with the greatest errors) were

| *Year* | *Rainfall* (in) |
|---|---|
| 1955 | 0·45 correctly suggesting a lower incidence. |
| 1956 | 4·28 correctly suggesting a higher incidence. |
| 1961 | 2·57. |
| 1964 | 1·28 correctly suggesting a lower incidence. |

### D. PARASITIC GASTRO-ENTERITIS IN SHEEP

The wide variety of species involved in outbreaks of parasitic gastro-enteritis in sheep makes it particularly difficult to approach the relationship between ambient climate and the disease on the basis of the epidemiology of the individual species concerned, even though much information on these species is now available. An examination of the Veterinary Investigation Officers reports on incidence in England and Wales, shows that many species may be involved, commonly in mixed infections; moreover there appear to be few months in any year when one or more outbreaks are not reported. On the face of it these observations would appear to preclude the early possibility of developing any relationship between weather and disease incidence.

An excellent account of an epidemiological investigation into parasitic gastro-enteritis in sheep mainly from the climatological aspect was reported as long ago as 1934 by Taylor. This investigation was undertaken as a result of widespread and heavy losses (averaging 15%) in the autumn and winter of 1933/1934 in England, Wales and Northern Ireland. Losses predominated in lambs, but older sheep and cattle were also affected. The losses occurred mainly during January, February, March and April. These outbreaks followed the exceedingly dry late summer and early autumn of 1933, and Taylor described some experiments designed to show the importance of dry weather in producing outbreaks of so called "winter scours" in sheep. Taylor also noted that similar outbreaks of disease followed the dry late summer and early autumn of 1911 (June had well above average rainfall in that year). Reference was also made to a report by M'Fadyean (1897) on outbreaks in 1895 and 1896. The outbreaks in 1895 occurred mainly in October following a dry May and June, a wet July and August, and a dry September. Those in 1896 followed a generally dry period lasting from April to August, but with a particularly wet September. With this last series of outbreaks losses started in October and extended into 1897.

In more recent years a high incidence of disease was reported in the winters and early summers of years following the dry late summers of 1955, 1959 and 1964. Disease seemed particularly common in yearlings and ewes. Whilst many of these losses occurred at the time of the "spring rise" as described by Morgan *et al.* (1951) and Crofton (1954), losses were reported as early as December, rather earlier than would be associated with the "spring rise". Clearly, losses in winters following dry summers parallel those in cattle. Losses in 1956, 1960 and 1965 continued through early summer and into autumn, but from June onwards the current year's lambs were also involved.

Outbreaks of disease in winter in yearlings and ewes tend to be regarded as unusual, but this may well be due to the fact that dry late summers are of some rarity in Britain. As with parasitic gastro-enteritis in cattle, the more normal occurrence of disease is in late summer and autumn. Again incidence tends to be high following the wettest summers, as in 1958, 1963, 1965 and 1966. There is evidence also that incidence at any given time is influenced by the previous level of disease.

Although there is an overall similarity between the incidence of disease in cattle and that in sheep, attention is drawn from time to time to differences in incidence. Generally outbreaks of disease in sheep are reported much more frequently than outbreaks in cattle. Moreover, there is a suggestion that the disease level in sheep seems to respond more rapidly to weather changes than it does in cattle.

Ross and Woodley (1968) in Northern Ireland have discussed the possibility of forecasting parasitic gastro-enteritis in sheep, though at present their approach is based on the examination of dung samples rather than on an analysis of weather. As with cattle a high incidence of disease is associated with two extremes of current climate in summer. Dry weather in late summer and early autumn produces disease in the following winter extending into early summer, and wet weather during summer produces disease at that time

and in autumn. The relationship suggested as a basis for forecasting the incidence of parasitic gastro-enteritis in cattle could be used as a general guide for assessing incidence in sheep. In due course, however, refinement of this approach will be possible. It appears that parasitic gastro-enteritis in sheep responds more rapidly to weather changes than parasitic gastro-enteritis in cattle, and it would seem that progress will come from a more detailed analysis of soil moisture deficits in the soil during summer. These may have to be determined on a weekly basis. In general terms, an increasing soil moisture deficit in the soil, i.e. drying out of the soil, will prevent herbage from becoming infected and give rise to a low incidence of disease at that time. The longer a deficit is maintained the greater the accumulation of eggs on the pasture, resulting in a correspondingly higher incidence of disease when soil moisture eventually returns to field capacity. If this is not achieved until October or November outbreaks are likely in winter extending to early summer. If a change from a high to a low soil moisture deficit occurs in late summer, outbreaks in lambs in autumn will follow. Where soil is maintained at or near field capacity (i.e. a low deficit) throughout summer, the greater the chance of eggs developing into infective larvae with a consequent high incidence of disease from mid-summer onwards. In these circumstances the important factors will be the duration of extremes of soil moisture deficits and the timing and rate of change from high to low deficits. Outbreaks following dry periods seem likely whenever a period of 2 consecutive months with an increasing or high soil moisture deficit is followed by 6 weeks during which there is a return to field capacity.

There is at least one species of nematode, *H. contortus*, which does not seem to conform to this pattern. Outbreaks of parasitic gastro-enteritis due solely to this species have been reported during very dry summers as in 1955 and 1959, though they are by no means confined to such years. Some observations on the relationship between current climate and the incidence of disease due to *H. contortus* in England and Wales have been reported by Rose (in press). He noted that outbreaks were much more prevalent in S.E. England than elsewhere and he concluded that this was due primarily to the occurrence of higher temperatures in S.E. England. Generally more outbreaks were reported in summer than in winter and in wet summers than in dry ones.

Much work on parasitic gastro-enteritis in sheep has been carried out in Australia. Problems studied have included seasonal incidence in relation to climate, resistance mechanisms, malnutrition, and drug therapy. Prominent among workers in the climatological field have been Gordon (1948, 1950, 1953, 1957, 1958), Forsyth (1953) and Pullar (1953). In many respects the situation in Australia might be regarded as ideal from the point of view of establishing basic relationships between climate and the incidence of parasitic gastro-enteritis. There are recognised summer and winter rainfall areas. Weather patterns tend to be more pronounced than in Britain so that variations in incidence following wet and dry sequences should be more easily recognised. More species of worms are economically important as a result of sheep being kept under a greater range of temperature. In spite of this no study has produced any quantitative relationship which might be used to forecast

the incidence of disease. Some investigators have suggested that malnutrition and a varying resistance make it difficult to develop precise relationships. Furthermore, nearly all the emphasis on climatology and parasitic gastro-enteritis in sheep in Australia has been centred on the use of the bioclimatograph, particularly in respect of the occurrence of *H. contortus*. As described earlier, this approach has its limitations, although Gordon (1958) was able to relate a number of features concerning the incidence of *H. contortus* to the occurrence and distribution of rainfall.

There is some information also to indicate that the occurrence of other species, for instance *Trichostrongylus* and *Ostertagia* spp., is also dependent on rainfall. Pullar (1953) in Victoria and Gordon (1958) in Western Australia (both winter rainfall regions) found a diphasic curve in the seasonal incidence of these species. A rise in infection in terms of losses (Pullar) and egg counts (Gordon) was noted in late summer, usually in February, followed by a decline in late autumn. The second peak occurred in late winter, usually in July. Pullar found the rise in July slightly more important than did Gordon, who noted disease in late summer as being more important.

Mean monthly rainfall figures for eight meteorological stations in Victoria for part of the period referred to by Pullar are given in Table V, which shows that normally February is the first month when any useful quantity of rain

TABLE V

*Average monthly rainfall (in) (eight stations) over Victoria, Australia, from* 1948–1952

| Year | Jan. | Feb. | Mar. | Apl. | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1948 | 0·84 | 1·49 | 0·39 | 2·93 | 3·13 | 2·13 | 1·74 | 1·46 | 1·68 | 4·21 | 2·05 | 1·98 |
| 1949 | 2·17 | 3·03 | 3·08 | 0·62 | 2·17 | 1·65 | 2·28 | 1·02 | 1·88 | 4·69 | 3·93 | 0·54 |
| 1950 | 0·49 | 3·41 | 4·30 | 1·80 | 2·69 | 1·13 | 2·02 | 1·98 | 3·12 | 3·06 | 1·78 | 1·63 |
| 1951 | 1·02 | 3·53 | 0·17 | 2·98 | 3·53 | 3·06 | 3·49 | 3·99 | 1·31 | 2·88 | 1·43 | 1·41 |
| 1952 | 1·32 | 1·04 | 1·42 | 2·98 | 3·98 | 5·13 | 2·59 | 2·80 | 2·77 | 3·64 | 4·20 | 2·16 |

falls following the summer drought, and this coincides with the start of the summer peak. In 1949 the rise occurred in January and the records show that the late spring and summer had above average rainfall. On the other hand in 1948 the drought persisted through February and March and in that year the rise did not occur until April. In 1952 the summer rise failed to occur. In some respects this season was similar to that in 1948; there was little rain until April, though none of the summer months recorded less than 1 in of rain. Other unusual features were noted in 1945 and 1946, but rainfall records for these years were not available.

Pullar considered that the summer rise was probably due to the effect of a falling plane of nutrition on hosts with existing infections. On this basis,

however, it is a little difficult to reconcile the delay in the commencement of losses in 1948. In this year the plane of nutrition was likely to decrease more rapidly than usual. Conversely the early occurrence of disease in 1949 occurred in a year when nutrition should have been better than usual. Gordon too was not able to relate the rise in infection in late summer with rainfall, though he considered the general seasonal pattern of infection to be related to the seasonal distribution of rainfall. He noted that the infection generally reached a peak before the "autumn break", which brought a flush of grass to pastures. In these circumstances the rise in egg output could be similar to that due to the "spring rise" in Britain, although the rise occurs much earlier than the lambing season which, according to Pullar, commences in May.

In spite of these difficulties there is good evidence that in the years 1948–1952 in Victoria, the onset of infection in sheep corresponded closely with the occurrence of rain ending a prolonged drought. In this respect there is a similarity with outbreaks of disease in Britain which also follow the cessation of prolonged drought.

Although there was considerable variation in rainfall in May and June (the years 1948–1950 being much drier than those in 1951 and 1952) the rise in incidence in July was fairly constant. Pullar found only a slight rise in 1946 and in 1950 it occurred a month early. Pullar also noted that July is the first month of the year when there is an excess of herbage over grazing requirements. The decreasing moisture deficit in the soil necessary to produce this excess is also likely to produce an increasing level of worm infection in the herbage. It also coincides with the period when the susceptible lamb population is consuming increasing quantities of herbage. It would seem that the general pattern of this infection corresponds with that which is seen in lambs in late summer in Britain.

There is some evidence that the occurrence of disease in both Britain and Australia tends to occur under similar sequences of climatic conditions. It arises under conditions which facilitate good herbage growth, or following a wet spell which ends prolonged drought. The fact that the distinction between wet and dry spells is more marked and that they occur more frequently in Australia than in Britain should facilitate much needed observations on this aspect of the epidemiology of parasitic gastro-enteritis.

### E. PARASITIC BRONCHITIS IN CATTLE

The epidemiology of parasitic bronchitis in cattle has been intensively studied by a number of workers and the subject has been reviewed by Poynter (1963) and Michel and Ollerenshaw (1963). The most important features of *Dictycaulus viviparus* in relation to climate are the high susceptibility of the free living larvae to desiccation, the rapid rate of development in summer temperatures, the inactivity of the larvae and their failure to migrate from the faecal pat. Michel and Rose (1954) showed that not more than $\frac{1}{2}\%$ of the larvae succeeded in moving from the faecal pat and none of these were more than 5 cm from the pat. They concluded that in order to become infected, cattle must ingest bovine faeces. Cattle usually avoid their own faeces when

grazing, but less successfully when the faeces are diarrhoeic than when they are of normal consistency. Undisturbed faecal pats can serve as a reservoir of infection provided that the faeces remained sufficiently moist to be spread (Rose and Michel, 1957). On this basis climatic conditions favouring rapid and lush growth of herbage will also favour the development of high herbage infections. Conversely, dry conditions are likely to reduce the level of infection on the herbage.

Examination of the Veterinary Investigation Officers reports over England and Wales in recent years confirms the association of a high incidence of disease with wet summers, as in 1954, 1958 and 1965. In these years losses were reported in late summer and autumn. Incidence was high also in 1960, a year when spring and early summer were dry, followed by above average rainfall from July onwards. Incidence in 1961 was much lower—in the below average category—though a number of cases were reported in July. In 1962 incidence was even lower than in 1961. Both these years had summer and early autumn with below average rainfall, though 1962 was slightly wetter than in 1961. Surprisingly, in 1955 and 1959, both years with a remarkably dry summer and early autumn, a high incidence of disease was reported from many centres both during the dry spell and later in autumn. It was suggested that this was due partly to the large number of carrier animals carrying over an infection from the previous year, and partly to the lack of grazing, forcing animals to graze in close proximity to faeces which they would normally avoid. At this stage it is not possible to suggest any quantitative relationship between weather and the incidence of parasitic bronchitis in cattle beyond the fact that a high incidence of disease occurs under the two extremes of soil moisture in summer. When more information on the incidence of disease and the timing of outbreaks is available, it should prove profitable to compare these with the moisture deficits in the soil. Owing to the rapid rate of development of larvae, saturation deficits will need to be measured on a weekly basis if a worthwhile relationship is to be developed.

## VI. Discussion

It will be appreciated that the relationships between climate and helminthic diseases discussed above are in varying stages of development. With fascioliasis much is known and the relationship has been treated in depth. Nematodiriasis occupies a middle position, while the relationships in respect of parasitic gastro-enteritis in cattle and sheep and parasitic bronchitis in cattle are much more tentative. Indeed, there may well be criticism that these last relationships are so tenuous that they should have been omitted. It must be remembered, however, that study of the quantitative relationships between climate and disease in general is in its infancy. It is not long since the relationship between climate and fascioliasis was couched in terms similar to those used here in respect of parasitic bronchitis. In this situation there is a need for some speculation in order to stimulate a wider interest in this aspect of epidemiology.

In extending this account to encompass the major helminthic diseases of

livestock, certain features are seen to be common to a number of diseases. Although the level of disease in any year may be influenced by the level of disease in the previous year, a feature of all the diseases under discussion is that disease levels may go from low to high, and revert to low in succeeding years. Moreover, when epizootics arise, heavy losses may be sustained over extensive areas, sometimes even in adjoining countries. Outbreaks occur on many farms with widely differing husbandry practices and varying levels of disease control. All these features are a reflection of the overwhelming importance of the prevailing climate in determining disease levels. The fact that similar disease levels arise under a wide variety of farming conditions and over extensive areas is also an indication of the overriding importance of large scale weather patterns, even though these may eventually operate on the parasite in terms of its own microclimate. The fact that some relationships between weather and disease can be based on monthly figures is a further indication of the overall importance of the macroclimate.

Equally important in assessing the role of climate on helminth parasites are the disease-free years when, seemingly, the parasite population is reduced to an innocuous level. In this context it is important to recognise that the level of the parasite population is determined by the interaction between the high reproductive potential of the parasite and the variable limiting factors (mainly meteorological) in the environment. Normally, these limiting factors maintain a high environmental pressure on the parasite, high mortality ensues and the population is maintained in check. Epizootics arise when there is a relaxation in the environmental pressure; there is an immediate response from the parasite resulting in a population explosion. A special feature of some of the parasites under review is their ability not only to withstand particular adverse climatic conditions, but to build up a reservoir of stages capable of taking advantage of a subsequent relaxation in the environment. The succeeding level of the parasite population on the herbage may be correspondingly higher, may occur at an unusual time, and may present a completely different set of problems from those concerned with disease control.

Although resistance and immunity may play an important role in determining the class of animal which becomes infected, such is the continual replacement of stock that susceptible animals are available each year. Where resistance mechanisms depend on a degree of exposure, the level of that exposure is often dependent on the climate. The extent to which this exposure occurs at any given time may have an important bearing on the subsequent occurrence of disease in animals normally regarded as resistant.

Generally, development of the free living stages of the parasites under review is possible in summer, but not in winter in cool temperate climates, and it is this feature which largely determines the seasonal incidence of disease. In Britain, where many cattle and an increasing number of sheep are in wintered, there has been much research to determine whether a particular parasite survives in carrier animals and/or on the pasture.

In controlling disease this may be an important factor, but in relation to climate a different emphasis is needed. First, it is enough to recognise that the parasite survives, even though this may be at a low level. Such is the repro-

ductive potential of helminth parasites that by early summer there is usually a sufficient output of eggs or larvae on to pastures to produce a high herbage infection later in summer. Second, there is a need to recognise the possibility that the parasite may overwinter on the pasture in quantities sufficient to produce serious disease in stock turned out to grass, without the need for a further build-up in the population. Where this possibility seems likely it is important to determine the weather patterns favouring this process.

Although temperature largely governs the rate of development of eggs and larvae on pasture, the number of parasites which actually develop to the infective stage is inextricably linked with the availability of moisture. It is this factor which is most important in determining the intensity of disease. Nematodiriasis appears to be an important exception chiefly because there is rarely a soil moisture shortage before mid-April. Extreme wetness facilitates development of the free living stages, enabling the build-up of large populations of the infective stage on the herbage with a consequent high level of disease. Dryness, on the other hand, prevents development and a low incidence of disease ensues. There is ample evidence, however, that not all free living stages are killed by dryness, and indeed, some eggs and larvae are remarkably resistant to desiccation. Prolonged dryness leads to an accumulation of these stages which may resume development following the onset of wet weather and again give rise to large populations of the infective stage on the herbage. Parasitic gastro-enteritis of sheep and cattle are the most important helminthic diseases to show increased levels of incidence following dry periods in summer. The same feature appears to be true of parasitic bronchitis, though no free living stage of *Dictyocaulus viviparus* is particularly resistant to desiccation. In special circumstances, fascioliasis also shows an increased incidence following a dry summer, but there is evidence that this increase is due to the consequent lack of herbage forcing animals to graze the wetter flukey areas.

When dry weather in late summer and autumn influences the incidence of parasitic gastro-enteritis in both sheep and cattle, stock which are affected in the following winter include those which would normally be regarded as resistant, e.g. cattle at grass during the summer and ewes. Although work in Glasgow suggests that the extent to which winter ostertagiasis occurs is dependent on what happens to the larvae and not to the host, any prolonged dry spell is likely to reduce the uptake of larvae at that time by the grazing animal, and this may interfere with the normal development of resistance. In the same way, the development of resistance to *Dictyocaulus viviparus* may be influenced by prolonged dry weather. Certainly the lowest overall incidence of both parasitic gastro-enteritis and bronchitis occurs in Britain in years when moisture conditions in the soil are intermediate between the extremes of wetness and dryness. These conditions might well permit enough development of the infection on the herbage to maintain resistance at a high level without producing clinical disease.

As might be expected in view of the rarity of dry summers in Britain, the epidemiology of helminthic disease at such times has received little attention. There is sufficient evidence, however, to warrant further work in respect of the occurrence of disease brought about by extremes of soil moisture, includ-

ing investigations into the occurrence of a low level of disease when soil moisture is between these two extremes.

The use of rainfall as the meteorological parameter on which to estimate soil moisture has its defects. Now that acceptable methods of estimating evapotranspiration are available much more meaningful estimates of soil moisture can be made. Much work has already been done on the growth of grass in relation to soil moisture deficits and it would be most useful if future detailed studies on the epidemiology of individual species could relate development of free living stages to soil moisture deficits. This in turn could be related to grass growth, which is already accepted as playing an important role in determining the microclimate where development of the free living stages occurs.

Although some suggestions have been put forward relating the incidence of helminthic disease with varying weather patterns, more detailed information primarily on incidence will be needed before precise relationships between the incidence of disease and soil moisture can be developed. With some diseases there is evidence that weekly rather than monthly assessments of soil moisture will be required.

A prominent feature of the studies relating the incidence of disease to current climate is the obvious practical benefits that accrue from the results. Depending on the time factor, it may be possible to forecast the incidence and expected intensity of a particular disease, and this provides time for the appropriate control measures to be put in hand. Even where the time factor is such that it is not possible to forecast the incidence of disease, recognition of key weather patterns is likely to provide farmers with useful background information. While some may deride traditional folklore regarding the weather and farming, such information can play a useful role if it stems from sound observation.

Recognition that helminthic diseases are weather sensitive offers two alternative approaches to control. The first is to devise and follow practices which will avoid the effects of key weather factors. For instance with fascioliasis drainage of wet land eradicates the fluke snail; with nematodiriasis, disease can be avoided by grazing lambs on fields not grazed by lambs in the previous year. In any given situation these solutions to controlling disease may or may not be practical. A farmer may not have the capital to carry out the necessary drainage improvements, or he may be too heavily stocked to have adequate worm-free grazing. In certain circumstances a farmer may decide that it is worthwhile to carry out a control programme as a routine each year, accepting the fact that this level of control is necessary only in years of high incidence. In doing so the farmer may be able to recover the cost of control by increased stocking rates. With all these solutions the farmer must sacrifice a degree of flexibility in his husbandry operations or add to the cost of production. The extent to which he is prepared to adjust his farming practice will depend largely on the magnitude and frequency of occurrence of each individual disease, the extent to which it interferes with, for instance, the maximum utilisation of herbage, and above all on the resulting profitability of the individual enterprise.

The other alternative approach is to recognise that not all farming attains a uniformly high standard, that disease levels vary, and that weather plays a dominant role in determining the level of that disease. Control aims at limiting the level of the parasite population when climate ceases to contain the population of the parasite. This means that the degree of control will vary from year to year. This approaches recognises that there are other desirable objectives such as the eradication of disease and control of sub-clinical parasitism, but suggests that for many farmers there must be an order of priority in disease control. This is to undertake control by the most efficient means at the most advantageous times appropriate to the expected intensity of disease. The means by which such assessments are made and the precise recommendations for control depend on an analysis of all the relevant factors; the more that is known about any relationship between climate and disease the more specific can be the recommendations for control. Although forecasts of incidence may serve as a timely reminder to inefficient farmers of the need to take some drastic action, its prime purpose is to keep the well informed farmer abreast of the ever changing disease situation. It demands some understanding of the life history of the parasite, of the relationship between disease processes and the weather, and of the integration of alternative methods of control. The farmer must recognise that when forecasts of incidence are issued, these refer to general trends over a wide area, but they must be interpreted in relation to conditions on his own farm. Individual farmers must be given time to satisfy themselves from their own experience that forecasts are sufficiently reliable for their purpose. Where it is not possible to produce forecasts either because of the time factor or because reliable relationships do not exist, it may be possible to make farmers aware of particular patterns of weather in relation to the occurrence of specific diseases. Many farmers can recall past disease experiences and it is not asking too much of them to associate particular disease situations with the appropriate weather sequence. This would extend traditional knowledge and encourage them to make their own assessments of the disease situation each year, a process which they already adopt with regard to the interaction of weather on their other farming operations.

The main advantage of this approach to control is that it allows the farmer a greater degree of flexibility with regard to the management of stock and the utilisation of his pastures. Limitations on husbandry practices and control of disease are made only when there is a real need. In this way more efficient control is achieved at less cost.

It is accepted that this approach to the control of helminthic disease is asking a great deal of all concerned. All the relationships discussed need refinement, and often a good deal of work before they can be of real benefit in controlling disease. Nevertheless, even where relationships are not precise or where forecasts are experimental, they have something constructive to offer whatever the standard of farming achieved by individual farmers. It is a situation where progress can be made not only through the collaboration of scientists of different disciplines, but also with the active participation of farmers. With continuing development there is ample evidence that this approach can make a worthwhile contribution to the control of animal disease.

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# SHORT REVIEWS

## Supplementing Contributions of Previous Volumes

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# The Biology of the Hydatid Organisms

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## I. Introduction

This brief review follows the general form of the earlier review in "Advances in Parasitology" Vol. 2 (Smyth, 1964) and attempts to summarize work since carried out, up to early 1968. The areas in which significant progress has been made have been discussed in some detail, but attention has also been drawn to those research areas which continue to be neglected.

The clinical, pathological and epidemiological aspects of hydatid disease are not dealt with here and immunity and diagnostic tests are treated only very briefly. A valuable work (in Rumanian with an English summary) by Lupaşcu and Panaitescu (1968) reviews much of the literature in these fields, and a special issue of the W.H.O. Bulletin (Vol. 39, No. 1, 1968) deals with problems relating to the hydatid organism.

## II. Speciation

Recent literature describing *Echinococcus* species in new or hitherto unreported hosts from different parts of the world emphasizes the complex nature of the speciation problem, as did my earlier review. New species, sub-species or "strains" have been described which have different morphological characteristics from the widely accepted species *E. multilocularis* and *E. granulosus*. Space does not permit a list of all the new definitive and intermediate hosts reported, but those with a bearing on the general speciation pattern are mentioned here.

Rausch (1967a) surveyed the geographical distribution of *E. granulosus* and *E. multilocularis* and traced their probable dispersal throughout the world in historical time. However, not all workers accept these two forms as valid species, and most Russian writers place the organism causing multilocular cysts in the intermediate host into a separate genus, *Alveococcus multilocularis* (see Abuladze, 1960; Kurashvili, 1964). Rico (1961, 1963) and Romero-Torres and Campbell (1965) considered *E. granulosus* as not specifically distinct from *E. multilocularis*. In reviewing current views on speciation, Rausch (1967b) discussed and evaluated the criteria used to determine a species and sub-species. He recognized the following species and sub-species as valid on the present available information.

### *E. granulosus*

Sub-species:

*E.g. granulosus*, nominate sub-species, infective to dogs and domestic herbivores. Probably introduced from Europe to other regions of the world by settlers in their domestic animals.

*E.g. canadensis*. The indigenous species of the boreal region of N. America, utilizing wolves and wild ruminants.

### *E. multilocularis*

Sub-species:

*E.m. multilocularis*, nominate sub-species, infective to dogs and foxes. Intermediate hosts small rodents. Distribution restricted to that of suitable rodent hosts in Europe, Russian and central North America.

*E.m. sibiricensis*, utilizing the arctic fox and rodents on St. Lawrence Island

*E.m. kazakhensis* (Shul'ts, 1961), larval stage in sheep and wild swine (*Sus scrofa*) in Kazakhstan. Adults in dogs. Further work needed to establish this as a separate sub-species.

### *E. oligarthrus* Diesing 1863

Adults of this cestode in South American felids (*Felis concolor* and *F. yagouroundi*) from Brazil. Thatcher and Sousa (1966, 1967) described adults from the same hosts in Panama. Intermediate host not known.

*E. PATAGONICUS* Szidat 1960

Adult taken from the Magellan fox (*Dusicyon culpaeus culpaeus*) in the Meuquén provence of Patagonia. Intermediate host not known.

In recognizing only two sub-species of *E. granulosus*, Rausch placed the sub-species described by Verster (1965) from South Africa into the nominate sub-species *E.g. granulosus* because of the lack of evidence of ecological segregation or marked host specificity between *E.g. granulosus* and the cestodes she described, namely:
*E.g. ortleppi*: Transvaal; hosts, dog and black-backed jackal (*C.* (*T.*) *mesomelas*), cysts in cattle.
*E.g. africanus*: Transvaal, Orange Free State; hosts Canidae, cattle and sheep.
*E.g. felidis*: Transvaal; definitive host, *Panthera leo*, intermediate host not known.
*E.g. lycaontis*: Transvaal; hosts, Cape hunting dog (*Lycaon pictus*), sheep.
On similar grounds, Rausch considered *E.g. equinus* (Williams and Sweatman, 1963) from horses in Great Britain, conspecific with *E.g. granulosus*: the adults can develop to maturity in the red fox, including the Australian red fox (Howkins *et al.*, 1965), but dogs are presumably the most important definitive hosts. *E.g. equinus* was reported by Dailey and Sweatman (1965) in donkeys from the Lebanon and Syria, where *E.g. granulosus* commonly infects dogs and domestic animals; here again, evidence of ecological segregation would be difficult to establish and a predator-prey relationship between dog and donkey seems unlikely.

The morphological differences used to differentiate between sub-species occurring in unusual hosts or in an isolated geographic situation may not in themselves have taxonomic significance. For example, Verster (1965) places the *Echinococcus granulosus* infecting dogs and domestic animals in Australia and New Zealand in the sub-species *E.g. newzealandensis* because it differs from the nominate sub-species from Germany in the number and arrangement of segments and in the number and distribution of the testes. Yet because an indigenous form of *Echinococcus* is not known to exist in this part of the world before the movement from Europe of people bringing domestic animals, this could represent the source of the present infection and without further evidence there is, according to Rausch (1967b), no justification for a separate sub-species.

Gill and Rao (1967) described a buffalo-dog infection of *E. granulosus* in India which differs from the classical descriptions of this species. The cysts in the buffalo were large and had a 90% viability rate, unlike those found in the zebu. The eggs were not infective to white rats, white mice, rabbits, golden hamsters, guinea-pigs or chicks. Secondary cysts, however, developed in white mice. The mature adult had either 2 or 3 proglottids in the dog and did not develop to maturity in the cat. Morphological characters peculiar to this form are discussed together with the question of speciation. The authors suggested that this cestode should be considered a "variation" or "mutant" of *E. granulosus* adapted to a different host and one more suitable than the

zebu. They concluded that this evidence added support to the broad view of speciation in *Echinococcus* put forward by Smyth and Smyth (1964).

Until the complete life cycles of the South American species of *Echinococcus* are known and experimental infections in a variety of hosts carried out, *E. oligarthrus* and *E. patagonicus* should probably be regarded as *species inquirendae*. Morphologically, the adults of these cestodes differ from each other and from *E. multilocularis* and *E. granulosus* in size, hook number and size, and number and arrangement of testes; both *E. oligarthrus* and *E. patagonicus* are described as having characters between *E. multilocularis* and *E. granulosus* (Thatcher and Sousa, 1966; Szidat, 1963), and each is regarded by these authors as more closely allied to *E. multilocularis*. Thatcher and Sousa (1966) suggested that *E. oligarthrus* was causative of a case of human multilocular hydatidosis in a native Panamanian who never left the Republic and had spent 30 years in jungle areas where there are wild felids. Support for this view comes from the large hook size found in man (39·9–43·7 μ), and the fact that the cestodes from two infected pumas and one infected jaguarundi had larger hooks than other known species of *Echinococcus*; the shape and number of hooks in protoscoleces from the human cyst corresponded, within the limits of variation, with those found in the mature and immature adults. *E. granulosus* has not been reported in Panama and other cases of human hydatidosis there are believed to be of foreign origin. Thatcher and Sousa (1967) reported a heavy infection (6000 worms) of non-gravid *E. oligarthrus* in a jaguar (*Felis onca* L.).

Szidat (1963) mentioned that several cases of autochthonous human alveolar hydatidosis were reported from South America—about 12 cases from Argentina by Vinas (1903), and one case from Uruguay by Dévé *et al.* (1936)—and he discussed the question of an indigenous causative organism. Interestingly, Szidat quotes Dévé's description of the cyst found in the Uruguay case as assuming an "intermediate position" between the European alveolar echinococcosis and the cyst of the common hydatid *E. granulosus*, while Thatcher and Sousa (1966) stated "it was indicated by Sousa and Lombardo Ayala (1965) that the Panamanian human hydatid resembled *E. multilocularis* in some respects and *E. granulosus* in others."

Szidat (1963) found only six of 50 foxes naturally infected with *E. patagonicus* and the worm burden was low, 30 being the maximum number found in one fox and only 1–12 worms in each of the others. He related the low percentage infection and the low worm burden to the changed diet of the Magellan fox. Since the introduction of the European hare and the increase in sheep farming, the small native rodents, which could serve as intermediate hosts for cestodes, are no longer a major part of the fox diet. It is known that domestic livestock in South America carry infestations of *E. granulosus*, with the dog as the definitive host, and unless it can be shown that there is a separate sylvatic cycle in indigenous animals, it would not be unreasonable to suggest that the relatively few wild animals found to be infected have "strains" of *E. granulosus* which have become adapted to new hosts. This agrees with the hypothesis of multiplicity of adaptive strains previously put forward (Smyth and Smyth, 1964). This suggestion was supported also by Blood and

Lelijveld (1969) for the *Echinococcus* sp. found by them. Of 848 carnivores (seven species examined from three localities in Argentina), infections included 16 of the 442 Pampas grey fox (*Dusicyon gymnocercus*), 56 of the 360 Patagonian grey fox (*D. griseus*), and one of six grison (*Galictis cuja*).

Infection in the 72 foxes varied from a few to 2500 worms in one fox. The grison contained three well developed worms without gravid proglottids. Of 223 animals examined as possible intermediate hosts, 104 were rodents (9species), 19 were European hare, 80 armadillos (3 species), 19 opossums and one quanaco. They were all from the districts where infected carnivores were found, but none had hydatid cysts. Attempts failed to infect golden hamsters, gerbils, albino rats, guinea-pigs, rabbits and Pampas cavies (*Cavia pamparum*) using two doses of 25–300 echinococcus eggs from the Pampas grey fox. None of the animals had hydatid cysts at necropsy 4–6 months later. A small sterile cyst was obtained in one of two cotton rats given a dose of 250 eggs, and in one of 18 rats, in another series, another small sterile pulmonary cyst was found. Intraperitoneal inoculations of eggs from Patagonian foxes into albino mice produced cysts which were sterile.

Structurally the *Echinococcus* sp. from the Patagonian grey fox and the Pampas grey fox differed slightly from the classical description of *E. granulosus*, but the results of infections of cotton rats and mice led to its tentative classification as *E. granulosus*, possibly as sub-species or "strain" which is enlarging its host range in South America. The Pampas grey fox was found capable of infection with *E. granulosus* from sheep cyst material, but no mature worms were recovered 60–100 days after infection, and specimens were smaller than those from dogs infected with the same material. Further work is needed to find out if the morphological variations found in adult cestodes of different sylvatic hosts in South America represent varieties of the *E. granulosus* from domestic livestock, or exist as distinct indigenous species dependent on a separate predator-prey relationship of hosts and able to produce in man a multilocular-type cyst.

## III. Host Specificity

### A. General

Morphological variations and partial or complete adaptability of *Echinococcus* to a wider variety of hosts is predictable on theoretical grounds. As the adult worm is hermaphrodite and undergoes polyembryony in the intermediate host, any genetic mutation which occurs and survives in an unusual intermediate host may, if it can become established and develop a viable cyst, give rise to a large population of the new variant (Smyth and Smyth, 1964). Detailed work on the chromosomes of *E. multilocularis* and *E. granulosus* has been neglected, but first indications are that there is no difference in the chromosome number in the two species. Sakamoto *et al.* (1967) cultured cells of the germinal layer of *E. multilocularis* and gave the chromosome number as 18, which agrees with that given by Lukashenko *et al.* (1965), and

the same number of chromosomes was found in *E. granulosus* by Smyth (1962). Both species have a pair of hooked (banana-shaped) chromosomes.

### B. DEFINITIVE HOSTS

The wider host range for the adult parasite may not be significant in the ultimate spread of echinococcosis unless the cestode can become fully adapted to the new host—i.e. become established in large numbers and produce viable eggs. For example, the report of Blood and Lelijveld (1969), on the number of *Echinococcus* sp. from the two species of fox in the Argentine, showed that the number of foxes with gravid worms was very low (12 of 72 infected foxes). Szidat (1963) also found relatively few worms in the naturally infected Magellan fox and only three of six infected foxes contained mature worms. Experimental infections of *E. granulosus* from sheep and pig material in the fox (*Vulpes v. crucigera*) similarly produced low worm burdens with few gravid worms (Matoff and Yanchev, 1965). Szidat related the maturity of the worms found in naturally infected Magellan foxes to sexual maturity of the host. These foxes were taken during the rutting season (July and August), whereas a series of foxes examined earlier (1960) from the same area and, presumably, at a different season, contained no mature worms. In this connection, Fay and Rausch (1964) commented that the seasonal cycle of *E. multilocularis* in the Arctic fox is related to the seasonal availability of the vole, the intermediate host. The peak infection rate of 90% was found in foxes in autumn, with a trough of 30% infection in spring. In voles, the infection rate was highest in spring and lowest in autumn and was directly related to age and composition of the population. The infection rate of voles increased logarithmically with age.

There was no suggestion by the authors that the maturity of the parasite was dependent on the endocrine activity of the fox host; they held that it was due to the short life span of the adult worm, together with the fact that, because of the larger numbers available, voles were eaten in greater quantities in summer and autumn. Experimental investigation of the effect of age, sexual maturity and strain differences of normal definitive hosts of both species of *Echinococcus* on the growth and development of the parasites should probably be carried out before any significant comparisons can be made with development in "abnormal" hosts. However, the enormous number of animals which would have to be used to obtain statistically significant results would make such an investigation difficult. If workers were encouraged to record, for natural or experimental infections, the approximate age or sexual maturity and sex of the hosts, some significant information would perhaps accrue.

The effect of the physiology of the host's intestine on the establishment and maturation of the parasite was dealt with in the earlier review (Smyth, 1964), but a more recent suggestion is that the structure of the host's intestine may influence the proper establishment and development of the cestode (Smyth and Smyth, 1968). In histological sections of the duodenum of the dog, cat and fox, the crypts of Lieberkühn and the villi differ in size and shape. In cross-section, the crypts appear mainly oval in the cat, wider, more compressed and slit-like

in the dog, and much smaller and almost round in the fox. The villi in the fox tend to be more widely separated, longer and thinner than in the dog and the evaginated protoscoleces of *E. granulosus* may not be able to attach to the mucosa as readily as in the dog, and many might be swept away by the movement of the gut contents. The smaller scolex of *E. multilocularis*, however, may be able to enter the smaller crypt of the fox more readily. Yamashita *et al.* (1958) compared the sizes and growth-rates of *E. multilocularis* and *E. granulosus* from 15 days post-infection and stated that at 15 days the hooks of *E. multilocularis* are rather larger (mean 23·0 μ) than those of *E. granulosus* at 16 days (20·8 μ). It would be interesting to know whether this difference is apparent in the freshly evaginated protoscolex and whether the hooks of *E. multilocularis* can be erected and used for attachment to the gut at an earlier stage than in *E. granulosus*, as this might be an added factor in helping the worm to become established in the fox. As discussed later, close contact with a solid substrate has been shown, by *in vitro* experiments with *E. granulosus*, to be important for the strobilar development of the worm, and the low worm burdens and few gravid proglottids found in foxes experimentally infected with *E. granulosus* may be partly related to the topography of the fox intestine.

### C. INTERMEDIATE HOSTS

Work on the larval stage of *Echinococcus*, in recent years, has been concerned mainly with the susceptibility of various pure and hybrid strains of mice to primary and secondary infections of *E. multilocularis*.

Using the Alaskan strain of *E. multilocularis*, Yamashita *et al.* (1963) orally infected five mouse strains and from their results distinguished two types of cyst development. Type 1 developed rapidly with protoscoleces appearing 1½–2 months after infection. Host tissue reaction to type 1 was slight. Type 2 developed more slowly: cysts were small and protoscoleces developed after 5–7 months; host tissue reaction was severe. Susceptibility in two of the five strains was 100% and 39%–82% in the other three. One of the strains displayed a sex difference in susceptibility; the males were 100% susceptible with a type 1 infection, the females 85% susceptible with a type 2 infection. Ohbayashi and Sakamoto (1966) also demonstrated sex differences in susceptibility in two strains of mice. The females of both strains were more resistant (45·4% and 75% infection), the males in both series showed 92·4% infection rate; protoscoleces developed only in brood capsules, which were found only in the larger cysts.

Lubinsky and Desser (1963) and Lubinsky (1964) demonstrated that certain strains of mice and their hybrids have differing degrees of resistance to growth of the vegetatively propagated strain of larval *E. multilocularis*. The resistance of the hybrids was not intermediate in amount; some hybrids showed a greater resistance than either parent while others showed as low a resistance against the cyst strain as the least resistant parent strain.

Lukashenko (1966) investigated the rate of growth and host tissue reaction to *Alveococcus* (= *Echinococcus*) *multilocularis* larvae in 19 species of rodents

(2000 + animals) and man. The most rapid development of cysts took place in the cotton rat (*Sigmodon hispidus*) and the host tissue reaction was slight.

Protoscoleces developed in the cysts in 43–45 days and the tissue reaction was a thin layer of connective tissue surrounding the cysts. In five species of voles investigated, the cysts developed more slowly, protoscoleces developing only after 4 months; the cysts were surrounded by a well defined connective tissue capsule. In white mice the cysts developed more slowly than in voles and mainly were either sterile or contained a single protoscolex. Host tissue reaction was also more marked than in voles with a fibrous connective tissue capsule or a large zone of granulation tissue around the cyst. A marked difference in the rate of development of cysts was found in different strains of mice. In rabbits, white rats, golden hamsters, grey hamsters, squirrels, field mice (*Apodemus agrarius*) and brown rats (*Rattus norvegicus*) no cysts developed although evidence of penetration of the oncosphere and development of a primary parasitic follicle was sometimes found.

Histological examination of infected human liver (18 cases) and brain (five cases) showed that cyst development was abnormal and host tissue reaction very marked. Vesicles were very small, irregular in shape with a poorly developed germinal layer; protoscoleces were rarely found. The development of a connective tissue capsule around young cysts appeared similar to that found in mice and voles, but later this was surrounded by intensive changes in the liver parenchyma and neuroglia. Giant cells were often found in necrotic tissue around dead vesicles; these are usually not found in rodents.

## IV. Establishment and Development of Adult in Definitive Host

The early establishment of *E. granulosus* in the dog's duodenum has been studied for periods of 6 h to 41 days after infection (Smyth *et al.*, 1969). Six h after infection, evaginated scoleces occurred between the villi of the duodenum, and although the suckers were flat and not cup-shaped in section, the villus epithelium near a sucker was usually worn or partly torn, so that already the sucker could make contact. The suckers remained evaginated but the scolex was sometimes re-invaginated. After 18 h most worms occurred at the base of the villi, and one worm within a crypt of Lieberkühn. The suckers were cup-shaped but the hooks were relaxed and close to the body of the worm. At 24 h after infection the picture was similar but more worms were found within the crypts. By 3 days, the hooks were erect and able to penetrate the epithelium and one worm had disrupted a crypt and lay partly in the lamina propria. In most larger worms only the scolex lay within a crypt, the suckers grasping the epithelium of the surrounding villi. The crypt wall became stretched and the epithelium near the scolex was flattened, sometimes broken. However, in all stages up to 41 days, a few worms had some or all of the suckers inside a crypt. The crypt wall was then usually broken down near the suckers. Thus, close contact is maintained with the host tissue throughout development and as microtriches cover the fore part of the worm,

some sort of physiological exchange may be taking place between the worm and the surrounding tissue (Smyth *et al.*, 1966; Jha and Smyth, 1969; Smyth, 1968).

Study of the rate of strobilar development and maturation for *E. granulosus in vivo* by Smyth *et al.* (1967, 1968) showed that development is not uniform throughout the population of cestodes in any dog examined. Taking the *maximum development* we can note that: segmentation begins at 14 days; the second proglottid appears at 18 days; scattered testes capsules appear in the posterior proglottid (early gametogeny) at 22 days; the genital pore is first seen on the 24th day; spermatozoa appear in the receptaculum seminis as early as the 26th day; and the uterus appears as a thin-walled sac in the centre of the maturing proglottid on the 28th day. At this stage, free ova in various stages of development lie within the uterus but embryonated eggs containing 6-hooked oncospheres appear only at 40 days.

## V. Cytology and Ultrastructure

Some studies on ultrastructure, though few cytological studies, have been carried out since 1964.

### A. ROSTELLAR GLAND

Further studies by light and electron microscopy have revealed the unusual situation of the secretion having its origin in the nucleus, not in the cytoplasm as in most gland cells (Smyth *et al.*, 1969). By electron microscopy, the secretory globules appear as intensely osmiophilic bodies within the nuclei (Fig. 1). The apparent "secretion", a knob-like formation outside the worm *in vitro*, may prove to be a precipitate formed as a result of an antigen-antibody reaction. Antibody released from the dog serum or from the intestinal mucus (or both) may interact with the secretion to form an antigen-antibody complex, but so far there is no unequivocal evidence for this (Smyth, 1969a,b).

### B. TEGUMENT (CUTICLE)

The ultrastructure of the tegument follows the pattern seen in other cestodes (Morseth, 1966a). The tegument is a cytoplasmic extension of cells lying in the parenchyma, its surface drawn out into minute projections (microtriches) (Jha and Smyth, 1969), and an odd reticulated surface pattern, not described for other cestodes, occurs in *Echinococcus*. The presence of microtubules was also reported.

### C. NERVOUS SYSTEM

Electron microscope studies of the nervous system (Morseth, 1967b) revealed a nervous system of typical platyhelminth pattern. The tegument is rich in sensory endings similar to those of the vertebrate type. Shield (1969) adapted the acetylthiocholine iodide technique (for cholinesterase) to demonstrate the distribution of nervous tissue in *E. granulosus*.

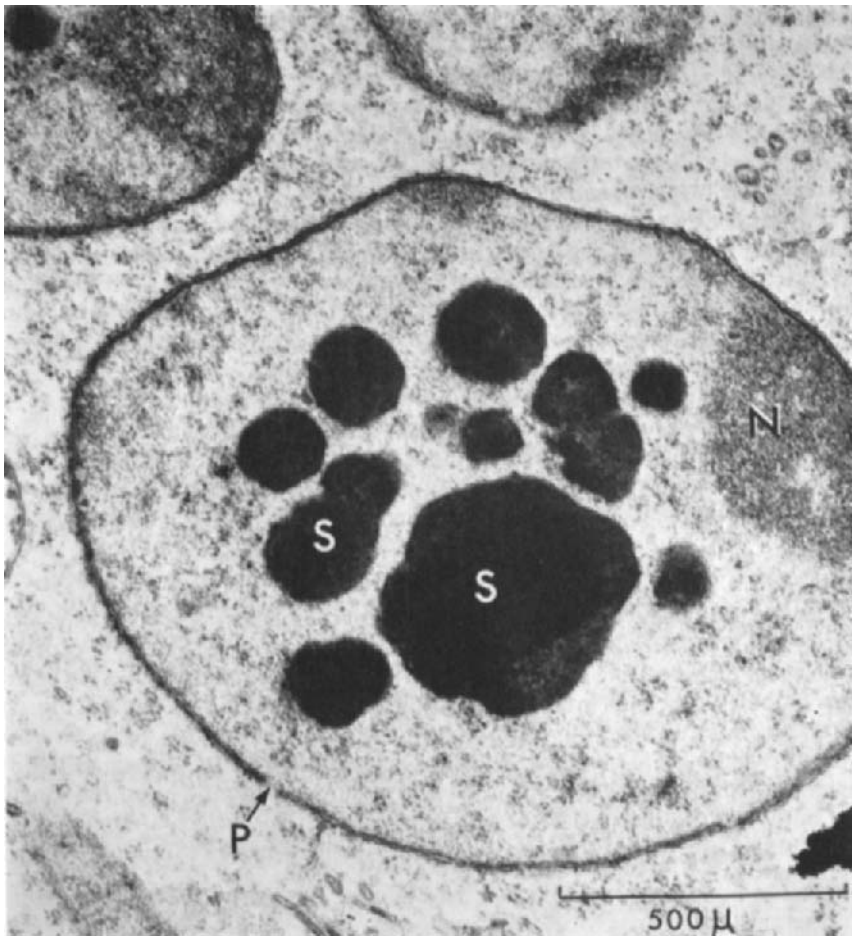


FIG. 1. Nucleus of secretory rostellar gland cell of *Echinococcus granulosus*, showing irregular shaped, osmiophilic (secretory?) globules. **N**, nucleolus; **S**, osmiophilic globules; **P**, pore in nuclear membrane. (Reproduced with permission from Smyth *et al.*, 1969.)

### D. CYST WALL

Cytochemical and ultrastructure studies show the hydatid cyst to be lined internally with PAS-positive materials. The laminated layer is a network of fine fibres with scattered dense granular material. The germinal membrane sends into the fibrous material of the laminated layer finger-like processes (Morseth, 1967a) which may prevent detachment of the germinal layer from the laminated layer, and possibly have a nutritive (i.e. placental) function.

### E. EGGS

Morseth (1965) studied the ultrastructure of taeniid embryophores and described the development of the embryophore blocks in the egg of *E. granulosus*. The egg has eight distinct layers and membranes, namely: the egg capsule, the vitelline layer, the outer embryophoral membrane, the embryophore, a granular layer lying just beneath the embryophoral blocks, a membrane at the inner limit of the granular layer, the oncospheral membrane, and the limiting membrane about the hexacanth embryo. The embryophore consists of blocks of electron-dense material containing spaces, or embryophoral lacunae. The blocks are irregularly polygonal in cross-section and fit together to form a protective layer about the enclosed embryo.

The chemical composition of the embryophore of *Echinococcus* sp. has not been investigated, but the embryophoric blocks of *T. hydatigena*, *T. ovis* and *T. pisiformis* eggs are believed to be a keratin-type protein (Morseth, 1966b). Laws (1968) has demonstrated that the embryophore is very sensitive to desiccation, few eggs surviving more than 3 days at 20% relative humidity, and he suggested that eggs carried by the wind would rapidly suffer damage by desiccation.

### F. HOOKS

The hooks of protoscoleces are also composed mainly of a keratin-type protein which differs somewhat from vertebrate keratins (Gallagher, 1964), having a higher content of aspartic acid and tyrosine and a lower content of glutamic acid, while the histidine content is two to three times the average value given for keratin. Glucosamine was not detected and this, together with the complete solubility of the hooks in alkali, eliminated the suggestion that chitin is present in any substantial quantity.

## VI. BIOCHEMISTRY

Only limited areas of biochemistry have been investigated since 1964, dealing with an analysis of succinate production (Agosin and Repetto, 1965), protein synthesis in the protoscoleces (Agosin and Repetto, 1967) and a study of the Krebs cycle in adult worms (Bryant and Morseth, 1968). The metabolism of the adult worms remains largely unexplored and this is clearly an area which calls for further work. With respect to protoscolex metabolism, Agosin and Repetto (1965) have demonstrated that various methods of $C^{14}O_2$ fixation are operative in *E. granulosus*. These are catalyzed respectively by phosphoenolpyruvic (PEP) carboxylkinase, pyruvate carboxylase, phosphophenolpyruvic carboxylase and "malic enzyme" according to the following equations:

$$\text{(1) PEP} + CO_2 + \text{(IDP)} \underset{\text{GDP}}{\overset{\text{PEP-carboxylkinase}}{\rightleftharpoons}} \text{OAA} + \text{(ITP)} \quad \text{GTP}$$

$$\text{(2) PEP} + CO_2 \xrightarrow{\text{PEP-carboxylase}} \text{OAA} + \text{Pi}$$

$$(3)\quad \text{PYRUVATE} + CO_2 + (\text{ATP}) \xrightarrow[\text{CTP}]{\text{pyruvate carboxylase}} \text{OAA} + (\text{ATP}) + \text{Pi} \qquad \text{CDP}$$

$$(4)\quad \text{PYRUVATE} + CO_2 + \text{NADPH} + H^+ \xrightarrow{\text{malic enzyme}} \text{MALATE} + \text{NADP}$$

Of the four systems listed above, only those involving PEP-carboxykinase and "malic enzyme" possess a sufficiently high activity to account for the production of succinate by the protoscoleces. Thus, 0·0963 μmol oxaloacetic acid/mg protein was formed in 3 h under incubation conditions appropriate for PEP-carboxykinase activity in the presence of inosine diphosphate (IDP). This figures a rate of production of 8·91 μmol succinate/g/FW/3 h. Since the production of succinate is only 7·0 μmol/g/FW/3 h under anaerobic conditions, this activity could more than account for the *in vivo* production of succinate (Agosin and Repetto, 1965). However, the activity of the "malic enzyme" is much higher and could produce 72 μmol succinate/g/FW/3 h which points to the "malic enzyme" being the primary enzyme system concerned in succinate production.

Evidence has been obtained by Agosin and Repetto (1967) that in protoscoleces protein synthesis takes place by a pathway involving amino acyl-adenylates and amino acyl-sRNA as intermediates. This system requires both ribosomes and "pH-5-precipitate" and is dependent on ATP, an ATP-generating system, GTP, magnesium and either potassium or sodium ions. The system is also inhibited by puromycin and ribonuclease but unaffected by chloramphenicol and actinomycin D. In contrast with the protein synthesizing systems reported in other organisms, polyuridylic acid does not stimulate either phenylalanine incorporation into microsomes or ribosomes or the amino acid moiety of phenylalanyl and RNA into microsomes. The system was shown to exhibit striking specificity characteristics in that *E. granulosus* ribosomes accept only the homogenous "pH-5 fraction" but not the rat liver fraction. However, the latter promotes the incorporation of amino acid into *E. granulosus* ribosomes in the presence of *E. granulosus* RNA. It was also shown that the above pathway was operative in intact protoscoleces. Preliminary studies by Bryant and Morseth (1968) have shown that the overall metabolic pathways in *adult E. granulosus* are essentially similar to those described in protoscoleces by Agosin and his co-workers.

## VII. Immunity

Investigation into the immune response of the intermediate host to the hydatid organism continues to receive much attention. A voluminous literature since 1964 mainly concerns (a) the development of new diagnostic tests and increase of the sensitivity and specificity of tests already in use, and (b) the analysis and purification of antigens of host and parasite origin in cyst material (fluid, cyst wall and protoscoleces). Both areas have been reviewed in some detail by Kagan (1968), Kagan *et al.* (1966), and by Lupaşcu and Panaitescu (1968), who discussed the eosinophilic response of the host to hydatid invasion and the dynamics of antibody formation. They distinguish

between peripheral eosinophilia, which is of inconsistent occurrence in hydatid disease but used in diagnosis, and the extensive pericystic eosinophilic infiltration, which occurs in response to hydatid "toxins" (= antigens?).

### A. DIAGNOSTIC TESTS

While new and promisingly sensitive methods of diagnosis are being developed (e.g. immunoelectrophoresis, double diffusion tests in agar gel, the fluorescent antibody test and the scoleoprecipitation test), the indirect haemagglutination (HA) test seems to be the most consistently sensitive at present. Kagan (1968) recommends HA as the test of choice but stresses that true sensitivity of any test is difficult to evaluate accurately from published reports since results often depend on so many variables. A modification of the HA test by Knierim and Saavedra (1966), the microhaemagglutination test, using microtitre methods, has proved useful for the mass screening of sera in an epidemiologic study of Brazilian recruits, and has given excellent correlation with known epidemiological distribution of hydatid disease in that country.

The Latex flocculation test, introduced by Fischman (1960a, b), uses Bacto Latex particles coated with diluted hydatid fluid antigen. When shaken with dilutions of suspected serum, these visibly clump (agglutinate) when antibody is present in the serum. A modification of this method was developed by Szyfres and Kagan (1963), who found the test as sensitive as the BF and HA tests with positive sera. Fischman (1965) compared the sensitivity of this test with that of complement fixation and found sensitivity was 8·5% lower in the complement fixation test; he also found that human hydatid fluid was better for this test than bovine or ovine fluids. Lupaşcu and Panaitescu (1968) suggested that negative results in CF test may be due to the fact that only the dialysable polysaccharide fraction of the cyst fluid, which does not induce complement fixing antibodies, can reach the host from an intact cyst.

Kagan *et al.* (1966) suggested that the high rate of false positives obtained with the intradermal (Casoni reaction) is probably related to the concentration of nitrogen in the antigens used. They found that when antigens over 100 γ-N/ml were used, at least 30–40% of the controls were positive, and the specificity of the test increased as the concentration of antigen nitrogen decreased. They used a technique patterned on the intradermal test recommended by the W.H.O. for the standardization of the bilharzia skin test, and they urged that a standardization of method along these lines should be used to obtain more accurate results.

Protoscoleces and the wall of cysts from human infections have been used as antigen in two further tests, namely the fluorescent antibody test and the "scoleoprecipitation" test. Both of these tests have been reported as having a high degree of sensitivity and may become very useful in diagnosis if further work confirms their sensitivity. Fraga de Azevedo and Rombert (1964), using protoscoleces from sheep as antigen, obtained fluorescence with all 14 sera (from infected lambs) which had been conjugated with fluorescein isothiocyanate; in 34 control sera, there was one false positive and ten doubt-

ful results. In six human cases of disease, only five sera gave positive fluorescence, while of the 20 sera tested as controls, seven gave doubtful results and the rest were negative. Pozzuoli *et al.* (1965) obtained 100% positive results with the sera of ten patients, conjugated with fluorescein isothiocyanate and tested against human cyst wall and sheep protoscoleces. The patients had hydatid cyst infection either in the lung or liver and both sites of infection gave positive results with this test. Panaitescu (1965) found that treatment of protoscoleces and cyst wall with Lugol's iodine prevented autofluorescence. This was observed to be strong in non-viable protoscoleces and in frozen sections of cyst wall; fluorescence was weak in viable protoscoleces. Autofluorescence may account for the false positives and doubtful positives found in the tests carried out by the workers cited above.

Shulz and Ismagilova (1962) demonstrated precipitates over the rostellum and around the excretory opening at the posterior ends of protoscoleces placed in immune serum. A similar immune reaction has been demonstrated by earlier workers for nematodes and trematodes. Protoscoleces were incubated in a few drops of immune serum in a hanging drop preparation and were examined on a warm stage at short intervals during the first 6 h and later once a day. Precipitation on the rostellum was observed as early as 4 min after placing the protoscoleces in the immune serum. After 24 h precipitation could be seen around the excretory opening, and after 48 h the precipitation around the hooks had become very heavy and in many cases the hooks and precipitation had separated from the scolex. No precipitation was found in protoscoleces placed in control serum. The precipitation reaction persisted in human immune serum as long as the cyst remained metabolically active. In artificially infected rabbits, the maximum reaction was reached on the 15th day; after this the reaction weakened and none was found by the 90th day. This follows the pattern of development of the cyst in the rabbit which is usually acephalic and does not develop further.

It is clear that greater specificity and accuracy of results in any of the diagnostic tests using hydatid fluid is dependent on the development of standard methods of procedure and standardization of the antigen used as suggested by Kagan (1968).

## B. HYDATID FLUID ANALYSIS

### 1. *Antigens*

Extensive work on the purification of *Echinococcus* antigens has continued, especially by Kagan and his group. Detailed description of this work is not possible here and the reader is referred to the review by Kagan (1968).

### 2. *Antibody*

Recent work in this laboratory has demonstrated that in addition to antigens, hydatid fluid contains small levels of antibodies which give precipitates in tubes and plates against both protoscoleces and adult tissue (Smith and Smyth, 1968). It is thus clear that some leakage of antibody from the host

into the cyst occurs, a fact that may account for the number of dead protoscoleces which are invariably present in cysts. It may also have some bearing on acephalic cysts, although the relationship of acephalic cysts and the amount of host antibody in the hydatid fluid has not been examined.

### C. VACCINATION

No major work on vaccination of the definitive host appears to have been published since the earlier review, but some further vaccination experiments in the intermediate host have been carried out. Gemmell (1966) showed that vaccination with activated embryos of *E. granulosus*, *Taenia hydatigena* and *T. ovis* (but not *T. pisiformis*) can induce substantial immunity against a challenge infection of *E. granulosus* in sheep. These results have been confirmed and experiments have shown that if viable, but non-activated, eggs of these species are used for vaccination, only the eggs of the homologous species *E. granulosus* can confer any protective immunity on the host (Gemmell, 1967). On this evidence, Gemmell supports earlier workers (see Gemmell and Soulsby, 1968) in the view that vertebrate intermediate hosts of cestodes may have two immune responses to a challenge infection following vaccination. The first immune response is due to antigen from the egg which, possibly by inhibiting penetration of the gut, prevents the establishment of the challenge infection. The second immune response is due to antigens from activated embryos of the same or heterologous species and is directed against the growth and development of the larvae at the site of election.

## VIII. *In vitro* CULTIVATION

### A. *In vitro* CULTIVATION OF THE ADULT

The problems of *in vitro* cultivation of *Echinococcus* protoscoleces to egg-laying adults were discussed in detail by Smyth (1968, 1969a) and Smyth *et al.* (1966, 1967). The problems to be overcome are: (a) successful evagination of the protoscoleces, (b) the development of a suitable medium, and (c) provision of culture conditions to initiate development in a strobilar direction. As is well known, the protoscoleces of this parasite have the potential to develop either into a cyst (as in secondary hydatidosis), or into the sexually mature adult within the intestines of a suitable definitive host. As emphasized before (Smyth, 1964), early attempts to culture the protoscoleces *in vitro* generally resulted in development in a *cystic* direction.

*Evagination of the protoscoleces*

Although some evagination will take place without preliminary treatment, the process is greatly accelerated by treatment with enzymes. Pepsin is said to play some part in bringing about evagination (Rycke and Grembergen, 1965), but further treatment with pancreatin and trypsin in the presence of bile serves the multiple role of accelerating evagination, stimulating activity, and removing cystic debris (Smyth *et al.*, 1966). Experiments on evagination

must be interpreted with caution, however, for there is evidence that evaginating solutions only *accelerate* evagination and do not increase the final percentage of evaginated protoscoleces (Smyth, 1967).

*Cultivation to sexually mature strobila*

Studies on the ultrastructure of the tegument of the freshly evaginated protoscoleces of *E. granulosus* (p. 335), together with the observation that, on reaching the duodenum in the dog, the protoscolex quickly made and maintained very close contact with the mucosa, led Smyth *et al.* (1966) to believe that contact with a solid nutrient substrate might prove to be the stimulus necessary for strobilar development to take place. A series of experiments designed to test this hypothesis was carried out. After evagination treatment, protoscoleces were placed in a diphasic medium consisting of a solid base of bovine or canine serum, coagulated by heat, in which a series of small holes had been made with a fine glass pipette; this was covered by a liquid phase of "Parker 199" plus 20% hydatid fluid. Initially a gas phase of 8·8% oxygen and 5% carbon dioxide in nitrogen was used. In this diphasic culture system strobilar development occurred and organisms with three proglottids were developed (Smyth, 1967).

A much improved medium was later developed (Smyth *et al.*, 1967) using the same coagulated serum base and a liquid phase of Parker 858 plus 20% hydatid fluid with a gas phase of 5% $CO_2$ plus 10% $O_2$. In this medium worms developed to sexual maturity: large numbers of spermatozoa were seen in the testes, vas deferens and in the cirrus; the uterus contained masses of free cells which appeared to be undeveloped ova. The receptaculum seminis, however, did not contain spermatozoa, from which it was concluded that impregnation and fertilization had not taken place. The authors suggested that either some physical requirement to fertilization was lacking in the culture system used, or the spermatozoa produced *in vitro* might be abnormal in some way. No evidence to support either hypothesis was found and more recent work (Smith, unpublished data) has suggested that an antibody present in the hydatid fluid may be forming an antigen-antibody reaction on the surface of the worm sufficient to block the genital pore. Investigation is in progress which will confirm or deny this hypothesis. It has been suggested that the high $pCO_2$ used in this cultivation system may be related to $CO_2$-fixation with subsequent formation of pyruvate or fumarate and its use in the oxidation of NADH. The optimum gas phase appeared to lie between 10–20% $O_2$, for below and above this level abnormalities developed (Smyth, 1968). The precise optimum $pO_2$ has not been determined, however, nor is the oxygen level within the dog gut known.

Significant progress, then, has been made towards the understanding of conditions required for the adult development of this cestode, and the achievement of insemination and fertilization *in vitro* is clearly the next goal.

Smyth *et al.* (1967) speculated that the close contact between the evaginated protoscolex and the protein substrate resulted in the release of a "strobilization organizer". The precise nature of the phenomenon taking place at the

host/parasite interface is not known, but they suggested that extracellular secretion and digestion may occur between microtriches covering the rostellum and the suckers and the protein substrate *in vitro* (or the intestinal mucosa *in vivo*); alternatively, the process may involve "membrane (contact) digestion"—as in intestinal cells. This process was reviewed by Ugolev (1965), who envisaged digestion at surfaces by enzymes absorbed from the intestine and/or enzymes structurally associated with the membrane. The resultant materials may provide some essential metabolites, or they may be stimulatory and initiate the process of strobilization by means of an "organizer", possibly through some neurosecretory mechanism. Both mechanisms could operate. That a digestive process is taking place at the interface is supported by the fact that if the parasite/substrate interface is "disturbed" continuously (Flask A, Fig. 2) strobilization does not occur. If, however, the circulating medium is "confined" (Flask B, Fig. 2) within cellulose tubing (i.e. so that nutrient exchange can occur but the interface remains undisturbed) strobilization and growth take place (Smyth, 1969a).

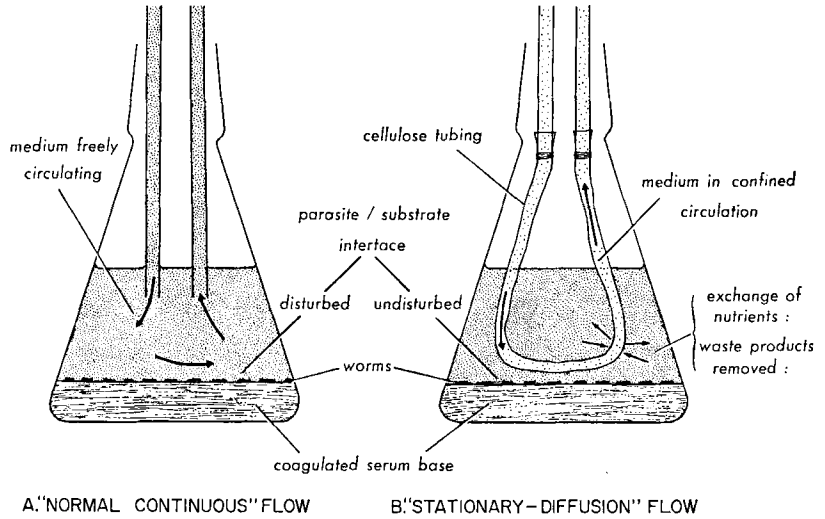


FIG. 2. Experimental demonstration of the role of the host (substrate)/parasite interface in inducing strobilization of *Echinococcus granulosus in vitro*. Culture flasks were connected to a continuously circulating medium; gas phase 5% $CO_2$+10% $O_2$ in $N_2$. Strobilization occurred only in Flask B, in which the interface was undisturbed; this provided environmental conditions under which digestion [=membrane (contact) digestion ?] could theoretically occur. (Based on Smyth, 1969a).

### In vitro *cultivation of partly developed worms from a dog*

Smyth and Howkins (1966) described a technique for culturing partly developed worms from the gut of an infected dog to maturity in diphasic medium similar to that used for cultivation of protoscoleces. Worms taken from the dog 28 and 35 days after the initial infection produced embryonated eggs on the 42nd day in both cases—2 days longer than was required *in vivo*.

This technique has the advantage that the dangerous egg-bearing stage can be handled with safety in a culture tube.

### B. *In vitro* CULTIVATION OF THE CYSTIC STAGE OF *Echinococcus*

As stated already, the protoscoleces of *E. granulosus* cultured in a monophasic medium produce posterior bladders or become vesicular. Smyth (1967) examined the effects of some physical factors on vesicularization *in vitro* and found that under anaerobic conditions or under high oxygen tensions (95%) protoscoleces rapidly became vesicular, whereas at 10–20% levels of oxygen this did not occur. A low pH (6·5) or a high pH (8·0) also induced vesicularization but not pH levels of 7·0–7·4. High levels of bile caused the same effect and it was concluded that the process of vesicularization is brought about by almost any abnormal conditions *in vitro*. This property could have a protective function, viewed against the life cycle of the worm.

Culture of miniature hydatid cysts of *E. granulosus* was not maintained long enough for protoscoleces to be formed. However, Lukashenko (1964) carried out *in vitro* culture experiments on protoscoleces and minced germinative tissue of cysts of *Alveococcus multilocularis* (=*E. multilocularis*) and obtained viable cysts which were invasive when fed to a puppy or inoculated into a cotton rat. Using a medium consisting of 199 plus cotton rat (*Sigmodon hispidus*) embryo extract, bovine serum and lactalbumin, protoscoleces developed into vesicles with lamellar and germinal membranes by the 38th day and at 119 days some of these had developed protoscoleces which were infective to a puppy. Development was slightly quicker when minced germinal membrane was used. Very minute (about 0·27 mm) vesicles were found in the culture after 27 days and by 38 days occasional vesicles could be seen with the naked eye; these had developed a lamellar membrane by the 54th day and contained protoscoleces with hooklets by the 99th day. Invasiveness of these scoleces was proved by inoculation of cotton rats by intraperitoneal injection.

### C. TISSUE CULTURE OF GERMINAL CELLS

Germinal cells released from a daughter cyst wall of *E. multilocularis* by trypsinization were grown as tissue culture cells by Sakamoto *et al.* (1967). The medium which produced the highest growth rate of cells consisted of three parts 199, one part 0·5% lactalbumin and 0·1% yeast extract in Hank's balanced salt solution combined with 20–30% calf serum and 20% heated cotton rat liver extract. Initially, cells were rounded, but most later became spindle-shaped. By the third day, the round cells were proliferating and bothryoid colonies of round cells developed from them. When subcultured, these round cells became spindle-shaped and several days later developed, like the spindle-shaped cells in the original culture, into asteroid cells with long branching processes. By the 12th day, a complete sheet of cells was established on the bottom of the culture bottle. The authors describe two other types of cells in the tissue culture; in one of them, unlike any cell found *in vivo*, a round cell body is surrounded by fine ciliary and spinous processes.

The other type of cell appeared about the 5th day as a large syncytial cell with many nuclei; the cytoplasm contained some large vacuoles which pushed the nuclei against the cell wall. These syncytial cells were considered to be the precursors of multilocular vesicles, although they did not develop into vesicles in the cultures.

Further work along these lines should be rewarding, and a comparison between the cell types found in tissue culture of the germinal membrane of *E. granulosus* and of *E. multilocularis* might possibly be of value. Sweatman *et al.* (1963) found that pieces of germinal membrane of *E. granulosus* did not develop into secondary hydatid cysts after transfer to immature mice, rabbits and sheep using the cerebral intraventricular and intraperitoneal routes. Protoscoleces appeared to be the only source of secondary cysts in *E. granulosus* although, as mentioned earlier, Lukashenko (1964) obtained viable cysts of *E. multilocularis* in *in vitro* culture of minced germinal membrane.

## Acknowledgements

Some of the studies of the author reported in this review have been supported by The Australian Wool Board, the World Health Organization and the National Institutes of Health Public Health Service Research Grant No. A 107452-02.

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# Advances in Veterinary Anthelmintic Medication

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## I. Introduction

When this subject was reviewed in Volume 2 of *Advances in Parasitology* we stood on the threshold of a period of great progress. In almost every field reviewed in 1963/4 further advances have taken place and many new anthelmintics have been launched. The majority of these have advantages over their predecessors in that they have a wider spectrum of activity, a lower dose rate or are less toxic. As far as cost goes, however, none of the newer anthelmintics has any advantage over the older ones and most of them are more expensive than those formerly used. Greater cost, however, is compensated for by higher efficiency. One dose of a drug which can remove both adult and immature worms will serve the same purpose as two doses of a less efficient compound which removes only mature worms, and although more expensive per dose may be cheaper per course of treatment. There seems to be no end to the ingenuity of the organic chemists and it is probable that new anthelmintics will come forward at least as rapidly as in the recent past. The present review, therefore, has the nature of an interim report which will require future modification.

## II. Parasitic Gastro-enteritis of Cattle and Sheep

### A. PHENOTHIAZINE

#### 1. *Drug resistance*

Drudge *et al.* (1957) isolated a strain of *Haemonchus contortus* which had been exposed for 10 years to phenothiazine, both in salt mixtures and periodic full doses, and compared sensitivity to the drug with that of a strain which had had minimal exposure to it. They found that inhibited development of *H. contortus* eggs in the faeces required only 0·5 g/day in the latter strain but 1·0 or 2·0 g/day for the same effect in the former. Later Drudge *et al.* (1959) showed the resistant strain to be also the more resistant to full therapeutic doses of the drug. There was, however, only a 2·5-fold difference between the thresholds of the two strains to full therapeutic doses whereas the difference was 4–8-fold with small daily doses. Bennett and Todd (1964a) investigated the effect of particle size and purity on the efficiency of phenothiazine against the resistant strain of *H. contortus*. They found that 15–30 g of purified phenothiazine of 2·3 μ particle size removed the worms but that a similar dose of 16 μ particle size National Formulary (N.F.) phenothiazine was ineffective. In a further experiment, Bennett and Todd (1964b) showed that both strains of *H. contortus* were effectively removed by 220, 250 and 1100 mg/kg of purified 2·3 μ phenothiazine. Both strains were less susceptible to 16 μ particle size N.F. phenothiazine but the resistant strain was the less susceptible. The effect of 1 g daily doses of 16 μ N.F. phenothiazine and 2·3 μ purified phenothiazine was investigated by Bennett and Todd (1965). The faecal egg count was reduced during an 80 day course of 2·3 μ purified drug but not when 16 μ N.F. phenothiazine was used. "Isolate W", a susceptible strain of *H. contortus*, was found to be susceptible both to the action of 2·3 μ purified phenothiazine and the 16 μ N.F. preparation, but less so to the latter.

This development of resistance to the action of phenothiazine by a strain of *H. contortus* is, therefore, less serious than was at first thought, in that resistant strains are susceptible to the newer pure micronised preparations of phenothiazine and may also be removed by giving larger doses of the older coarse preparations.

### B. ORGANO-PHOSPHORUS COMPOUNDS

The majority of the organo-phosphorus compounds discussed in the previous review are now no longer in general use as anthelmintics, although some of those preparations mentioned are used as systemic insecticides. Two organo-phosphorus compounds, haloxon and naphthalophos, are in common use as anthelmintics.

#### 1. *Haloxon O,O di-(2-chloroethyl) O-(3-chloro-4-methyl coumarin-7yl)phosphate*

(a) *Anthelmintic action in sheep.* Armour *et al.* (1962) assessed the activity of haloxon in laboratory tests. Given at the rate of 50 mg/kg high efficiency was obtained against mature *Ostertagia* spp. but only 75% of 14-day-old worms and 44% of 7-day-old worms were removed. A similar level of efficiency was recorded against all these stages of *Nematodirus spathiger*, but with *Trichostrongylus colubriformis* efficiency was high against all stages. 7- and 14-day-old *Haemonchus contortus* and *Cooperia curticei* were completely eliminated. Field trials on 600 lambs confirmed the value of haloxon on the species already mentioned and showed also that *Strongyloides papillosus* was effectively removed. Ross (1963) used haloxon successfully to control worm infection in 100 hogs running at pasture. Lower faecal egg counts and better weight gain were seen in the haloxon treated sheep than in a similar group treated with phenothiazine. Nunns *et al.* (1964) also carried out field trials with haloxon in the autumn on four flocks of sheep in which parasitic gastro-enteritis had been diagnosed. In the spring a further five trials were made on lambs which had high faecal egg counts, but none of which had died. A dose of 30–55 mg/kg was employed and good results were obtained as judged by faecal egg counts, clinical effects and worm counts, except that the immature forms of *Nematodirus* spp. were only partially removed. Trials carried out by Kingsbury and Curr (1967) in Australia confirmed the efficacy of doses of 35 mg/kg. It was also demonstrated that 20 mg/kg would remove 99% of adult, 4-day-old and 10-day-old *H. contortus*, for which purpose the drug was particularly recommended in Australia.

(b) *Anthelmintic action in cattle.* Armour (1964) carried out a controlled test on calves artificially infected with *Ostertagia ostertagi.* At a dose rate of 40 mg/kg no effect was observed on 7-day-old worms but 28% of 14-day-old worms, 64% of 21-day-old worms and 97% of 28-day-old worms were removed. Seven-day-old worms were also unaffected by a dose of 100 mg/kg. Hart (1964) extended these studies to other species of worm. Fifteen mg/kg was found to be highly effective against *Haemonchus* spp. but 30 mg/kg was required against *Cooperia* spp. and *Trichostrongylus* spp., whilst 50 mg/kg was

required for adequate removal of *Oesophagostomum radiatum*. The drug was ineffective against *Bunostomum phlebotomum* even at a dose rate of 50 mg/kg. While 30 mg/kg effectively removed 7- and 14-day-old *Haemonchus* spp., 50 mg/kg removed only 46% and 73% respectively of 7- and 14-day-old *Trichostrongylus axei*. With *Cooperia* spp. 30 mg/kg was highly effective against 14-day-old worms but 50 mg/kg removed only 43% of 7-day-old worms. No action was observed with 50 mg/kg against 7- and 14-day-old *Oesophagostomum radiatum*. Bosman (1965) in South Africa also reported high activity against the adult stages of most nematodes of cattle, except *Bunostomum phlebotomum*, using a dose rate of 38 mg/kg.

(c) *Toxicity*. Malone (1964) made extensive studies on the toxicity of haloxon and found therapeutic doses to be well tolerated by sheep. The therapeutic index for aged ewes was 3–4 and for young lambs 5–7. Stress factors did not increase toxicity. At high dose rates hind-leg ataxia was observed in a proportion of sheep but no adverse effects were observed with 3–4 times the therapeutic dose. Hart and Lee (1966) found that cholinesterase activity was inhibited for long periods in nematodes susceptible to its action but that in sheep spontaneous regeneration of cholinesterase was rapid. Armour *et al.* (1962) dosed 29 000 sheep at dose rates of 30–55 mg/kg without ill effects. In Australian trials involving 4000 sheep, Kingsbury and Curr (1967) confirmed the safety factor as five. Hind-leg ataxia was noted in 0·5% of sheep given doses of 150 mg/kg so that the safety factor in this respect was about 3 for lambs. Carbon tetrachloride should not be given within a few days of treatment with haloxon.

Neither Armour (1964) nor Hart (1964) reported any signs of toxicity when giving cattle doses of up to 50 mg/kg. Bosman (1965) tested haloxon for toxicity in over 3000 cattle in different regions of South Africa and under different conditions of nutrition and husbandry without noticeable ill effects. Doses as high as 100 mg/kg failed to cause toxic effects.

2. *Naphthalophos O,O-diethyl-O-naphthaloxymido phosphate*

(a) *Anthelmintic action in sheep*. Federmann (1964) carried out controlled tests with naphthalophos on artificially infected sheep. Very high efficiency was recorded against *H. contortus* and *T. colubriformis* but against *Ostertagia circumcincta* efficiency was 80–90% and *Oesophagostomum columbianium* was unaffected. 85–100% of the fourth stage larvae of *Trichostrongylus* spp. and fifth stage of that species and of *H. contortus* were removed. Fourth stage *H. contortus* were effectively removed in four of six animals. In naturally infected animals good results were obtained with all these species and also with *Cooperia* spp. but *Chabertia ovina* was unaffected. Thomas (1964) reported briefly on trials in New Zealand. A dose of 50 mg/kg was found to be effective against *H. contortus*, *O. circumcincta*, *T. axei*, and *T. colubriformis*. Immature stages of *H. contortus* were removed by a dose of 50 mg/kg but 75 mg/kg was required to remove immature *O. circumcinta*, *T.axei* and *T. colubriformis*. Hebden and Hall (1965) also tested the drug on artificially and naturally infected sheep in Australia. *H. contortus*, *T. axei*, *T. colubriformis* and *Oster-*

*tagia* spp. were satisfactorily removed by a dose of 50 mg/kg but activity against *Nematodirus* spp. was irregular. Stampa (1965) carried out critical tests using doses of 50 and 60 mg/kg with similar results to those already given. Zettl (1965) used naphthalophos at a dose rate of 50 mg/kg in 1700 sheep from flocks affected with parasitic gastro-enteritis. As judged on egg count data and the clinical picture, including weight gain and wool quality, satisfactory control of the worms was achieved.

(b) *Anthelmintic action in cattle.* Controlled tests were carried out by Rubin and Hibler (1967) on 38 calves experimentally infected with *Ostertagia ostertagi, Cooperia oncophora* and *C. punctata.* When given at a dose rate of 50 mg/kg activity was indifferent against all species. At 75 mg/kg activity was good against mature *O. ostertagi* but poor against larval forms. 6·7% activity was observed against both the immature and mature stages of both *Cooperia* spp. Cox *et al.* (1967) successfully controlled gastro-intestinal helminths in feed lot cattle using doses of 50 and 75 mg/kg; 10 or 20 mg/kg incorporated in the food given daily for 6 days was not effective in control.

(c) *Toxicity.* Smith and Thomas (1964) found that 150 and 250 mg/kg could be given to housed animals without ill effects, but two of three sheep given 200 mg/kg died. Other dose rates were given in field trials under various conditions and it was concluded that the safety margin was adequate to cover errors of dosing. Animals fed concentrated dry food were more susceptible, but this and stress factors did not appreciably increase the hazard. Hebden and Hall (1965) gave doses of 100, 200 and 400 mg/kg. Scouring in the latter group was the only untoward sign observed. Hall (1965) carried out trials on the safety of naphthalophos under Australian conditions. Groups of 20 sheep were given doses varying from 50–300 mg/kg and a further ten sheep received 750 mg/kg. Death occurred in only one animal given 750 mg/kg and there was evidence that aspiration of the drench into the lung was the cause of death. No ill effects were observed in sheep grazing lush pastures or in animals starved for 6, 24 or 48 h before treatment. Two ml of carbon tetrachloride given a week before, a week after or at the same time as a dose of 50 mg/kg of naphthalophos, caused no visible ill effects. Five sheep were given 50 mg/kg for 21 days. One animal showed anorexia on the 11th day and two others were similarly affected on the 15th. Under field conditions 12 000 sheep were given doses usually of 50 mg/kg, but occasionally of 100 mg/kg, with only three deaths which were attributed to the natural hazards of drenching.

### C. THIABENDAZOLE

The anthelmintic activity of thiabendazole was summarized in the previous review and work since that time has confirmed the high efficiency of the drug against gastro-intestinal worms in sheep and cattle, although in common with other anthelmintics its action is indifferent against nematodes inhibited in their development, particularly the inhibited form of *Ostertagia* spp.

One interesting aspect of the use of thiabendazole has been the development of strains of *Haemonchus contortus* resistant to its action. An indication of this phenomenon was given by Conway (1964), who found it necessary to

give a dose of 80 mg/kg to remove *Haemonchus contortus* from his experimental sheep, the usually recommended dose of 50 mg/kg being without noticeable activity. It was, however, first noted by Drudge *et al.* (1964), who found, when giving monthly doses of 44 mg/kg to sheep under conditions where reinfection occurred, that the *H. contortus* present at the end of a 20-week period were indifferently removed by a dose of 44 mg/kg. After three treatments with thiabendazole the worms became resistant to its effects. The fourth treatment in week 12 was associated with a higher than average count, the fifth treatment reduced the average egg count about 70%, while the sixth produced practically no change. The phenomenon has also been reported in Brazil by Santos and Franco (1967), who described a strain resistant to 66 mg/kg of thiabendazole, and in Australia by Smeal *et al.* (1968), who established laboratory infections of a resistant strain by infection of lambs with larvae cultivated from sheep obtained from farms where the action of thiabendazole had been reported to be indifferent. Raising the dose increased efficacy against the resistant worms but even at 150 mg/kg some sheep retained 5–10% of the worm burden of untreated sheep. Smeal *et al.* found morphological differences in the resistant strain and quote a personal communication from Santos to the effect that the resistant strain in Brazil is morphologically different from the more common susceptible strain.

Resistance of *Haemonchus contortus* to thiabendazole seems to be similar to that developed to phenothiazine, in that an increased dose of the drug will still remove the resistant nematodes. From the practical point of view it is, however, undesirable to have to give high doses of an expensive compound. A surprising feature of the strain of *Haemonchus* resistant to thiabendazole is the extraordinary rapidity with which it was selected out from the population.

### D. TETRAMISOLE

dl 2,3,5,6-tetrahydro-6-phenyl-imidazo(2,1–b) thiazole hydrochloride

#### 1. *Anthelmintic action in sheep*

Walley (1966c) gave an account of laboratory and field trials with tetramisole. In the laboratory trials high efficiency was recorded against the adult worms of species of *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Bunostomum*, *Nematodirus*, *Oesophagostomum* and *Chabertia*. Raising the dose level to 15 mg/kg virtually removed all adult worms of these genera. At low dose rates the drug is less active against worms up to 14 days old than against mature forms. At 5 mg/kg some action against immature forms was noted but not against *Ostertagia* spp. At 10 mg/kg a fair proportion of the immature worms except *Ostertagia* spp. were eliminated; the 10-day-old *Ostertagia* were particularly resistant to the action of the drug. At 15 mg/kg almost all the immature worms except Ostertagia were removed. 92% of the six-day-old and 87% of the 10-day-old *Ostertagia* spp. were expelled. These results have been confirmed in laboratory tests by Ross (1966) and Gibson (1966) in England, by Reinecke (1966), Pretorius (1967) and Shone and Philip

(1967) in South Africa, by Forsyth (1966a) in Australia, and by Graber (1966a) in Tchad. The workers in South Africa and Tchad have reported the successful removal of *Gaigeria pachyscelis* in addition to the species mentioned above. Graber (1966a), however, recorded irregular action against *Buckleyuris ovis* and *B. globulosa.*

Walley (1966c) confirmed his results in field trials and Pankhurst and Sutton (1966) also used tetramisole in field trials involving 2399 lambs in 16 flocks. In the latter trial the lambs were 4–12 weeks old, and judged on egg count data and clinical response high efficiency was obtained. Behrens (1967) used the drug to control gastro-intestinal nematodes in 1615 sheep in nine flocks. He found 10 mg/kg to be effective but there was indifferent action against some immature worms and 15 mg/kg was preferred.

2. *Anthelmintic action in cattle*

Walley (1966a) summarized the results obtained in tests on 642 cattle and found doses below 12 mg/kg to be unsatisfactory. Fifteen mg/kg, however, was highly efficient against 3-, 10- and 14-day-old and adult worms of the following species: *Haemonchus placei, Trichostrongylus axei, Cooperia* spp., *Trichostrongylus* spp. and *Nematodirus helvetianus.* The results were less satisfactory with *Ostertagia ostertagi,* with which species 22% of 3-day-old, 42% of 10-day-old, 77% of 14-day-old and 95% of mature worms were removed. Some evidence was produced that 20 mg/kg was more satifactory against the immature worms. Graber (1966b), however, obtained good results with 5 mg/kg against the mature and immature stages of *Bosicola radiatum, Bunostomum phlebotomum, H. contortus, Cooperia pectinata* and *C. punctata.* The fourth stage larvae of *B. radiatum* were not affected by treatment. Enigk *et al.* (1966) tested tetramisole in artificially infected calves using a dose rate of 10 mg/kg given subcutaneously. Ninety-nine per cent of the mature *Ostertagia* spp. and *Cooperia* spp. were removed, and 19% of the immature fifth stages of *Ostertagia* spp. and 97% of the same stage of *Cooperia* spp. With fourth stage larvae 84% of *Ostertagia* spp. and 98% of *Cooperia* spp. were removed. Forsyth (1966b) carried out a critical test using artificially infected calves and found 10 mg/kg given orally to be effective against mature *Ostertagia* spp. and *T. axei* but ineffective against larval forms. Improved action against immature worms was achieved with a dose of 13·2 mg/kg given orally. Supperer and Pfeiffer (1966) gave 10 mg/kg by subcutaneous injection and reported excellent action against *Ostertagia* spp. and *Cooperia* spp. on both artificially and naturally infected animals.

In field trials Forsyth (1966b) treated 3500 cattle at a dose rate of 13·2 mg/kg and noted a rapid clinical improvement in all animals. Wood and Ramirez-Miller (1966) carried out field trials in Brazil and found a dose of 10 mg/kg effective in controlling gastro-intestinal worms.

3. *Toxicity*

Supperer and Pfeiffer (1966) found the drug to be well tolerated but Enigk *et al.* (1966) reported that animals showed excitement and slight colic after

treatment. Forsyth (1966b) noted that side effects were more frequent in cattle than in sheep and included head shaking, lip licking and salivation, skin tremors and increased excitability. These effects, which were more severe in animals in good condition, were transitory and disappeared within 1 or 2 h. Kaemmerer and Budden (1966) described similar symptoms of short duration following the administration of 10 mg/kg, and Enigk *et al.* (1966) also reported that animals showed excitment and colic after treatment.

### E. PYRANTEL TARTRATE

trans-1-methyl-2[2-(a-thienylvinyl)]1,4,5,6-tetrahydropyrimidine tartrate

Cornwell (1966a) carried out laboratory trials on artificially infected sheep and showed the drug to be highly efficient against mature and immature stages of *T. colubriformis* and *N. battus* in tests on artificially infected animals, and in further tests on naturally infected animals efficacy against species of *Ostertagia*, *Cooperia* and *Trichuris* was demonstrated. Gibson and Parfitt (1968a) used a dose of 25 mg/kg in a controlled test with artificially infected lambs and reported high activity against 2-, 7-, 14- and 28-day-old *H. contortus* and *T. colubriformis*. In a series of field trials, Cornwell (1966b,c; 1967a,b) demonstrated the value of pyrantel tartrate for the control of nematodiriasis and parasitic gastritis under farming conditions.

### F. PARBENDAZOLE

methyl 5(6)-butyl-2-benzimidazolecarbamate

Actor *et al.* (1967) gave a preliminary account of the activity of this compound and reported that 15 mg/kg given orally or intra-rumenally to sheep removed 93–100% of *Haemonchus*, *Ostertagia*, and *Trichostrongylus* from the abomasum, and *Strongyloides*, *Cooperia*, *Trichostrongylus*, *Nematodirus*, *Oesophagostomum* and *Chabertia* from the small and large intestines. In cattle 20 or 40 mg/kg doses gave good results against *Haemonchus*, *Ostertagia*, *Trichostrongylus axei*, *Strongyloides*, *Cooperia* and *Oesophagostomum*. These results were confirmed by Theodorides *et al.* (1968), who also demonstrated that in sheep 12·5–15 mg/kg could remove 4-day-old *Haemonchus*, *Ostertagia* and *Trichostrongylus* but was only moderately active against *Oesophagostomum*. High efficiency was obtained against 7-day-old and 14-day stages of these nematodes. A single oral dose of 500 mg/kg was well tolerated by cattle.

Ross (1968) carried out controlled tests on sheep artificially infected with *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Ostertagia circumcincta* and *Nematodirus battus*. Using a preparation having 24% of the particles less than 10 μ and 11% less than 4 μ, a dose of 15 mg/kg removed 100% of 3-day-old, 10-day-old and adult *H. contortus* and *T. colubriformis* as well as 84, 96 and 100% of the same stages of *O. circumcincta*. Against 3-day-old *N. battus* 90% activity was achieved but there was no action against 10-day-old and adult worms. A second preparation having 64% of the particles less than 10 μ and 49% less than 4 μ, when given at 15 mg/kg, was 100% active against all stages of the first three nematodes except the 3-day-old *O. circumcincta* against which activity was 94%. Against 3-, 10- and 20-day-old *N. battus*

activity was respectively 95%, 80% and 48%. A third preparation having 95% of the particles less than 10 μ and 80% less than 4 μ was tested only against *N. battus*. Using a dose of 22·5 mg/kg activity was 96%, 0% and 24% against 3-, 10- and 20-day-old worms respectively, but increasing the dose to 30 mg/kg resulted in the removal of 99, 97 and 89% respectively of the three developmental stages.

### G. CONCLUSION

There are now several excellent anthelmintics which may be used for the removal of gastro-intestinal worms from sheep and cattle, and the selection of which compound to use is largely a matter of personal choice. Thiabendazole has been very widely used in sheep in the last few years with very satisfactory results, but if resistant *H. contortus* became widespread it would lose much of its attraction in favour of compounds such as tetramisole, pyrantel tartrate and parbendazole which have not as yet shown that phenomenon. Where lungworms are also a problem tetramisole has considerable advantages since it will efficiently remove both gastro-intestinal and lungworms from sheep.

In cattle methyridine and thiabendazole have been widely used in the recent past. Methyridine had the advantage of possessing some action against lungworms as well as gastro-intestinal worms, but the local reactions frequently produced when the drug was given subcutaneously made its use unpopular. Tetramisole is, however, superior to methyridine in several ways; it has a wider therapeutic index, it is equally effective against gastro-intestinal worms and is more efficient against lungworms. It may well become the anthelmintic of choice for use against nematodes in cattle.

## III. Parasitic Bronchitis

### A. INTRODUCTION

In 1963 the two specific drugs available for the removal of lungworms from domestic animals were cyanacethydrazide and diethylcarbamazine. Of these cyanacethydrazide was active against adult lungworms and diethylcarbamazine against immature worms, especially the juveniles. Cyanacethydrazide has not, in practice, equalled its early promise but diethylcarbamazine has been widely and successfully used to treat parasitic bronchitis. Since 1963, the action of methyridine against lungworms has been described and the new drug tetramisole has also been shown to be effective.

### B. METHYRIDINE

The drug is active against lungworms when given orally, subcutaneously or intraperitoneally. Walley (1963) found that in cattle given 200 mg/kg the early migratory stages are little affected but 50% of 10-day-old worms are removed. About 75% of 15-day-old worms were eliminated and activity against adult worms ranged from 86–99%. A number of natural infections were also

successfully treated. In sheep Vodrázka and Berecký (1965) found that 200 mg/kg removed 98% of mature *Dictyocaulus filaria* and 74% of *Protostrongylus* spp. Walley (1963) used methyridine in artificially infected sheep and found activity against *D. filaria* to be similar to that recorded above for *D. viviparus*. In sheep naturally infected with *Protostrongylus rufescens* 80% of the worms were removed.

### C. TETRAMISOLE

#### 1. *Anthelmintic action in cattle*

Walley (1966a) found that doses of 10 mg/kg removed 6% of 3-day-old worms, 85% of 10-day-old worms, 95% of 14-day-old worms and 95% of the adult worms from cattle artificially infected with *Dictyocaulus viviparus*; 15 mg/kg was 80% effective against 3-day-old worms, 92% effective against 10-day-old worms and virtually 100% effective against 14-day-old and adult worms. Walley found that 10 mg/kg gave excellent results in the field. Enigk *et al.* (1966) gave 10 mg/kg by injection and found 88% of the mature and 84% of the immature fifth stages were removed. High activity was also recorded against the fourth stage. Supperer and Pfeiffer (1966) and Reinders (1966) obtained good results in treating parasitic bronchitis in cattle. Forsyth (1966b) found that 10 mg/kg of tetramisole would remove over 90% of *D. viviparus* from cattle. Behrens (1967) found the drug to be effective against lungworms in a field trial but the worm burdens of the cattle concerned were low. McCulloch *et al.* (1968) treated 249 animals on 22 farms with 9 mg/kg and 374 animals on 26 farms with 15 mg/kg; clinical improvement followed during the next 4 weeks.

#### 2. *Anthelmintic action in sheep*

Walley (1966c) carried out laboratory studies in sheep and found that a dose of 10 mg/kg removed 94% of the mature *D. filaria* and 83–87% of the immature stages. No action was observed against *Muellerius capillaris*. Pretorius (1967) carried out a controlled test on artificially infected sheep and found that 15 mg/kg removed 77% of 7-day-old worms and 98% or more of 14-, 21- and 28-day-old worms; 5 mg/kg was effective against adult worms. Gibson and Parfitt (1968b) also carried out a controlled test on lambs artificially infected with *D. filaria*, giving a dose of 15 mg/kg. The drug was given to different groups of sheep when the worms were 24 h, 5 days, 14 days and 32 days old; the percentage of worms removed was respectively 95·3%, 73·6%, 98·1% and 94%.

#### 3. *Anthelmintic action in pigs*

Walley (1967) tested tetramisole in pigs artificially and naturally infected with *Metastrongylus apri*. A dose of 10 mg/kg removed 63% of 3-day-old worms, 86% of 10-day-old worms and 90% of 28-day-old worms. At 15 mg/kg the corresponding percentages were 58%, 96% and 99%. The drug may be

administered in the food or in the drinking water and has a 5-fold safety margin in pigs.

### D. CONCLUSION

Of the first two specific drugs for parasitic bronchitis, cyanacethydrazide has now fallen into disuse and, although diethylcarbamazine acts mainly on immature adult worms, it has been widely and successfully used in the field. The action of methyridine against immature worms is somewhat indifferent and when given by subcutaneous injection frequently results in local reaction at the injection site. Tetramisole, however, is active against both mature and immature lungworms and has a good safety margin. It is likely to become the anthelmintic of choice for the elimination of lungworms and it has the added attraction of considerable activity against gastro-intestinal worms.

## IV. FASCIOLIASIS

### A. HILOMID

A mixture of equal parts 3,4'5-tribromosalicylanilide and 4'5-dibromosalicylanilide

Boray *et al.* (1965) tested hilomid on sheep artificially infected with *Fasciola hepatica*. 30 mg/kg was highly effective against 12-week-old flukes and 60 mg/kg was highly effective against flukes 6 weeks old. No signs of intoxication were seen in 50 infected sheep which received doses of 30–60 mg/kg or in ten uninfected sheep which received 120 mg/kg. Three of ten uninfected sheep, which were given 150 mg/kg, died. Boray and Happich (1966) carried out more extensive tests and found that a dose of 30 mg/kg removed 29%, 36%, 59%, 86% and 98% of 4-, 6-, 8-, 10- and 12-week-old flukes. With a dose of 60 mg/kg the percentages were 45, 90, 98, 100 and 100 respectively. 8000 sheep were treated with 30 mg/kg and 250 were given 60 mg/kg without ill effects. When doses of 120 mg and 150 mg/kg were given, deaths occurred amongst the treated animals. In poisoned sheep extensive subcutaneous haemorrhages were seen and petechial haemorrhages were seen in the epicardium and myocardium. These results were confirmed by Boray *et al.* (1967), who also showed that a dose rate of 120 mg/kg was 90% efficient against 4-week-old flukes. Boray and Happich (1968) carried out a further laboratory evaluation of hilomid and summarized their results as follows. Ninety per cent or more of the 12-week-old flukes are removed by a dose of 20 mg/kg with a safety index of 3; 90% or more of the 10-week-old flukes are removed by a dose of 30 mg/kg with a safety index of 2; 98% or more of 6-week-old flukes are removed by a dose of 60 mg/kg with a safety index of 1; and 98% or more of 4-week-old flukes are removed by a dose of 120 mg/kg with a safety index of 0·5. Hildebrandt (1968) carried out a controlled test on sheep artificially infected with *Fasciola gigantica* using dose rates of 30 mg/kg and 60 mg/kg. The latter dose given to sheep carrying flukes 6, 8, 10, 12 and 16 weeks old removed 39·1, 92·2, 100, 99·9 and 100% respectively. The dose 30 mg/kg removed 79·4% of 10-week-old flukes, 99·4% of 12-week-old

flukes and 100% of 11-week-old flukes. No toxic effects were observed, and 60 mg/kg is recommended for the treatment of acute fascioliasis and 30 mg/kg for the chronic disease.

Leiper (1968, *in litt.*) states that of the two compounds present in the mixture the 3,4'5-tribromosalicylanilide is the more active; 15 mg/kg of this compound having approximately the same activity as 30 mg/kg of the mixture. It has about the same toxicity as the mixture. Preparations of hilomid containing only the 3,4'5-tribromosalicylanilide are now available commercially.

### B. MENICHLOPHOLAN

2,2'-dihydroxy-3,3'-dintro-5,5' dichlorodiphenyl

Kuttler *et al.* (1963) used 3 mg/kg of menichlopholan in naturally infected sheep and on the basis of egg count data concluded that the drug was effective. Knapp *et al.* (1965) confirmed its efficacy in critical tests carried out on naturally infected animals. Lee *et al.* (1966) used menichlopholan at a dose rate of 5 mg/kg in naturally infected sheep. High efficiency was reported against mature flukes, and some activity against immature forms. 40–57% of flukes up to 10 mm were eliminated and 53–100% of immature stages bigger than that. All the above workers found the drug to be well tolerated. Lane and Stewart (1967) treated 1299 sheep in flocks suffering from fascioliasis and found doses of 4–7 mg/kg generally well tolerated, but some deaths were recorded in severely infected flocks. Pregnant ewes were dosed without effect on the subsequent lambing. Boray *et al.* (1967) tested the drug on artificially infected sheep and found 8 mg/kg removed 92·5% of 4-week-old flukes, 99·7% of 6-week-old flukes, and 99·7% of eight-week-old flukes. 16 mg/kg was 100% effective against 4-week-old flukes and 4 mg/kg removed 58·4% of 8-week-old flukes and 100% of 12-week-old flukes. Boray and Happich (1968) confirmed these results and summarized the efficacy and safety of menichlopholan as follows: 2·7 mg/kg will remove 12-week-old flukes with a safety index of 4·4, 6 mg/kg will remove 6-week-old flukes with a safety margin of 2·0, and 8 mg/kg will remove 4-week-old flukes with a safety margin of 1·5.

### C. DISOPHENOL

2,6-diiodo-4-nitrophenol

Boray *et al.* (1967) tested disophenol in artificially infected sheep giving doses of 25 or 50 mg/kg by subcutaneous injection. 50 mg/kg removed 98·9% of 4-week-old flukes and 100% of 6-week-old flukes. 25 mg/kg was 99·3% effective against 12-week-old flukes. Six of 10 sheep given 50 mg/kg died within 2 days of treatment. Boray and Happich (1968) assessed the efficacy of doses between 10 and 50 mg/kg and their results may be summarized as follows: high efficiency is obtained against 12-week-old flukes using 15 mg/kg with a safety margin of 2·7; against 6-week-old flukes using 34 mg/kg with a safety margin of 1·2; and against 4-week-old flukes using 50 mg/kg with a safety margin of 0·8.

### D. OXYCLOZANIDE

3,3′, 5,5′, 6-pentachloro-2,2′-dihyroxybenzanilide

Walley (1966b) tested this compound in both sheep and cattle, using mainly naturally infected animals. Nine hundred and eighteen sheep were used in the tests, which indicated rather poor action against immature flukes with safe doses. 60 mg/kg will, however, remove 89% of 6-week-old flukes, 10 mg/kg will remove 75–81% of mature flukes, while 15 mg/kg is 91–97% efficient. In tests on 121 cattle 10 mg/kg removed 80% of the flukes and 15 mg/kg 92%. In sheep doses up to 25 mg/kg were without significant ill effects, but at 30 mg/kg animals became dull and showed varying degrees of looseness of the faeces. These effects increased with higher doses and death occurred at 60 mg/kg. No enhanced toxicity was observed in animals receiving concentrates. In cattle doses of 30 mg/kg produced scouring, inappetance, depression and loss of weight. Toxic effects increased markedly with doses above 60 mg/kg but only two of 30 animals died. With higher dose rates death was frequent. Vaughan (1966) and Kelsey (1966) tested oxyclozanide in both sheep and cattle in the field with satisfactory clinical results and infrequent side effects. Boray *et al.* (1967) carried out some tests in artificially infected sheep. 60 mg/kg removed 97% of 6-week-old flukes while 15 mg/kg removed 93·4% of 12-week-old flukes. These results were extended by Boray and Happich (1968) and they recorded high efficiency against 12-week-old flukes using a dose of 15 mg/kg with a safety index of 4·0, and against 6-week-old flukes using a dose of 40 mg/kg with a safety index of 1·5. A dose of 60 mg/kg will remove 62% of 4-week-old flukes and the safety index is 1·0.

### E. NITROXYNIL

4-cyano-2-iodo-6-nitrophenol

Lucas (1967) tested nitroxynil in artifically infected sheep and calves. The drug may be administered either orally or by subcutaneous injection, which is more effective. When given by injection the minimum effective dose was 8 mg/kg in both sheep and calves and by the same route 10 mg/kg was highly effective against mature flukes. Increasing the dose to 20 or 30 mg/kg increases activity against immature flukes. The maximum tolerated dose, given by injection to sheep and calves, is 40 mg/kg, at which dose level transient hypernoea and hyperthermia were observed; 50 mg/kg caused death without the production of characteristic post mortem lesions. Boray and Happich (1968) in controlled tests on artifically infected sheep found the following dose levels would remove 90% or more of flukes of the age stated: 6·7 mg/kg removed 12-week-old flukes with a safety index of 6·0; 13·5 mg/kg removed 6-week-old flukes with a safety index of 3; and 30 mg/kg removed 4-week-old flukes with a safety index of 1·3.

Colegrave (1968a) carried out field trials on sheep affected with fascioliasis, giving the drug by subcutaneous injection. 20 mg/kg was used in flocks affected with acute fascioliasis and this prevented deaths if applied early in

the outbreak. In flocks where treatment was given after considerable mortality had occurred the death of some severely infected sheep was hastened. A dose rate of 10 mg/kg was used in more than 6000 sheep with satisfactory clinical response and without ill effects. Colegrave (1968b) carried out controlled field trials with cattle using 10 mg/kg by subcutaneous injection. Satisfactory reduction in faecal egg output was achieved. In other trials involving more than 1300 cattle, including bulls and pregnant cows, the drug was well tolerated and produced a satisfactory clinical response. The drug persists for a considerable time in the tissues and animals should not be slaughtered for human consumption within 30 days of treatment. It should not be given to lactating cows whose milk is to be used for human consumption. The solution stains and when injecting it care should be taken not to spill any on the skin of the animal.

### F. CLIOXANIDE

2-acetoxy-4′-chloro-3,5-diiodobenzanilide

Boray and Happich (1968) tested clioxanide using doses of 10–135 mg/kg given by intra-rumenal injection. The maximum tolerated dose was found to be 100 mg/kg. Doses of 10, 15 and 22·5 mg/kg were 68, 93 and 97% effective respectively against 12-week-old flukes. Against 6-week-old flukes 30, 45 and 67·5 mg/kg were used with the percentage efficiencies 74, 99 and 97. Doses of 60, 90 and 135 mg/kg were used against 4-week-old flukes and the percentage efficiencies were respectively 53, 75 and 98. From their results, Boray and Happich (1968) concluded that 15 mg/kg is highly effective against 12-week-old flukes with a safety index of 6·7, 40 mg/kg against 6-week-old flukes with a safety margin of 2·5, and 135 mg/kg against 4-week-old flukes with a safety index of 0·7.

### G. COMPARATIVE VALUE OF FASCIOLICIDES

Boray and colleagues conducted a series of assays of fasciolicides in sheep, the results of which have been collected and summarized by Boray and Happich (1968). Some of these results have already been referred to above but they are summarized for easy reference in Table I, from which it is seen that all the drugs are active in lower doses against mature flukes and that in some instances 4-week-old flukes can be removed only if the dose is increased to a toxic level. On the basis of Boray and Happich's data, carbon tetrachloride is as efficient and safe as any fasciolicide yet discovered for the removal of both mature and immature flukes. Although total efficiency over the whole range of fluke sizes is important in choosing a fasciolicide, activity against two stages is essential. Fatal liver damage occurs between the 6th and 8th week so that in treating acute fascoliasis activity on flukes 6 weeks old is important. Death from the chronic disease does not occur until 16 weeks after infection and egg production by the flukes begins 9–13 weeks after infection. For the treatment of chronic fascioliasis activity against 12-week-old flukes is important. From Table I it is clear that nitroxynil is the safest drug to use in

TABLE I

*Showing the dose in mg/kg required to remove* 90% *of* F. hepatica 4, 6, 8, 10 *and* 12 *weeks old, and the safety index (S.I.) of fasciolicides, as estimated by Boray and Happich* (1968)

| | 4 weeks | | 6 weeks | | 8 weeks | | 10 weeks | | 12 weeks | |
|---|---|---|---|---|---|---|---|---|---|---|
| Drug | Dose | S.I. | Dose | S.I. | Dose | S.I. | Dose | S.I. | Dose | S.I. |
| Carbon tetrachloride | 640 | 1·3 | 480 | 1·7 | 240 | 5 | — | — | 80 | 10 |
| Hexachloroethane | 1800 | 0·7 | — | — | 600 | 2·0 | — | — | 300 | 4 |
| Hetol | — | — | 1200 | 0·5 | — | — | — | — | 150 | 4 |
| Hexachlorophene | 30 | 1·0 | 20 | 1·5 | — | — | — | — | 15 | 2·0 |
| Hexachlorophene monophosphate | 40 | 1·0 | 25 | 1·6 | — | — | — | — | 20 | 2·0 |
| Hilomid | 120 | 0·5 | 60 | 1·0 | — | — | 30 | 2·0 | 20 | 3·0 |
| Menichlopholan | 9 | 1·5 | 6 | 2·0 | — | — | — | — | 2·7 | 4·4 |
| Oxycloxanide | — | — | 40 | 1·5 | — | — | — | — | 15 | 4·0 |
| Disophenol | 50 | 0·8 | 34 | 1·2 | — | — | — | — | 15 | 2·7 |
| Nitroxynil | 30 | 1·3 | 13·5 | 3·0 | — | — | — | — | 6·7 | 6·0 |
| Clioxanide | 135 | 0·7 | 40 | 2·5 | — | — | — | — | 15 | 6·7 |

acute cases, clioxanide following closely. For chronic fascioliasis carbon tetrachloride is by far the best, then clioxanide and nitroxynil in order. As Boray and Happich used only 200 metacerceriae to infect sheep, the resulting liver damage would be less than that seen in many cases of acute fascioliasis. Their results may not, therefore, be applicable directly to the field but they are, nevertheless, a useful guide to the comparative value of the fasciolicides available for use in sheep.

## *V. DICROCOELIUM DENDRITICUM*

### A. HETOLIN

*β,β,β-tris* (4-chlorophenyl) propionic acid-4′-methyl piperazine hydrochloride

Lämmler (1963) carried out tests with hetolin in laboratory animals infected with *D. dendriticum* and found it to be highly effective against mature and immature forms. Enigk and Düwel (1963) carried out controlled tests with 231 sheep using doses of 15–30 mg/kg; 55% of animals receiving 15 mg/kg were cleared of infection and 68% of those given 16 mg/kg. Doses of

19–22 mg/kg removed the flukes from 90% of treated animals. Further tests on 1945 sheep in which egg count data were used as criteria of efficiency, confirmed these results. The drug was well tolerated even by pregnant animals. Behrens and Horn (1963) tested the safety of hetolin in 1993 sheep. Doses of 17·5–20 mg/kg were safe and occasional doses of two to three times that level produced no ill effects. Doses of more than 160 mg/kg resulted in toxic symptoms but sometimes only after repetition of the dose. Euzéby and Gevrey (1964) found 30 mg/kg to be completely effective in ten of 16 treated sheep. In a critical test in artifically infected sheep, Güralp and Oguz (1966) found dose levels of 40, 50, 60 and 70 mg/kg to be 36·8, 61·8, 79·4 and 88·1% effective respectively. No adverse effects were produced by doses of 120–170 mg/kg but 200 mg/kg invariably caused death.

In cattle Gebauer (1964) recommended a dose rate of 25 mg/kg. Ruosch (1966) treated 37 animals with hetolin and 55 mg/kg and 70 mg/kg did not result in clearance of all animals. In a group given 80 mg/kg only one of 13 treated animals continued to pass eggs after treatment. Ruosch and Zimmermann (1967) gave doses of 60–80 mg/kg to 61 cattle and found 41% still expelling eggs 1 month later. Two to five treatments were required to completely clear all animals of infection. Gründer (1963) found that doses of 3–180 mg/kg produced no ill effects in treated cattle but 240–800 mg/kg caused liver damage and indigestion which passed off in 2–4 weeks. In lactating cows the smell and taste of the milk is affected for 30 h after treatment.

### B. THIABENDAZOLE

Guilhon (1962) gave doses of 50–500 mg/kg to 12 sheep infected with *D. dentriticum* and found that post-treatment egg counts were zero. 0–12 flukes were recovered at autopsy compared with 3000 in a control animal. Šibalić *et al.* (1963) carried out controlled tests on sheep infected with *D. dendriticum* giving doses of 50, 100, 200 and 300 mg/kg. The reduction in worm burden was respectively 0, 43%, 96% and 98%. Doses of 200 or 300 mg/kg were recommended for use in practice.

### C. HEXACHLOROPARAZYLOL

Fetisov (1964) used doses of 800 mg/kg, either as an emulsion or with polyethylene glycol monostereate in 49 sheep. Five days later faecal egg counts were reduced or negative and at autopsy 20 sheep were free from flukes and the rest had negligible worm burdens compared with controls.

## VI. TAPEWORMS

### A. NICLOSAMIDE

5-chloro-N-(2-chloro-4-nitrophenyl)salicylamide

The use of niclosamide for the removal of *Moniezia* spp. from sheep was reported in the previous review. Since that time Hall (1966) recorded its successful use in field trials using a dose rate of 75 mg/kg.

Allen *et al.* (1967) tested the efficacy of niclosamide for the removal of *Thysanosoma actinioides* from naturally infected sheep. The drug was given as a drench or tablets in dose rates of 200–600 mg/kg; 600 mg/kg removed 97% of the worms from 15 sheep, 14 being completely freed of tapeworms. Doses of 400 and 500 mg/kg greatly reduced the worm burden but doses of 200 and 300 mg/kg were erratic in their action. A transient softening of the faeces was noted in three sheep given 600 mg/kg but in general the drug was well tolerated.

The drug has also been used in birds. Terblanche (1965) treated 21 pigeons carrying tapeworms with 86·25 mg of niclosamide, the dose rate varying between 158–243 mg/kg. All the treated birds passed specimens of *Raillietina* spp. and in only one were tapeworms found at autopsy. In toxicity trials doses of 300–1045 mg/kg were given. Only three birds showed prostration 2–2½ h after administration, but this effect had disappeared after 6 h. Boisvenue and Hendrix (1965) gave 25–500 p.p.m. in the food to chickens and after 3 days the birds were artificially infected with *Raillietina cesticillus*. After 12 days the birds were killed. Dose levels of 20 mg/kg per day removed 90% of the tapeworms but below that level control was less satisfactory.

Niclosamide has also been tested in dogs for the removal of *Dipylidium caninum*, *Taenia* spp. and *Echinococcus granulosus*. Kurelec and Rijavec (1961) gave doses of 100 mg/kg to three dogs infected with *D. caninum*, and faecal examination was negative subsequently. Forbes (1963) fasted dogs infected with *Taenia hydatigena* for 24 h and then gave doses of 50, 100 or 150 mg/kg. At autopsy 5–14 days later it was found that 50 mg/kg was completely effective in four dogs but not in two others. 100 and 150 mg/kg completely cleared all dogs. Gregor (1963) gave a report of a clinical trial involving 73 dogs and cats. The drug was given after 18 h fast. The dose for dogs varied from 0·5 g to 2 g and for cats from 0·5 g to 1 g according to size. Sixty-seven dogs were clinically cured by one treatment and the rest after repetition of the treatment. Thirty-two cats were cleared by the first treatment but one cat required two treatments to free it from tapeworms. Kurelec and Rijavec (1961) gave 100 mg/kg of niclosamide to four dogs experimentally infected with *E. granulosus*. Three dogs were cleared of infection but the fourth was found at autopsy to be carrying 2500 worms. Delak *et al.* (1963) found 300 mg/kg to be effective in one third of the dogs treated and 500 mg/kg was effective in 76·9%. Forbes (1963) found doses of 50, 100 and 150 mg/kg to be almost without effect in dogs artificially infected with *E. granulosus*. The drug was given after a 24-h fast, usually in gelatin capsules but sometimes inserted into horse meat. Forbes suggested that the difference between his results and those of Kurelec and Rijavec may have resulted from the use of milk as the vehicle in the three dogs successfully treated. Niclosamide is, therefore, very effective against *D. caninum* and *Taenia* spp., but more erratic against *E. granulosus*. All workers agree that the drug is well tolerated.

In cats artificially infected with *Hydatigera taeniaeformis*, Westcott (1967) gave niclosamide in doses of 100 and 200 mg/kg. Both dose rates were 100% effective. Two hundred and seventy-three tapeworms were removed from 40 cats in 2 days and at autopsy 2 weeks later no tapeworms were recovered.

### B. BUNAMIDINE

N,N-di-*n*-butyl-4 hexyloxy-1-naphthamidine

Czipri *et al.* (1968) carried out controlled tests on lambs naturally infected with *Moniezia* spp. and found doses of 25 and 50 mg/kg to be 100% effective. In light infections 12·5 mg/kg gave good results. In a small field trial 12·5–25 mg/kg was only moderately effective but 25–50 mg/kg was completely effective. Doses up to 200 mg/kg produced no side effects but scouring occurred in animals given 400 mg/kg.

McCulloch and Kasimbala (1967) tested bunamidine in chickens carrying natural infections of *Raillietina* spp. and *Amoebotaenia sphenoides*. 200 mg/kg was 60% efficient, 300 mg/kg 80% efficient and 400 mg/kg 94% efficient. No toxic effects were observed at any dose level. 200 mg/kg was administered to laying flocks without any significant effect on egg production.

Hatton (1965) tested bunamidine against tapeworms in dogs and cats. Doses of 25–50 mg/kg were effective against *Taenia pisiformis* in dogs but action against *Dipylidium caninum* was uneven. In cats 20–25 mg/kg removed *Hydatigera taeniaeformis*. A few animals vomited or passed fluid faeces but otherwise treatment was well tolerated. Repeated large doses given as a drench caused inflammation of the lips and gums but this passed off after dosing ceased. This effect can be avoided by administration of the drug as lactose-coated tablets. Burrows and Lillis (1966) gave tablets containing 200 mg of bunamidine hydrochloride to dogs and cats infected with tapeworms. *D. caninum*, *H. taeniaeformis* and *Spirometra mansonoides* were effectively removed from cats by one half tablet given before or after feeding. In dogs one tablet given before or after food was effective against *D. caninum*. Two tablets were required for effective action against *H. taeniaeformis* and a better effect was obtained when the drug was given on an empty stomach. Some animals vomited after treatment. Forbes (1966) treated puppies which had been artificially infected with *Echinococcus granulosus* at a stage before the worms became gravid. 100 mg/kg was completely effective and 50 mg/kg significantly reduced the worm burden. Hatton (1967) tested three salts of bunamidine on dogs artificially infected with *Taenia hydatigena*. In doses of 25 mg/kg the hydrochloride and *p*-toluene sulphonate were highly effective when given in rice-paper cachets on an empty stomach. Bunamidine hydroxy-naphthoate was ineffective when given on an empty stomach but 25 mg/kg given in the food was highly effective. The hydrochloride given on an empty stomach and the hydroxynaphthoate mixed with the food were highly effective against *Taenia hydatigena* at a dose rate of 25 mg/kg. The hydrochloride was also effective against *Multiceps multiceps*. Bunamidine hydrochloride and *p*-toluene sulphonate both produced vomiting and diarrhoea but the hydroxy-naphthoate produced diarrhoea in only two of 27 dogs treated. This latter salt, therefore, has considerable advantages for use in practice.

## VII. Ascariasis

### A. TETRAMISOLE

Walley (1967) tested doses of 5–20 mg/kg against 3-, 10- and 28-day-old *Ascaris suum* in artificially infected animals. All dose levels were highly effective against the 28-day-old worms but doses of 15 mg/kg were required to remove 10-day-old worms. The 3-day-old worms were more difficult to remove and 15 mg/kg removed only 69% of the burden. Some 530 naturally infected pigs were also treated and similar results were obtained. The drug may be given orally or incorporated in the food or drinking water. It is taken readily in the food and by that route has a five-fold safety margin.

## VIII. Equine Strongyliasis

### A. HALOXON

Bosman (1966) carried out critical tests on five naturally infected horses giving one a dose of 56 mg/kg and the other 75 mg/kg. 56 mg/kg almost completely expelled both *Trichonema* spp. and *Oxyuris equi*; 75 mg/kg also removed 100% of *Triodontophorus* spp., *Oesophagodontus* spp., *Craterostomum* spp., *Parascaris equorum* and *Probstmaryia vivipara. Habronema muscae* and *H. microstoma* were unaffected. Infections of *Strongylus* spp. were low in number but the observations suggest that the drug is highly efficient against *S. vulgaris* and about 50–70% effective against *S. equinus* and *S. edentatus.* One hundred and fifty-six horses were routinely treated with doses of 60–80 mg/kg without ill effects. Pregnant mares foaled normally after treatment. A few emaciated animals showed some sluggishness 3–5 days after treatment, but they improved in condition following treatment. Doses of 200 mg/kg were given to two horses without noticeable effect. Dipping in organophosphorus dips after treatment produced no untoward symptoms.

### B. PYRANTEL TARTRATE

Cornwell and Jones (1968) carried out critical tests in six horses to which 12·5 mg/kg of pyrantel tartrate had been given. Ninety-seven per cent of *Strongylus vulgaris*, 100% of *Triodontophorus* spp., 90% of *Trichonema* spp. and 96% of mature *Oxyuris* were removed. Only 27% of fourth stage *Oxyuris* were eliminated but 62% of immature *Trichonema* spp. and 84% of a small number of *Parascaris equorum* were removed. Nine New Forest ponies were given 50, 75 and 100 mg/kg in toxicity tests. 75 mg/kg produced no ill effects but one of three horses receiving 100 mg/kg died.

## IX. Porcine Oesophagostomiasis, Hyostrongyliasis and Strongyloidiasis

### A. THIABENDAZOLE

Taffs (1966) tested thiabendazole in artificially infected pigs using the critical test. 44 mg/kg removed 80% of *Oesophagostomum* spp. while 66 mg/

kg removed 98%. Given at 66 mg/kg the drug had little effect on larvae in the intestinal wall 5 days after infection. Shanks (1963, 1965) produced evidence that 100 mg/kg would remove *H. rubidus* from pigs and Karlovic *et al.* (1964) agreed with that finding. In field trials Davidson and Sutherland (1966) successfully controlled mixed *Oesophagostomum* spp. and *H. rubidus* infections, using a dose of 66 mg/kg given incorporated with the food. Low level administration of thiabendazole was used by Taffs and Davidson (1967) to control *Oesophagostomum* spp. and *H. rubidus* in pigs; 0·05% of the drug was incorporated in the diet from 3–8 weeks of age and 0·01% from 8 weeks to 10 days before slaughter. Supperer and Pfeiffer (1964) controlled *Strongyloides* infection in four sows using a dose of 50 mg/kg of thiabendazole. Up to 200 mg/kg was administered without ill effect. Enigk and Flucke (1962) also found 50 mg/kg of thiabendazole to be 100% effective against *Strongyloides* in pigs.

## X. Capillariasis in Poultry

### A. THIABENDAZOLE

Long and Wakelin (1964) gave 0·1% in the food and found it effective against the larval stages of *Capillaria obsignata* up to 13 days old. Mature worms were unaffected. Norton and Joyner (1965) also found the drug effective against mature worms when given in single oral doses of 500 mg/kg. Hendriks (1965) found that 1000 mg/kg removed 36% of mature *C. obsignata* and 2000 mg/kg removed 88%. 1000 mg/kg was also 93% effective against immature worms. All treated birds were dull for some hours after treatment and were diarrhoeic. At effective dose levels the treatment is not likely to be economic in poultry.

### B. HALOXON

Hendriks (1964) treated experimentally infected chicks with haloxon. Given in the food 50 mg/kg consumed over 2 or 3 days was 87·6% efficient and 100 mg/kg was 92% efficient. Immature worms were less susceptible to treatment and 100 mg/kg removed only 19%. Norton and Joyner (1965) found doses as low as 10 mg/kg to be active and 50–60 mg/kg to be fully effective against adult worms. Clarke (1962) carried out laboratory experiments showing that 25–100 mg/kg was highly efficient against adult worms but that 10 mg/kg was only 41% efficient. In the field good results were obtained by the administration of 25 or 50 mg/kg. Gacongne (1964) administered 50 mg/kg in the drinking water to seven flocks of from 700 to 1400 birds with satisfactory results. Beech (1967) treated 11 650 birds on four farms with 50 mg/kg and a further 300 were treated twice at 75 mg/kg. Birds slaughtered after treatment revealed an efficiency of 96% on three farms. On the fourth farm efficiency was 88·5 and 70% in two flocks, a result attributed to emaciated birds having consumed little of the medicated food. Clinical improvement was seen in birds on three farms but not on the fourth.

## XI. Syngamiasis in Birds

### A. DISOPHENOL

Kelley (1962) gave 8·7 mg/kg of disophenol to seven heavily infected pheasants by subcutaneous injection. Two of the birds died but the rest were cleared of their infection. Sixteen per cent of 96 pheasants given 10·5 mg/kg died but satisfactory removal of the worms was achieved in the rest. Subsequently 691 pheasants were treated at the same dose rate and 11% died but the rest were completely or almost completely freed from infection. Clinical improvement quickly followed treatment. Boisvenue (1963) carried out experiments with artificially infected turkey poults. Disophenol was given in capsule or by subcutaneous or intramuscular injection at dose rates varying from 3·76–12·4 mg/kg; 7·9 mg/kg given in capsule was the most effective but 50–75 mg/kg incorporated in the food for 3 days also resulted in excellent control without signs of intoxication. Worms 1 week old as well as mature worms were removed. Doses of 17·79 to 109·03 mg/kg were given in the food to various groups of birds. 17·79 mg/kg was 90% and 24·13 mg/kg was 93% efficient, but at the high dose levels some unpalatability of the food was observed although efficiency remained high. For treating syngamiasis in turkeys, 8 mg/kg per day for three days was recommended as a satisfactory regimen. Boisvenue (1965) continued experiments with artificially infected birds giving disophenol in the food at 0·005%, 0·008% or 0·0125%. After 25 days 76·5, 97·3 and 99·6% respectively of the birds were free from infection. 0·0125% in the food caused yellow coloration of the flesh and some discolouration was seen at 0·008%. No colouration was seen in birds receiving 0·005%.

### B. THIABENDAZOLE

Leibovitz (1962) carried out tests on six severely infected pheasants giving them 0·286% of thiabendazole in the food for 6 days. The worm burden was reduced to negligible proportions. Following this 440 pheasants were given 0·05% of the drug in the food for 3 weeks, during which time the number of gapeworm eggs passed in the faeces decreased and clinical signs of disease disappeared. McGregor (1963) attempted to control syngamiasis in pheasants by incorporating the drug in the drinking water, without success, but the incorporation of 0·05% in the diet was effective in 14 days. Horton-Smith *et al.* (1963) carried out extensive tests on chickens artificially infected with *Syngamus trachea*, and found that doses between 300 and 1500 mg/kg were effective when administered to individual birds. Fourth stage larvae, immature worms and adults in the lung were all affected by treatment but the worms were most susceptible to treatment 9 days after infection. 0·1%, 0·2% and 0·5% of the drug incorporated in the food were effective in reducing the gapeworm burden but the best effect was observed when treatment began before the birds were infected. Sharpe (1964), treating pheasants, found that the continuous feeding of 0·1% of the drug in the food killed mature gapeworms in 10 days. 0·05% was almost equally effective. Single doses of 1 g also effectively reduced the

faecal egg count of infected birds. Wehr (1964) gave 0·5% in the food for 20 days to birds carrying infections 2–15 days old. Autopsy demonstrated high efficiency, in many groups 100%.

## XII. Conclusion

This paper brings up to date the review published in 1964 and has to report much progress since that date. As the number of new anthelmintics increases it becomes increasingly difficult to select the ideal compound for use against the various parasitic diseases of livestock. The final choice depends upon personal preference amongst the compounds available. The majority of the newer anthelmintics are expensive compared with their predecessors and this may limit their use in those countries where farmers are living at subsistence level. The efficiency of some of the newer anthelmintics in removing immature as well as mature stages means that one dose will do the work of two of the older, less efficient compounds. In countries where labour is costly one dose of an expensive anthelmintic may be better than two doses of a cheaper one, so that there may be a financial advantage in using some of the dearer modern drugs.

For the treatment of parasitic gastritis in ruminants thiabendazole is undoubtedly the most popular of the newer compounds, although methyridine has been preferred by some workers for use in cattle. If thiabendazole-resistant strains become widespread, thus necessitating the use of an increased dose of thiabendazole, it may well become uneconomical to use. Tetramisole, which is also active against lungworms and has a useful safety margin, seems to be the most likely drug to replace thiabendazole in these circumstances.

Diethylcarbamazine has been the drug of choice for use in parasitic bronchitis for a number of years but it acts mainly on the immature adult worms. Tetramisole has a much wider range of activity and if its use in practice supports the results obtained in the initial laboratory and field trials, it will become the drug of choice for the treatment of lungworm disease.

Although the number of fasciolicides has multiplied rapidly in the last decade none of them is outstandingly better than carbon tetrachloride. Of the newer compounds nitroxynil comes nearest to equalling the activity of carbon tetrachloride, but the unpredictable toxicity which accompanies the use of carbon tetrachloride is a distinct disadvantage. So far as the treatment of chronic fascioliasis is concerned, oxyclozanide, which is active only against adult flukes, has considerable advantages because it is safe in use.

It is encouraging to see that anthelmintics having specific action against *Dicrocoelium dendriticum* are now available, but there is insufficient evidence at present for a decision to be made between hetolin and thiabendazole as the drug of choice.

The advent of bunamidine has added a useful compound to the range of taeniacides. It shows great promise as a safe drug for the elimination of *Echinococcus granulosus* from dogs.

Other useful advances which have been made in the last 5 years are the use of thiabendazole for oesophagostomiasis in pigs, the use of thiabendazole

and haloxon for capillariasis in poultry, and the use of disophenol and thiabendazole for syngamiasis of birds.

In spite of the extraordinary progress which has been made over the last decade there are many facets of anthelmintic medication still in need of improvement. The toxicity of fasciolicides is one of these and it is hoped that further progress in this field will be made in the near future. Another likely development is the discovery of compounds with an even wider range of activity than those at present available. Such compounds will greatly simplify the control of parasites in the field.

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# *Paragonimus* and Paragonimiasis*

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## I. SPECIES OF *PARAGONIMUS* AND THEIR GEOGRAPHICAL DISTRIBUTION

Species of *Paragonimus* hitherto reported number 31 (including *Euparagonimus*) (Table I), some of which may not be valid species. The species whose life cycles are fully known are *P. westermani*, *P. kellicotti*, *P. ohirai*, *P. iloktsuenensis*, *P. skrjabini*, *P. miyazakii* and *P. sadoensis*.

Fourteen species in China are: *P. westermani*, *P. ohirai*, *P. iloktsuenensis*, *P. skrjabini*, *P. yunnanensis*, *P. szechuanensis*, *P. macrorchis*, *P. fukiensis*, *P. cheni*, *P. heterotremus*, *P. proliferus*, *P. tuanshanensis*, *P. mengalensis* and *Euparagonimus cenocopiosus*. Among them *P. szechuanensis*, *P. tuanshanensis* and *P. mengalensis* are considered to be identical with *P. skrjabini*, *P. heterotremus* and *P. proliferus*, respectively. (Chen, 1963; Hu, 1963; Tung, 1963; Wang *et al.*, 1964; Yin and Liu, 1964; Miyazaki, 1968). *P. szechuanensis* and *P. tuanshanensis* are also known as an aetiological agent of human paragonimiasis in addition to *P. westermani* (see Chung and Ts'ao, 1962; Chung *et al.*, 1964).

In Japan five species of *Paragonimus* occur, i.e. *P. westermani*, *P. ohirai*, *P. iloktsuenensis*, *P. miyazakii* and *P. sadoensis*. The life cycles of all of these species were clearly elucidated in Japan, and *P. westermani* is the only agent of human paragonimiasis, although *P. miyazakii* and *P. sadoensis* may infect man, because the crab host, *Potamon dehaani*, is often eaten uncooked or pickled.

In Korea only one species is known, *P. westermani*, but paragonimiasis patients may total 1 000 000–1 500 000. A fresh-water shrimp, *Macrobrachium nipponensis*, was added recently to the list of crustacean hosts of *P. westermani* (see Ahn and Lee, 1964; Soh *et al.*, 1966).

In Taiwan there are two species, *P. westermani* and *P. iloktsuenensis*. The

* This review should be read in conjunction with Yokogawa's article in "Advances in Parasitology" Vol. 3 (1965), pp. 99–158.

snail host of *P. iloktsuenensis* is *Tricula chui* and its crab host is *Potamon miyazakii*, which also serves as a crab host of *P. westermani* (see Chiu, 1962a,b; Miyake and Chiu, 1965).

In Malaysia there is no record of autochthonous human infection in paragonimiasis, although *P. westermani* has been confirmed in animals. Rohde (1963) examined about 20 lung flukes obtained from the intestine of a tiger that was shot in Raub (Pahang), and reported that these worms seemed to be *P. westermani* (?) although the location of the worms is unusual. Lee and Miyazaki (1965) re-examined 90 adult specimens of *Paragonimus* from two tigers and a crab-eating monkey in Malaya, and identified all of them as *P. westermani*. The same writers found the metacercariae of *P. westermani* in *Potamon johorensis*. Miyazaki *et al.* (1968a) reported in the same locality

TABLE I

*Species of* Paragonimus (proposed by)

*P. rudis* (Diesing, 1850)
*P. compactus* (Cobbold, 1859)
*P. westermani* (Kerbert, 1878)
*P. ringeri* (Cobbold, 1880)*
*P. pulmonis* (Nakahama, 1883)*
*P. pulmonalis* (Baelz, 1883)*
*P. kellicotti* (Ward, 1908)
*P. edwardsi* (Gulati, 1926)*
*P. ohirai* (Miyazaki, 1939)
*P. iloktsuenensis* (Chen, 1940)
*P. macacae* (Sandosham, 1953)*
*P. skrjabini* (Chen, 1959)
*P. yunnanensis* (Ho *et al.*, 1959)
*P. miyazakii* (Kamo *et al.*, 1961)
*P. szechuanensis* (Chung and Ts'ao, 1962)
*P. macrorchis* (Chen, 1962)
*P. fukiensis* (Tang and Tang, 1962)
*P. cheni* (Hu, 1963)
*P. heterotremus* (Chen and Hsia, 1964)
*P. proliferus* (Hsia and Chen, 1964)
*P. tuanshanensis* (Chung *et al.*, 1964)
*P. mengalensis* (Chung *et al.*, 1964)
*E.* †*skrjabini* (Chen, 1965)
*P. siamensis* (Miyazaki and Wykoff, 1965)
*P. africanus* (Voelker and Vogel, 1965)
*P. uterobilateralis* (Voelker and Vogel, 1965)
*P. bankokensis* (Miyazaki and Vajrasthira, 1967)
*P. harinasutai* (Miyazaki and Vajrasthira, 1968)
*P. sadoensis* (Miyazaki *et al.*, 1968)
*P. caliensis* (Little, 1968)
*P. mexicanus* (Miyazaki and Ishii, 1968)

* Appears to be identical with *P. westermani*
† *Euparagonimus*

another crab, *Parathelphusa maculata*, as a new host of *P. westermani* in Malaysia. *P. kellicotti* from a Malaysian tiger and *P. macacae* from a Malaysian crab-eating monkey were regarded by Lee and Miyazaki (1965) as misidentified. The snail host is as yet unknown in this country

In Indonesia *P. westermani* has been recorded in the dog, cat and tiger, but no human autochthonous cases of paragonimiasis have ever been found. Kwo and Miyazaki (1968) examined 5096 adult lung flukes obtained from ten tigers shot in N. Sumatra and identified them as *P. westermani.* The snail and crab hosts of *P. westermani* have never been recognized in Indonesia. Fischthal and Kuntz (1965) briefly reported *P. westermani* from the lungs of a tiger in N. Borneo.

In Thailand, some cases of human paragonimiasis and an endemic area of the disease were recorded, but the adult worms or larval stage of *Paragonimus* were not identified until 1964, when Daengsvang *et al.* (1964) first found adult *P. westermani* in two leopards captured in S. Thailand. Six species of *Paragonimus* have now been reported: *P. westermani, P. siamensis, P. heterotremus, P. bankokensis, P. macrorchis* and *P. harinasutai.*

Miyazaki and Harinasuta (1966) identified two immature worms which were removed from subcutaneous nodules in a 13-year-old boy in N.E. Thailand as *Paragonimus heterotremus.* Miyazaki and Vajrasthira (1967b) obtained *P. heterotremus* from cats and dogs and found the metacercariae in *Potamon smithianus.* Miyazaki and Vajrasthira (1967c) found adult *P. macrorchis* in two kinds of bandicoots. The crabs known at present to harbor *Paragonimus* metacercariae belong to four species: i.e. *Potamon smithianus, Parathelphusa germaini, P. dugasti* and *P.* sp., the first of which is most important because it is often eaten raw. The snail host of *Paragonimus* in Thailand is still unknown.

India was the first known locality of the lung fluke in Asia, but the snail and crab hosts of *Paragonimus westermani* are still unknown there. In Nepal, Iwamura (1964) reported human paragonimiasis in the Tansen district, Palpa State, but there is no report of finding either adult or larval stages of *Paragonimus.* In Ceylon, Dissanaike and Paramananthan (1962) first reported *P. westermani* and *P. compactus.* Snail and crab hosts have not been found.

In the Philippines only one species is known, *P. westermani,* and its snail and crab hosts are *Brotia asperata* and *Parathelphusa grapsoides,* respectively.

In Africa, Voelker and Vogel (1965) found a new species, *Paragonimus africanus,* in the Cameroons and Congo, suggesting that it is the causative agent of human paragonimiasis in W. Africa. They also found a few adult worms of another new species, *P. uterobilateralis,* in dogs from Lower Bakossi and in the swamp mongoose, *Atilax paludinosus,* from Liberia.

In Mexico, human paragonimiasis was undetermined until 1961, when Martinez Baez and Jimenez Galan (1961) found *Paragonimus* eggs in lung tissue excised from a male Mexican patient. Mazzotti and Miyazaki (1965) examined for the first time in Mexico adult lung flukes obtained from an opossum, *Didelphys marsupialis* captured in Colina on the Pacific coast. Miyazaki and Ishii (1968) described *Paragonimus mexicanus* as a new species in Mexico. They considered that the eggs found in the lung tissue from the

Mexican patient appeared to belong to this new species, but no larval stages of *Paragonimus* have ever been found.

Thatcher (1967) reported that he found *P. rudis* in some wild and domestic animals in Panama. However, his opinion that *P. rudis* is the only species of the genus *Paragonimus* cannot be accepted.

In Costa Rica an unidentified metacercariae of *Paragonimus* was found by Sogandares-Bernal and Smalley (1965) in a fresh-water crab, *Pseudothelphusa tristani.*

Little (1968) described *Paragonimus caliensis* from the lungs of the opossums *Didelphys marsupialis* and *Philander opossum,* in Colombia. The metacercaria was found in the liver of crabs, *Strengeria* (*Strengeria*) sp.

In Peru several cases of paragonimiasis have been reported, all from the coastal area north of Lima. They were assumed to be due to *P. westermani,* but the discovery of another species in animals of the endemic area of Peru (Ibanez and Miranda, 1967) and the finding of *Paragonimus* eggs unlike those of *P. westermani* in the sputum of the patients (Miranda *et al.*, 1967) make it doubtful that *P. westermani* occurs at all in Peru.

In Ecuador, human paragonimiasis was first observed by Heinest in 1921 (see Heinest, 1947), and has since been found throughout the coastal region of that country (Cevallos and Segovia, 1957; Rodriguez, 1963). It is suspected now that human paragonimiasis in Ecuador may also be due to some species other than *P. westermani.*

In Honduras the eggs of *Paragonimus* sp. were found in the sputum of a patient by Larach (1966).

## II. Species Differentiation

It is generally accepted that the arrangement of spines, the shape of ovary and testes, the nature of eggs and the relative size of oral and ventral suckers are good criteria for differentiating adult *Paragonimus* species. However, whether or not these variable characters can define specific or subspecific differences is still unsolved; no one of them can be used as the sole criterion. A thorough study must be made of the morphology, life history and pathogenesis before we can settle the question of the possible plurality of species from different hosts in different geographical areas. The characteristics of the species of *Paragonimus* which have been found recently, are described below.

### 1. *Paragonimus skrjabini* Chen, 1959, or *P. szechuanensis* Chung and Ts'ao, 1962

The number of flame-cells of the metacercaria of *P. skrjabini* found in *Potamon* sp. is 72, which are arranged in the following pattern: $2(3+3+3+3+3+3)+(3+3+3+3+3+3)=72$. *Assiminea lutea,* the intermediate host of *P. iloktsuenensis,* can be experimentally infected with *P. skrjabini.*

Yin and Liu (1964) described a cercaria from naturally infected *Tricula* snails collected in Szechuan province as that of *P. skrjabini.* Chen (1965) observed 12 pairs of flame cells in the cercariae of *P. skrjabini* from naturally infected *Tricula* snails.

Chen (1964, 1965) proposed a new taxonomic system of *Paragonimus* trematodes in the genus *Paragonimus* and he transferred *Paragonimus skrjabini* which was found in *Paguma larvata* to *Pagumogonimus*, a new genus, found in the subfamily *Paragoniminae*, for the reception of forms with 72 flame-cells in the metacercaria as differentiated from the forms with 60 flame-cells. However, his proposal has not been generally accepted. The definitive hosts are *Paguma larvata*, cat and dog.

2. *Paragonimus miyazakii* Kamo *et al.*, 1961

The adult worms are closely similar to those of *P. kellicotti. Potomon dehaani* is a second intermediate host. The snail host is a tiny fresh-water snail, *Bythinella* (Moria) *nipponica akiyoshiensis* (see Hatsushika *et al.*, 1966a, b). The final hosts are weasels, martens, wild boars and dogs. Rats, cats and rabbits may be experimentally infected. Yoshida and Nishimura (1968) reported that *Potamon dehaani* is highly susceptible to *P. kellicotti* from North America.

3. *P. macrorchis* Chen, 1962

Characterized by having extraordinarily large testes and singly spaced cuticular spines. The ovarian lobes are not clear-cut and moderately branched. The metacercaria is small ($282 \times 259$ μ) and has 60 flame-cells. The crab host is *Potamon sinensis.* Rats are naturally infected.

4. *P. cheni* Hu, 1963

The metacercaria was first found in *Potamon denticulatus.* The encysted larva is contracted and slightly bent within the cyst. The number of flame-cells of the metacercaria is 60 (Hu, 1963). The snail host and definitive host are not yet known. Rats and cats may be experimentally infected.

5. *Paragonimus heterotremus* Chen and Hsia, 1964

Characterized by having a very large oral sucker, almost twice as large as the ventral sucker, singly spaced cuticular spines, profusely branched ovary and small uterine mass. The metacercaria (266 μ long) of *P. heterotremus* is common in certain unidentified stone crabs, *Potamon* sp. in China. Miyazaki and Harinasuta (1966) reported that *Potamon smithianus* harboured many metacercariae characteristic of *P. heterotremus* in Thailand. Rats, dogs and and cats are naturally infected.

6. *Paragonimus proliferus* Hsia and Chen, 1964

Characterized by having a voluminous uterine mass, branched ovary, and cuticular spines in groups. The metacercaria is found in *Potamon* sp. Rats may be experimentally infected. The snail host is still unknown.

7. *Paragonimus tuanshanensis* Chung *et al.*, 1964

The adult worm is morphologically indistinguishable from *P. heterotremus*. The metacercaria is small (214 × 246 μ). The first intermediate host is *Tricula gregorina* and the second intermediate host is *Potamon* sp. in China. Cats, dogs and leopards are naturally infected.

8. *Paragonimus mengalensis* Chung *et al.*, 1964

This species is considered to be identical with *P. proliferus* (Chen, 1965). The metacercaria is the largest among all known species of *Paragonimus* (860 × 717 μ). The first intermediate host is probably *Tricula gregorina* (see Chung *et al.*, 1964). The second intermediate host is *Potamon* sp. Naturally infected mammals have not yet been found. Cats may be experimentally infected.

9. *Euparagonimus cenocopiosus* Chen, 1965

This species is probably the most interesting, being the only species with an excretory bladder not extending behind the intestinal bifurcation but ending at the region of the ventral sucker. The metacercaria has 72 flame-cells. The cuticular spines are arranged in groups. The testes are star-shaped with a distinct central body. The metacercaria is found in *Potamon denticulatus*, often co-existing with *P. skrjabini*. Dogs may be experimentally infected.

10. *Paragonimus siamensis* Miyazaki and Wykoff, 1965

The adult worm is very similar to *P. westermani*, but the cuticular spines of *P. siamensis* are always arranged in groups. The metacercaria of *P. siamensis* in *Parathelphusa* (*Parathelphusa*) *germaini* resembles that of *P. ohirai* in shape but its cyst is much the larger. The snail host is unknown. The definitive host is the cat.

11. *Paragonimus africanus* Voelker and Vogel, 1965

Characterized by having an oral sucker which is larger than the ventral sucker, a profusely branched ovary with five lobes, and strikingly large antler-shaped testes. The cuticular spines are arranged either singly or in groups. The second intermediate hosts are *Sudanautes africanus* and *S. pelli*. The snail host is unknown. The definitive hosts are man, mongoose, dogs and cats.

12. *Paragonimus uterobilateralis* Voelker and Vogel, 1965

Characterized by small eggs, the uterus extending into both halves of the body, profusely branched ovary and singly spaced cuticular spines. The first and second intermediate hosts are unknown. The final hosts are mongoose in Liberia and dogs in West Cameroon.

13. *Paragonimus bankokensis* Miyazaki and Vajrasthira, 1967

The adult worms are similar to *P. skrjabini* but the cuticular spines are arranged in groups. The crab host is *Potamon smithianus*. The snail host is unknown. The definitive host is the Indian mongoose, *Herpestes javanicus*, and cats. The bandicoot, *Bandicota indica*, may be experimentally infected.

14. *Paragonimus harinasutai* Miyazaki and Vajrasthira, 1968

Characterized by having singly spaced cuticular spines and moderately branched ovary. The metacercaria is almost as large as that of *P. yunnanensis*, measuring 533–666 × 513–649 μ. The second intermediate host is *Potamon smithianus*. The first intermediate host and the definitive host are unknown. Cats and dogs may be experimentally infected.

15. *Paragonimus sadoensis* Miyazaki *et al.*, 1968b

Both the metacercariae and adult worms are morphologically similar to *P. ohirai* in all respects. However, the metacercariae of this species are found in the fresh-water crab, *Potamon dehaani* (Kawashima *et al.*, 1967) and the cercariae are also found in a small fresh-water snail, *Tricula minima* (see Hamajima *et al.*, 1968) (hosts of *P. ohirai* live in brackish water). In addition, the number of the flame-cells of the cercaria of *P. sadoensis* is more than 50, but the exact number and pattern of the flame-cells are not specified. The natural final host is a weasel in Japan. Dogs and rats may be experimentally infected.

16. *Paragonimus caliensis* Little, 1968

*P. caliensis* differs from *P. kellicotti* in having shorter ovarian lobes with fewer branches, unbranched testicular lobes, and a single thin metacercarial cyst membrane. The metacercariae of *P. caliensis* are similar to that of *P. iloktsuenensis* but the latter species differs from *P. caliensis* in that the cuticular spines of the adult worms are arranged in groups and the ovary is profusely branched. The number and pattern of flame-cells in the metacercariae of *P. caliensis* are 96 and $2[(3 \times 8)+(3 \times 8)]$; the number differs from that in other species, i.e. 60 in *P. westermani*, *P. ohirai*, *P. iloktsuenensis*, *P. miyazakii* (see Komiya and Tomimura, 1964), *P. cheni* (see Chen, 1965) and *P. macrorchis* (Chen, 1965), and 72 in *P. skrjabini* (Chen, 1965).

17. *Paragonimus mexicanus* Miyazaki and Ishii, 1968

The adult worms are closely similar to *P. kellicotti* and *P. miyazakii* but can be separated from the former by the size of the oral and ventral suckers and the shape of ovary, and from the latter by the size of the two suckers and the geographical distribution. The definitive host is opossum in Mexico. The first and second intermediate hosts are not yet known.

## III. Symptomatology

Clinically, *Paragonimus westermani* has been considered as the only species that causes paragonimiasis in man, but recently, evidence was presented to show that *Paragonimus skrjabini* (*P. szechuanensis*), *P. tuanshanensis*, *P. heterotremus* or *P. africanus* were other causative agents of human paragonimiasis (Chung and Ts'ao, 1962; Chung *et al.*, 1964; Vogel and Crewe, 1965).

The most noticeable symptoms of pulmonary paragonimiasis westermani are cough and blood-stained sputum, with typical *Paragonimus* eggs. The most characteristic clinical features due to *P. skrjabini* are migrating subcutaneous nodules and high eosinophilia, but cough is not common and often not severe; sputum is generally scanty and is more blood-streaked than rusty, indicating that *Paragonimus* eggs are, as a rule, not present. According to Wang *et al.* (1964), among the 45 cases of paragonimiasis skrjabini, 37 (82·2%) had subcutaneous swellings which were different in consistency and definitely larger than the nodules in paragonimiasis westermani.

Chung *et al.* (1964) suspected that *P. tuanshanensis* is pathogenic to man and responsible for the occurrence of most, if not all, human cases of paragonimiasis in China. They considered that although it is true that clinical cases in the area have symptoms of chronic cough, expectoration, chest pain, hemoptysis, general weakness, etc., resembling those due to paragonimiasis westermani, yet the absence of *Paragonimus* eggs in the sputum as well as the absence of central nervous system symptoms in the cases seen so far would be different from symptoms of paragonimiasis westermani. Most recently, the immature worms removed from the subcutaneous swellings of a patient in Thailand were identified as *P. heterotremus* by Miyazaki and Harinasuta (1966). The patient was a 13-year-old boy who was suffering chiefly from chest pains for 1 year. At physical examination a subcutaneous swelling (about $5 \times 5 \times 3$ cm) was found in the left infrascapular region, but no sign of inflammation. Eosinophils were 48%. *Paragonimus* eggs were not found in the faeces. Chest X-ray films of the patient showed bilateral minimal infiltration with bilateral effusions. The first swelling in the left infrascapular disappeared and 2 months later migratory swellings appeared over the chest, abdomen and back of the patient. Vogel and Crewe (1965) reported that in West Cameroons all those patients who indicated eggs of *Paragonimus africanus* suffered from chronic coughs and discharged blood in sputum.

Although the lung is the primary site of infection with *P. westermani*, involvement of the brain, spinal cord, subcutaneous tissue, abdomen, eyes and genital organs may be found. Among them cerebral paragonimiasis occurs with the highest frequency. Oh (1967b) estimated that there were about 5000 cases in South Korea in January 1968. Recently, Oh (1968) studied 62 cases of cerebral paragonimiasis in Korea. He reported that ophthalmological signs are common in cerebral paragonimiasis, homonymous hemianopsia, optic atrophy and papilloedema being most frequent. Impaired visual acuity is caused mostly by optic atrophy, which is usually primary.

Galatius-Jensen and Uhm (1965) reviewed the radiological findings in 37 cases of cerebral paragonimiasis. In almost half of the cases calcifications of

typical appearance were found. Further information about the localization of the disease was obtained by means of pneumo-encephalography, which also gave an impression of the severity of the brain atrophy usually associated with this disease. Carotid angiography proved valuable in those cases where pneumo-encephalography was contra-indicated, and where an attempt at encephalography gave no delineation of the cerebral ventricles.

## IV. Sero-immunological Diagnosis

A reliable diagnosis of paragonimiasis depends on the recovery of *Paragonimus* eggs from the sputum or faeces, and the intradermal test, complement fixation test and other serological tests are all supplementary diagnostic methods. However, the sero-immunological techniques are now playing an important role in the diagnosis of paragonimiasis, especially extrapulmonary paragonimiasis, in the evaluation of the therapeutic effect and in the differential diagnosis between the species of *Paragonimus*, etc. The results of the complement fixation test are closely correlated with the active infection, but the positive reaction in the intradermal test does not imply an existing infection because the positive dermal reaction persists for long periods after recovery from the disease.

Yokogawa *et al.* (1962a,b) reported that in epidemiological surveys the intradermal test should be applied first and the complement-fixation test then performed on individuals who showed *positive* reactions. However, a problem in the performance of these procedures is how to treat the cases which are positive for complement-fixation test but negative for *Paragonimus* eggs. A quite interesting result was obtained by Yokogawa *et al.* (1967a) in the mass treatment of both suspected and confirmed paragonimiasis cases in the endemic areas. Most (86·3%) of those suspected cases which were positive both for the intradermal test and the complement-fixation test, but negative for *Paragonimus* eggs, became negative for the complement-fixation test within 6 months after treatment with bithionol, like cases which showed *Paragonimus* eggs in sputum or faeces. From these results it can be said that the cases positive for the complement-fixation test should be treated even if *Paragonimus* eggs are *not* found. Recently, many studies have been reported on the analysis of parasitic components in the immunology and serology of paragonimiasis. Sawada *et al.* (1964) found that the cathodic antigen isolated by starch zone electrophoresis from adult worms of *P. westermani* gave higher titres in the precipitin test on sera of paragonimiasis patients than the non-migrating or the anodic antigen, but the fraction migrating towards the anode provoked the most intense reaction in the intradermal test for paragonimiasis.

Sawada *et al.* (1968) described the fractionation procedures to isolate the antigenically active substance from the adult worms of *P. westermani* for the intradermal test. The antigen was isolated through Sephadex G-100 column, CM-cellulose column and DEAE-cellulose column.

Seed *et al.* (1966) detected a minimum of five soluble precipitating antigens

in the extracts prepared from the adult worms of *P. kellicotti* by means of the agar diffusion test (Ouchterlony test), utilizing serum from cats infected with *P. kellicotti.*

Nomoto (1967) described the urine precipitin test for paragonimiasis, utilizing the urine extract of the patients and immune rabbit serum immunized with the adult antigen from *P. westermani* with Freunds' adjuvant. The results of the serum precipitin test and urine precipitin test for paragonimiasis showed close correlation, and antibody titres of both reactions were also high in those which showed high antibody titres in the complement fixation test.

Biguet *et al.* (1965) first demonstrated the possible application of immuno-electrophoretic methods to the diagnosis of paragonimiasis. They detected two to seven specific bands in the sera of paragonimiasis patients with 0·1% NaCl extract antigen of the adult worms of *Paragonimus westermani.* Capron *et al.* (1965) conducted immuno-electrophoresis on 57 samples of serum from paragonimiasis patients and suspected cases; if more than two precipitin bands were found, they could confirm, immunologically, an infection of paragonimiasis. Yogore *et al.* (1965) reported that, by means of micro-ouchterlony and micro-immuno-electrophoresis, two to five precipitating bands were detected between the antisera of cats infected with *P. westermani* and the adult antigen of *P. westermani.*

Tada (1967a) reported that in agar double diffusion analysis there was an increase of antibody systems in sera from rats infected with *P. miyazakii* during the course of infection; however, in the last stage, from the 90th to the 150th day, the number of precipitin bands was reduced. Tsuji *et al.* (1967) applied the immuno-electrophoretic technique to the differentiation of three species of *Paragonimus: P. westermani, P. ohirai* and *P. miyazakii.* The different precipitin patterns were shown in respective antigen-antibody systems. The above workers also proved the specific band of each species by the saturation method. Yokogawa *et al.* (1968) reported that the different patterns of *P. sadoensis* from those of *P. westermani, P. ohirai* and *P. miyazakii* were proved by immuno-electrophoresis.

Tran Van Ky *et al.* (1968) reported that 14 enzyme activities were characterized at the level of the antigen fractions of the immuno-electrophoregram with fresh antigen prepared from the adult worms of *P. westermani.* Mills *et al.* (1966) studied the activities of acid and alkaline phosphomonoesterase in *P. kellicotti.* The enzymes, partially purified by differential centrifugation and column chromatography on p-60 polyacrylamide gel, exhibited maximal activity at pH 4·5 and 9·0 against a variety of substrates. Mills *et al.* concluded that a complex group of enzymes is involved in the breakdown of phosphomonoesterases by *P. kellicotti.*

Tada (1967) studied the effects of worm burden and of cortisone on the *Paragonimus* infection in rats. The average number of worm cysts in the lungs and the size of worms of *P. westermani* recovered from the rats given cortisone were significantly larger than those of worms from the control rats on the 30th day of infection. Tada also reported that by agar diffusion, no precipitin bands were detected in sera from the cortisone-treated rats.

Recently, Sogandares-Bernal (1965) reported that apparent age immunity

was demonstrated in the snail *Pomatiopsis lapidaria* from Louisiana to infection with *P. kellicotti* from Louisiana.

It is of interest to note that susceptibility of the 1·1 to 3·0 mm size class is approximately double that of the 3·1–4·0 mm size class.

## V. Treatment

Bithionol, 2,2′-thiobis (4,6-dichlorophenol) was introduced by Yokogawa *et al.* (1961, 1962b). Reports of clinical cure rates of pulmonary paragonimiasis after treatment with bithionol vary from 84·0% (Wang *et al.*, 1964) to 100% (Kang *et al.*, 1963). Bithionol thus became the drug of choice for the treatment of pulmonary paragonimiasis. As for the internal treatment of extrapulmonary paragonimiasis, especially cerebral involvement, many workers have suggested that good results can be expected with bithionol (Kitamura and Nishimura, 1963; Shim *et al.*, 1964; Wang *et al.*, 1964; Oh, 1967b). These results showed that one course of bithionol, 30–50 mg/kg every other day for ten to 15 doses, in the treatment of pulmonary paragonimiasis is usually adequate, but that two courses or more are sometimes required for maximum benefit in the acute stage of cerebral paragonimiasis, although it affords no benefit in chronic stabilized cases. As the mortality rate of cerebral paragonimiasis is highest during the early phase, and bithionol is most effective during this period, early diagnosis and treatment are imperative.

Bithionol is also effective for cutaneous paragonimiasis. Wang *et al.* (1964) treated the 24 cases with subcutaneous swelling due to *P. skrjabini* with a dose of 3·0 g (50 mg/kg) of bithionol orally every other day for 20 days, with a total dose of 30 g. They reported that subcutaneous swellings in all 24 cases gradually diminished in size and finally disappeared after completion of treatment, and that 3 cases relapsed 4, 5 and 9 months after treatment, but all of them were completely cured after the second course of treatment.

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# Author Index

*Numbers in italics refer to pages in the References at the end of each article*

C

I



J

N

S

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# Subject Index

L

P

T

U



V



X