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VOLUME 18

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PARASITOLOGY

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PREFACE

We indicated in the Preface to Volume 17 what we thought should be our policy as Editors of *Advances in Parasitology*. It was, firstly, to interpret parasitology in its widest sense, regarding the parasitic mode of life as the unifying principle, and so consider for inclusion *any* organism group which adopts that mode of life and, secondly, to try to select subjects for review, of which our knowledge has indeed advanced significantly up to the time of publication. We feel that we have progressed towards these aims in the present volume. Two reviews are concerned with arthropods (ticks) in relation to their hosts, one deals with helminths and two with *Protozoa*. Although we are ourselves all "medical" parasitologists, only two reviews directly concern human medicine. We hope to continue, and broaden, this mixture in future volumes.

We shall welcome comments and suggestions on the policy which we propose for *Advances in Parasitology* so that we can maintain the stimulus to the subject which the series so excellently provided under the editorship of the late Professor Ben Dawes.

May 1980

W. H. R. LUMSDEN
J. R. BAKER
R. L. MULLER

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Seasonal Occurrence of Helminths in Freshwater Fishes

Part III. Larval Cestoda and Nematoda*

JAMES C. CHUBB

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* This review will be completed by Part IV to appear in "Advances in Parasitology" Vol. 19.

I. Introduction

This third part of the review was originally intended to complete the series. However, as the search for literature progressed it became increasingly clear that the amount of material made it impossible to achieve this aim within the time available. In this event, it was decided to consider the seasonal occurrence of larval cestodes and nematodes in Part III, so that the final part will cover the adult cestodes, nematodes and acanthocephalans of fishes. One exception to this division has been made. In the cestodes of the Order Proteocephalidea the larval and adult worms can occur in the same fish individual, so that for this order the larval and adult stages will be treated together in Part IV.

The life cycles of all the larval cestodes and nematodes considered here are complex, and mostly require three hosts, an invertebrate first intermediate host, the fish second intermediate host and a suitable definitive host which, according to parasite species, can be a fish, an amphibian, a bird, or a mammal. Information about the life cycle for each species is briefly summarized in Section III. Many of the larval stages in fishes are of economic importance, for example, *Triaenophorus crassus*, *Diphyllobothrium dendriticum*, *Ligula intestinalis*, *Digramma interrupta* and *Eustrongylides* species, or present a public health problem if eaten by man in uncooked fish, for instance, *Diphyllobothrium latum*.

The terms used follow the style of the first two parts of this review (Chubb, 1977, 1979). Incidence is used to indicate the percentage infection of the fish hosts and intensity of infection to show the numbers of parasites found in each host. In this part of the review as adult parasites are not considered no maturation stages are defined. The term invasion is used to describe the actual process of acquisition of the parasites by the hosts.

As was indicated by Chubb (1979), in an ideal study of the host–parasite relationship, the fishes should be stratified into age classes or length groups because parasitization is not uniform through the host population. This ideal is achieved in some investigations of larval cestodes and nematodes. For larval stages of these parasites, owing to their longevity in the fish host, it is suggested in Section V A that the most meaningful assessments of the dynamics of the host and parasite relationships are possible if host age is used as the parameter of stratification.

Section II of the review indicates the classification used. Section III reports the seasonal studies of the cestode and nematode larvae under species. In Section IV the seasonal information is related to the major climatic zones of the world. The general conclusions in Section V attempt to gather the data into meaningful divisions, thereby to facilitate our understanding, and to suggest areas where further investigations could help in the appreciation of the complexities of the seasonal dynamics of larval cestodes and nematodes.

As far as possible the relevant literature has been abstracted and presented to make the review as comprehensive as possible. If material has been omitted, it is for three reasons: it was not regarded as relevant; the literature was not available at the time the review was written; or the publication was

not known to the author. It is hoped, however, that a fairly wide coverage of the literature has been achieved.

II. Classification

The classification of Wardle *et al.* (1974) is used for the cestodes. The arrangement of the nematodes follows that of the series of keys to the nematode parasites of vertebrates which commenced publication in 1974 (see Anderson *et al.*, 1974) and are still in progress.

The species and their seasonal biology are presented in alphabetical order under families.

III. Seasonal Studies

A. Class Cotyloda

1. *Order Pseudophyllidea*

The adult tapeworms of the species considered below occur in fishes. The seasonal material for the adults will be considered in Part IV of the review in each appropriate instance.

(a) *Family Bothriocephalidae*

Bothriocephalus species.

Juvenile *Bothriocephalus* species are found in the intestines of a wide variety of freshwater fishes that eat zooplankton, some species of which serve as intermediate hosts. Often, as in *Perca flavescens* in Lake Opeongo, Ontario, Canada (Cannon, 1973), no seasonal pattern of incidence of *Bothriocephalus* juveniles can be seen owing to their low incidence. However, Noble (1970) noted them most commonly from April to June in *P. flavescens* in Lake Oneida, New York, U.S.A. Bogitsch (1958) provided data for *Bothriocephalus* sp. from *Lepomis macrochirus macrochirus* occurring in ponds in Albemarle County, Virginia, U.S.A. Although his species showed evidence of maturation in the spring, May and early June, he concluded that *L. macrochirus* was not the definitive host, but either served as an intermediate host or was accidentally parasitized. The annual pattern of occurrence seen in *L. macrochirus* was of invasion from late June or early July, by worms about 0·4 mm long, of an increase in average worm length to 1·3 mm by August–September, to 5·4 mm by December–February and ultimately to reach 8·6 mm in April–May, but to be lost from *L. macrochirus* by the middle of June. The incidence pattern was: June, 0; August–September, 14%; December–February, 37%; April–May, 17%. Presumably the pattern of occurrence in *L. macrochirus* followed that of the worm in its unknown definitive host, except that in *L. macrochirus* maturity was never achieved.

(b) *Family Triaenophoridae*

Triaenophorus amurensis Kuperman, 1968 (Adult worms see Part IV of review).

This species was noted by Dubinina (1964c) and Kuperman (1965, 1967b) as a geographical form of *T. nodulosus*, but recognised as a distinct species by Kuperman (1967a) and described as such by Kuperman (1968).

Kuperman and Monakov (1972c) found that full development of the procercoid occurred only in *Acanthocyclops languidoides*, although three of five species of copepods could be experimentally infected. Kuperman (1973b) provided a list of the cyprinid second intermediate hosts which carried the plerocercoids throughout the year. As the adult worms left the definitive host at the end of May the invasion of the cyprinid hosts occurred sometime thereafter, in early to mid-summer. Kuperman (1973b) showed that in experimental infections of fish the first indications of scolex hook formation were seen 24 to 28 days after invasion and fully developed plerocercoids were recovered by the forty-second day.

Triaenophorus crassus Forel, 1868 (Adult worms see Part IV of review).

The life cycle of *T. crassus* has been studied in considerable detail. The eggs are expelled from adult worms in a pattern related to environmental water temperature. Thus, in Canada the majority of the eggs were released during the period 1–15 May; in Lake Winnipeg, Manitoba, Lake Nipissing and Lake of the Woods, Ontario (Ekbaum, 1937), in Lesser Slave Lake, Alberta (Miller, 1943a), in Baptiste Lake, Alberta (Libin, 1951) and in Square Lake, Alberta (Miller, 1952). In Nesslin Lake, Saskatchewan, egg release was somewhat later, 25 May and 13 June (Rawson and Wheaton, 1950). Miller and Watkins (1946) noted that the maximum spawning of *T. crassus* at Baptiste Lake, Alberta was about 1 week later than at Lesser Slave Lake, Alberta. In the U.S.S.R., Kuperman (1967a, 1973b) found that *T. crassus* was more sensitive to increasing temperatures than *T. nodulosus* and the embryos developed *in utero* owing to increasing spring water temperatures. The same temperature of 8–12°C was significant for *T. crassus* development in the northwest European U.S.S.R. (Lake Ladoga and Rybinsk Reservoir), Siberia (River Yenisei) and Chukotka (River Anadyr). He observed that the time of embryonic development *in utero* in the European U.S.S.R. was May and early June, but in Siberia and Chukotka, owing to the colder climatic conditions, embryonic development was delayed until late June or even the middle of July.

Temperature also controlled the rate of embryonic development and the time of hatching of *T. crassus*. Thus Watson and Lawler (1963) found that at 2°C embryonation occurred, but not hatching, whereas at 20–25°C hatching commenced at 4 days. Watson (1963b) kept control cultures of eggs at 24–26°C, and showed that at temperatures at or above 30°C the hatching of embryonated eggs was retarded with an increased rate of death of the embryos within the eggs. Kuperman (1973b) obtained comparable results with *T. crassus* in the U.S.S.R., mass emergences of coracidia were obtained at various temperatures: 5–7°C, 35–40 days; 8–12°C, 12–13 days; 13–17°C, 6–11 days; 17–20°C, 4–7 days; 21–22°C, 3–4 days; 23–24°C, 3–4 days. At 26–28°C a mass emergence did not occur, and at 28°C all the eggs died. He concluded that throughout its geographic range *T. crassus* had the same optimum temperatures for embryonic development, 17–20°C.

Infection experiments by a number of authors (see for instance, Watson and Price, 1960; Kuperman and Monakov, 1972a, b, c) have demonstrated the range of copepod intermediate hosts for *T. crassus*. Kuperman (1973b) showed that temperature influenced the speed of procercoid development in the copepod host, so that the number of days post infection when the cercomer was formed were: at 4–5°C, after 30; 9–10°C, 11–12; 16–20°C, 7–9 days.

Watson (1963a) found the low frequency of cyclopid copepods in the diet of *Coregonus clupeaformis* noteworthy, as no more than 1% of all stomachs contained these, and none had more than a few individuals. Nevertheless, he considered that the small numbers of *Cyclops* eaten seemed sufficient to establish infections of *T. crassus*. The incidence of procercoids of *T. crassus* was about 1% in spring on average, but copepod samples with up to one-third infections were seen, and these aggregations improved the chance of infection of the fish intermediate host. Miller (1943b, 1945b) considered the peak infection of fishes to occur sometime during late June or July. Arnason (1948) found that in Heming Lake, Manitoba, Canada, between 0 and 50% of *Cyclops bicuspidatus* were infected by procercoids of *Triaenophorus* during June. The pattern of occurrence was that the procercoids were found during early June, their incidence declined during late June, to fall to zero in July and a zero incidence continued through August.

Rawson and Wheaton (1950) found procercoids (?*T. crassus*) penetrating via the stomach of fishes from 5 July to the last week in August in Nesslin Lake, Saskatchewan, Canada.

The newly established plerocercoids in the flesh of the coregonine hosts have been reported as follows: Lesser Slave Lake, Alberta, Canada, July (Miller, 1945b); Baptiste Lake, Alberta, July to October (Miller and Watkins, 1946); Heming Lake, Alberta, 21 July and 29 August (Lawler, 1950); and Nesslin Lake, Saskatchewan, Canada, first week in August (Rawson and Wheaton, 1950). Miller (1952) concluded that the establishment of the new generation of capsules containing plerocercoids of *T. crassus* occurred in Canada in the July–August period, but was a function of climate and limnological conditions at each habitat. In the Rybinsk Reservoir, U.S.S.R., in *Coregonus lavaretus*, by 2 August all plerocercoids already had the scolex clearly outlined, although the hooks were incompletely developed. By 30 August all plerocercoids had fully formed hooks (Kuperman, 1973b).

The features of the morphology of the plerocercoids of *T. crassus* have been detailed by Scheuring (1929) and Miller (1945b). Miller showed that the large capsules contained young plerocercoids, recently arrived in the flesh of the fish, whereas the smaller capsules were those containing older worms in the process of degeneration and the smallest contained the final stage before death. A cauda beginning at the posterior end of the ageing plerocercoids represented the progressive decline of the worms. Miller (1945b, 1952) deduced the length of life of the plerocercoids to be at least 3 years, and probably 4 to 5 years. During the life of the coregonine fishes in Canada there was a gain of plerocercoids during the first five summers of life

little or no increase during the sixth summer, with a tendency for a decrease in numbers thereafter. In the present context, it is clear that infective plerocercoids were present in the fish intermediate hosts at all times of the year. It is noteworthy that Keleher (1952) reported that the incidence rate of four species of *Coregonus* in Lake Winnipeg, Manitoba, Canada, varied from year to year in the same locality and in different localities in the same year.

Triaenophorus meridionalis Kuperman, 1968 (Adult worms see Part IV of review).

Originally considered a southern form of *T. crassus* (Kuperman, 1965, 1967a) but described as a separate species by Kuperman (1968). In this species the plerocercoids were found in *Esox lucius*, *Silurus glanis*, *Mesogobius batrachocephalus* and *Neogobius kessleri* (Kuperman, 1968, 1973b).

Optimal temperatures for embryonic development of *T. meridionalis* (22–24°C) were higher than for *T. crassus* (17–20°C) (Kuperman, 1973b). In southern European parts of the U.S.S.R., the development of the embryos occurred in May and early June at the following temperatures: RiverDnepr, 11–18°C; River Kuban, 16°C; Lake Paleostomi, 15–22°C (Kuperman, 1973b). As in the other species of *Triaenophorus*, the proceroids develop in copepods.

The plerocercoids of *T. meridionalis* were presumably available during all months of the year for the invasion of the definitive host, *Esox lucius*.

Triaenophorus nodulosus (Pallas, 1760) (Adult worms see Part IV of review).

Miller (1943b) observed that in Lesser Slave Lake, Alberta, Canada the eggs of *T. nodulosus* were mostly laid in June, about 1 month later than for *T. crassus* in this habitat. In Llyn Tegid, Wales, Chubb (1963) found peak egg presence to be in April and May, and by June all the mature worms were lost. Such differences between localities were probably a function of water temperature. Kuperman (1973b) found that the eggs developed *in utero* in the adult worm, even when within the intestine of the definitive host, if the spring water temperatures reached high enough levels.

The effect of increasing temperature was to increase the rate of embryonic development and decrease the time needed for the hatching of the eggs. Michajłow (1951) showed that all eggs frozen for 24 h died, but if kept at 1°C for 15 minutes, thereafter they developed normally at higher temperatures. At 2–5°C first hatching occurred in 36 days, whereas at 15–16°C hatching occurred from day 7 onwards. Watson (1963b) showed that at high temperatures, above 30°C, the hatching of *T. nodulosus* eggs was retarded and there was an increased rate of death of the embryos within the eggs. Watson and Lawler (1963) also found that although eggs could embryonate they would not hatch at temperatures below 2°C.

Guttowa (1955, 1958) determined not only the time of development of the eggs of *T. nodulosus* at various temperatures, but also the invasiveness of the coracidia to *Cyclops strenuus strenuus*. In summary, at 0–2°C, all eggs died within 42 days, at 2–4°C 58–61% of the larvae hatched in 21 to 23 days, and had a 66% invasive ability, whereas between 5 and 20°C 75–99% hatched, with an 85–100% invasive ability. It was concluded that a temperature of 4–5°C was critical at the lower level, above this development occurred

normally. Guttowa (1958) concluded that *T. nodulosus* eggs and coracidia were adapted to conditions found at the bottom of (European) lakes during the ovulation months February, March and April.

Kuperman (1973b) obtained similar hatching data with material from the Rybinsk Reservoir, U.S.S.R.; mass emergences of coracidia from the egg cultures were obtained at various temperatures: 5–7°C, 29–35 days; 10–13°C, 10–13 days; 13–15°C, 6–11 days; 17–20°C (optimum), 4–7 days; 23–24°C, 5 days. At 26–28°C no mass emergence of coracidia occurred and the eggs died, whereas at 28°C no development took place and all the eggs died.

Factors other than temperature, including light, oxygen availability, pressure and salinity have been demonstrated to influence the hatching of *T. nodulosus* eggs (Michajłow, 1933, 1951), although Kuperman (1973b) has disputed some of Michajłow's conclusions. These factors are unlikely to have any significant influence on seasonal development in *T. nodulosus*.

The length of life of the coracidia also varied with temperature. Michajłow (1951) found that at 2·5–5°C 50% lived for up to 10 days and 5% to 13 days. At 15–16°C, 70% survived for 4 days, at room temperature they lived for 2–3 days, but at 29°C 30% died in 40 minutes, and at 1½ h only 10% survived and by 3 h all were dead.

The rate of growth of the procercoid in the copepod host was also influenced by temperature. Michajłow (1951) found that at 2–5°C development was greatly inhibited, but at 15–16°C typical procercoids were obtained by 7 to 9 days. Kuperman (1973b) obtained similar results, at 4–5°C cercomer formation occurred after 30 days, at 9–10°C after 11–12 days, and 16–20°C after 7–9 days.

Experimental studies to identify the copepod hosts of *T. nodulosus* have been carried out by Michajłow (1932), Miller (1943b), Watson and Price (1960), Lawler and Watson (1963), Halvorsen (1968), Kuperman and Monakov (1972a, b, c). A summary of these investigations and a list of principal and other copepod hosts for *T. nodulosus* can be found in Kuperman (1973b). Plasota (1970) compared the mortality of control and infected populations of *Cyclops vicinus* infected with *T. nodulosus* using Weibull's distribution. In the infected population it was shown that there was intensified mortality in the terminal parts of the experiment.

Halvorsen (1968) collected plankton samples in Bogstad Lake, Norway, from April to June, but did not find any natural infections by procercoids of *T. nodulosus*. Nonetheless, from a knowledge of the rate of procercoid maturation it is possible to forecast the time when early invasions of the fish intermediate hosts should occur. In the conditions of Lesser Slave Lake, Alberta, Canada, Miller (1945a) predicted that early infections should be available in *Lota lota maculosa*, the fish intermediate at that locality, from mid-June to mid-July.

Rosen (1919a) has described procercoid penetration in *Perca fluviatilis* and Vogt (1938) studied invasions of procercoids in *Salmo gairdneri* fry and yearlings, in which he showed that procercoids must pass rapidly through the stomach and enter the intestine in a short time to survive. The rapid passage

of food, and procercoids, through the stomach was facilitated by higher water temperatures, thereby favouring survival of the procercoids.

Early developmental stages of *T. nodulosus* plerocercoids in fishes have been reported from natural infections by Miller (1945a), Chubb (1964), Lawler (1969b), Lien (1970) and Andrews (1977a, b). At Llyn Tegid, Wales, Chubb (1964) found developing plerocercoids in *P. fluviatilis* from March to June, and Andrews (1977b) found a similar invasion period in the same habitat and host species some years later. A later time of invasion was seen in *P. fluviatilis* in Heming Lake, Manitoba, Canada, by Lawler (1969b). New worms were apparent in July, when 18% of 1-month-old *P. fluviatilis* were infected: invasion was considered to have occurred in June. A somewhat similar situation was noted by Lien (1970) at Bogstad Lake, near Oslo, Norway. Immature plerocercoids were found in July, August and September, so that first invasions of the fishes were also likely to have occurred in June. Pronin *et al.* (1976), at the River Bol'shaya Rechka, U.S.S.R., found an incidence of 78·2% of plerocercoids in young of the year *Esox lucius* at the end of July. Again invasions would have first occurred in late June or early July.

It was noted above that Miller (1945a) predicted that in Lesser Slave Lake, Alberta, Canada, *Lota lota maculosa* would be invaded by procercoids from mid-June to mid-July. Miller found some young plerocercoids free in the liver tissues of these fishes on 11 July, and already the worms were 2–3 cm long, in a transitional state between procercoids and plerocercoids. At this date the larvae were in tunnels in the liver tissue. By 25 July some fully formed scolices were seen, with hooks, and thin connective tissue capsules were formed around the plerocercoids: the worms were arranged in loose folds inside. Compact capsules with tightly coiled plerocercoids within were seen on 6 September, and this condition was maintained on 10 June the following year, by which time the *L. lota maculosa* were entering their second summer of life. By the latter date, the development of the cauda (discussed under *T. crassus* p. 5) had occurred, which implied a progressive degeneration of the plerocercoid larvae which would lead to their ultimate death. At Lesser Slave Lake, Miller (1945a) did not observe any new invasion of *L. lota maculosa* during their second summer, as these fishes had changed from a planktonic to a benthic diet. The *T. nodulosus* capsules probably disappeared by the third summer in the life of this host species. It is relevant to note that in Lesser Slave Lake *L. lota maculosa* was the principal fish intermediate host for *T. nodulosus*. *Perca flavescens* were present, but did not serve as a host, owing to their late spawning date, June, so that the fry were not hatched until July, by which time they were not exposed to invasion by procercoids of *T. nodulosus* (Miller, 1945a).

Kuperman (1973b) carried out infection experiments with fry of *Perca fluviatilis* at Rybinsk Reservoir, U.S.S.R. The fry, which were hatched from spawn obtained 13–16 May, were fed on plankton from 3 days post-hatching, and the first developing plerocercoids were seen in 8-day-old fry. Completely formed hooks on the scolices of the *T. nodulosus* were first observed 27 June–2 July, at a fish age of 41–44 days. Kuperman noted plerocercoids during June and July with the scolex hooks at various stages of development but by

the middle of July the hooks were fully formed in the majority of plerocercoids. The last instance of developing hooks was seen on 19 August. Accordingly, he concluded that invasion of the fishes had continued until 5–10 July. During June to August the developing plerocercoids were usually free within the liver parenchyma, to become encapsulated thereafter. Observations of natural infections at the Rybinsk Reservoir (Kuperman, 1973a) confirmed Kuperman's laboratory data (1973b).

The success of establishment of each generation of procercoids in the fish intermediate host may vary from year to year. Thus Lawler (1969a) at Lake Mälaren, Sweden, found that 1965 had been a good year, as shown by a considerably higher proportion of capsules of *T. nodulosus* per infected *Perca fluviatilis* in fishes which had started life in 1965. He concluded that annual differences may exist in the incidence of invasion in the second intermediate hosts. In 1965 at Lake Mälaren the water level was high, assuring more favourable spawning habitats for *Esox lucius*. Therefore the production of *E. lucius* was good, and the evidence was that the establishment of *T. nodulosus* in *P. fluviatilis* that year was also high. In summary, ecological conditions at that time were generally favourable for the early stages in the life cycle of the cestode (Lawler, 1969a).

It has been indicated above (Miller, 1945a) that the plerocercoids of *T. nodulosus* can live in the intermediate fish host for up to the third year. The presence of developing, mature and degenerate plerocercoids within the liver of one fish, representing at least three generations of invasion, confirm this fact (Chubb, 1964; Mishra, 1966; Lawler, 1969b; Lien, 1970; Andrews, 1977b).

The length of life of the plerocercoids explains why there were little or no seasonal variations in periodicity of plerocercoids in the following hosts and habitats: *Perca fluviatilis*; Lake Leman (Geneva), Switzerland (Zschokke, 1884), Rybinsk Reservoir, U.S.S.R. (Izyumova, 1958), River Svratka, Czechoslovakia (Vojtkova, 1959), Lake Konche, Karelia, U.S.S.R. (Malakhova, 1961), Llyn Tegid, Wales (Chubb, 1964), Rostherne Mere, Cheshire, England (Rizvi, 1964), Lipno Reservoir, River Vltava, Czechoslovakia (Ergens, 1966), Shropshire Union Canal, Cheshire, England (Mishra, 1966), Bogstad Lake, near Oslo, Norway (Borgström, 1970; Lien, 1970), Lake Dusia, Lithuania, U.S.S.R. (Rautskis, 1970a), Lake Dargin, Poland (Wierzbicki, 1970, 1971), Lake Võrtsjärv, Estonia, U.S.S.R. (Tell, 1971), Llyn Tegid, Wales (Andrews, 1977a, b), Serpentine, London, England (Lee, 1977) and Lakes Galstas, Obelija and Shlavantas, Lithuania, U.S.S.R. (Rautskis, 1977): *Perca flavescens*; Heming Lake, Manitoba, Canada (Lawler, 1969b): *Cottus gobio*; River Avon, Hampshire, England (Rumpus, 1965): *Esox lucius*; Lake Dusia, Lithuania, U.S.S.R. (Rautskis, 1970b), Lake Võrtsjärv, Estonia, U.S.S.R. (Tell, 1971), and Lake Leman (Geneva), Switzerland (Zschokke, 1884): *Gymnocephalus cernua*; Rybinsk Reservoir, U.S.S.R. (except winter), Izyumova (1959): *Lota lota*; Lake Konche, Karelia, U.S.S.R. (Malakhova, 1961), Lake Võrtsjärv, Estonia, U.S.S.R. (Tell, 1971): and *Lucioperca lucioperca;* Rybinsk Reservoir, U.S.S.R. (Izyumova, 1958). No doubt if sufficient examples were examined of all the numerous other species

of fishes that have been recorded as second intermediate hosts for *Triaenophorus nodulosus* (see Kuperman, 1973b for list) the occurrence of plerocercoids in these too would be found to show no seasonal periodicity.

Wierzbicki (1971) examined seasonality of occurrence of *T. nodulosus* plerocercoids in littoral, shallow and deep water conditions in Lake Dargin, Poland. Some seasonal variations were seen in littoral and shallow waters, but not in the deep zone, where the plerocercoids were most common. The increased abundance in the deeper area was attributed to the facts that the copepod intermediate hosts were mainly pelagic, and here *Perca fluviatilis* fed intensively on zooplankton. Rautskis (1977) studied the seasonal dynamics of *P. fluviatilis* parasites in the shallow eutrophic Lake Obelija compared with the deep mesotrophic Lakes Galstas and Shlavantas in Lithuania, U.S.S.R. Although in general the incidences and intensities of parasite infection were higher in Lake Obelija, as compared with the two deeper lakes, there appeared to be no clear difference for the patterns of occurrence of the plerocercoids of *T. nodulosus*. Andrews (1977b) did not find any variation of infection of *P. fluviatilis* by *T. nodulosus* within Llyn Tegid, Wales. In this habitat the *P. fluviatilis* populations showed marked seasonal migrations. They occurred in deep water, 10–12 m and deeper, from late September until March–April, at which time they moved into shallower conditions for the summer months. By July to mid-September, they were very common in shallow water, 1 to 3 m in depth. Such migrations may occur in many deeper lakes.

It has been shown above that mature plerocercoids of *Triaenophorus nodulosus* were present at all seasons. As is discussed under the section dealing with the adults of *T. nodulosus* (see Part IV of review), the plerocercoids were invasive to the definitive host *Esox lucius* at all times of the year. Chubb (1964), at Llyn Tegid, Wales, and Borgström (1970), at Bogstad Lake, near Oslo, Norway, have shown that *Perca fluviatilis* form part of the diet of *E. lucius* through the year, although clearly seasonal variations in the rate of consumption and speed of digestion of prey fishes by *E. lucius* occur. Indeed Borgström speculated that the higher intensity of infection of female *E. lucius* in Bogstad Lake might be directly attributed to their greater overall food consumption.

The dynamics of the process of recruitment of procercoids from copepods, maintenance of a plerocercoid population in the intermediate fish hosts, and of loss of plerocercoids through their natural death in the body of the fish host have been discussed by Chubb (1964) and Lawler (1969b), among others. The details will vary considerably from water to water, but it is important to realise that the strength of any generation of plerocercoids in a fish population in a particular year may be determined over a rather short time, perhaps as little as 30 to 45 days. Lawler (1969b) has pointed out that the strength of the year class of *T. nodulosus* in *Perca fluviatilis* fry may be determined in their first month of life, even though older *P. fluviatilis* which feed on plankton will also acquire invasions.

Owing to the variations that occur from year to year with respect to local weather conditions, thereby potentially influencing the year class (generation) strength of the plerocercoids in a habitat, it is no surprise to find that annual

variations over a period of time have been reported. Thus Lawler (1969b), at Heming Lake, Manitoba, Canada, found a high incidence of *T. nodulosus* plerocercoids in 1956, followed by a steady decline until 1960, with an increase again to 1965, with a drop in incidence in 1966. These changes were related to fishing of *E. lucius* in the lake. However, in 1966 there was a poor year class (generation) of adult worms in *E. lucius*, but the following year the conditions must have been ideal because there was a high (61%) incidence of plerocercoids in *P. fluviatilis*. Andrews (1977a, b) considered that there were significant changes in the plerocercoid population in *P. fluviatilis* in Llyn Tegid, Wales, between 1957 and 1958 (see Chubb, 1964) and the period of his own study 1975–76. Andrews considered that the rate of recruitment in adult *P. fluviatilis* was higher in 1957–59. However, there were higher incidences and mean intensities of infection in 1975–76, but with a greater occurrence of degenerating plerocercoids. The feeding regimes of the *P. fluviatilis* were the same in both 1957–58 and 1975–76.

The question of the development of immunity in fishes to infections of *Triaenophorus nodulosus* has not been satisfactorily resolved. Scheuring (1919) could not reinfect *Salmo trutta* which were already infected by *T. nodulosus* plerocercoids. Therefore he concluded that immunity occurred. Vogt (1938), however, using *Salmo gairdneri*, carried out further experiments which showed that successive invasions by procercoids were possible, so that he decided that an immunity did not exist. Bauer (1959) considered that the experiments of Vogt were incorrectly planned, so that the conclusion was without proper basis. Kuperman (1973b), after some experimental invasions, invoked a host defence mechanism on the part of the fishes to explain the low levels of infection by plerocercoids. It is clear that if an immunity or other host defence mechanism exists, it confers only a partial protection. Thus Chubb (1964) at Llyn Tegid, Wales found some *Perca fluviatilis* with concurrent infections of developing (recent invasions), mature (1-year-old) and degenerate (probably 2 years, or older) plerocercoids. The low intensity of infection of *P. fluviatilis* at Llyn Tegid by plerocercoids was attributed to an apparent absence of copepods from the food of the fishes during the months in which invasion could occur, and not with an immunity effect. Scheuring's (1923) observation of massive infections by *T. nodulosus* plerocercoids were considered as evidence in support of the above hypothesis.

Stromberg and Crites (1974) have described infections of plerocercoids in *Morone chrysops* from Lake Erie, U.S.A. The incidence of infection increased with size and age of the fishes. Since it had been shown elsewhere that adult *M. chrysops* were chiefly piscivorous, they speculated that these fishes might have acquired the infection by eating other fishes containing plerocercoids, rather than by invasions from copepods. Such a transmission pathway has been demonstrated experimentally by Halvorsen and Wissler (1973) for plerocercoids of *Diphyllobothrium latum* from *Esox lucius* and *Perca fluviatilis* fed to *Lota lota*, and Grimaldi (1974) and others have considered that the visceral infections of *D. latum* had all arisen by secondary transfer of plerocercoids from other fishes, whereas the muscle infections had resulted from primary ingestion of procercoids from the zooplankton.

However, as far as is known to the author, no evidence of secondary transfer of plerocercoids of *T. nodulosus* has been reported elsewhere.

Mortalities of fishes attributed to *Triaenophorus nodulosus* have been noted. An epizootic in *Perca fluviatilis* of Lake Kandry-Kul, Bashkir Republic, U.S.S.R., was reported by Markevich (1943, quoted from Petrushevskii and Shul'man, 1958) in May–June. More recent mortalities, also in *P. fluviatilis* in natural waters, from Lake Zug, Switzerland, in May 1957 and January 1958 were described by Matthey (1963). The May–June mortalities might be attributed to invasion of the fishes by heavy infections of procercoids, but some other stress factor such as cold must have been involved in the January mortality. Sous (1968) observed the effect of winter mass mortalities on the parasite fauna of fishes in forest-steppe lakes of western Siberia, U.S.S.R. In 1965 the incidence of *T. nodulosus* in *Perca fluviatilis* was 50%, but after the mortalities by 1967 incidence was only 30%. In the definitive host, *Esox lucius*, the incidence fell from 100% in 1965 to 63% in 1966, and after the destruction of *E. lucius* in 1967 plerocercoids were no longer present in *P. fluviatilis*.

Triaenophorus orientalis Kuperman, 1968 (Adult worms see Part IV of review).

This species was observed by Dubinina (1964c) and Kuperman (1965, 1967b) as a geographical form of *T. crassus*, but recognized as a distinct species by Kuperman (1967a) and described and named by Kuperman (1968). It occurs in the Amur Basin and the Maritime Provinces of the U.S.S.R.

The adult worms in *Esox reicherti* leave this host at the end of May. The eggs may already have embryonated within the uterii of the worms in the host intestine if water temperatures have reached 15°C. In this event a mass emergence of coracidia can occur from eggs removed from worms only 3 h before. At 17–20°C mass emergences of eggs normally occurred in 1 day, and at 11–17°C in from 6 to 2 days (Kuperman, 1971, 1973b). The only copepod intermediate host in which full development of the procercoid occurred in experimental conditions was *Acanthocyclops languidoides* (Kuperman and Monakov, 1972c). The plerocercoids in experimentally infected *Perccottus glehni* had the hooks of the scolex completely developed by the 39th day (Kuperman,1973b). The plerocercoids presumably retain an invasive capability for 1 or more years. The overall pattern of seasonal events in *T. orientalis* follows a similar sequence to that of the other species of the genus.

Triaenophorus stizostedionis Miller, 1945 (Adult worms see Part IV of review).

The adult worms occur in *Stizostedion vitreum* and the plerocercoids in *Percopsis omiscomaycus*. In Lesser Slave Lake, Alberta, Canada, the gravid adult worms passed out during the first 2 weeks of June. Procercoids developed in *Cyclops bicuspidatus* in 10 to 14 days. The plerocercoids from *P. omiscomaycus* must be invasive at all seasons, as the definitive hosts *S. vitreum* were never free of worms, and newly established individuals occurred during the period of maturation of the adult generation (Miller, 1945c). The biology of the plerocercoids was similar to that of *T. crassus* and *T. nodulosus* in Lesser Slave Lake.

Triaenophorus species.

The following comments are included together here, as they apply to more than one of the species described above.

Kuperman and Monakov (1972b) stressed, as a result of their experimental studies, that individual species of copepods should not be regarded as universal intermediate hosts for the same species of *Triaenophorus* (or helminth) over a wide geographical area.

The natural infections of cyclopid copepods with procercoids of *Triaenophorus* species, probably mostly *T. nodulosus*, but *T. crassus* and *T. stizostedionis* also present, were studied in Heming Lake, Manitoba, Canada, by Watson and Lawler (1965). *Cyclops bicuspidatus thomasi* was established as the first intermediate host in this locality. However, the incidence of the procercoids of *Triaenophorus* species was confined to only one brood of *C. biscuspidatus thomasi* hatched in early May and which matured in mid-July. Such as event markedly limits the season of occurrence of the procercoids of these species.

Dr. W. A. Kennedy (quoted from Miller, 1952) first pointed out the similarity of the southern limits of distribution of *Triaenophorus* species in North America to an isothermal line, and he postulated therefore that temperature was involved. From published work and some of his own on seasonal movements of coregonine fishes, Kennedy reasoned that north of the line the water warmed slowly and the coregonines lingered in the richer, shallow waters until well into the summer. However, south of the line the more rapidly warming water drove the coregonines into deeper water much earlier in the year, too early to contact infected *Cyclops*. This was a simple, logical explanation of the northern preference of *Triaenophorus* and of its southward extension only into large lakes which warmed slowly in the spring (Miller, 1952). Lawler and Scott (1954) postulated that spring or early summer temperatures corresponding to a 70°F (21·1°C) July isotherm were critical for successful development or otherwise. Kuperman (1973b) speculated that the above explanation was probably applicable only to the distribution of *Triaenophorus crassus* in the great lakes of southern Canada.

Markevich *et al.* (1976) noted that hot effluent from power plants had a marked effect on an ecosystem. The development of some planktonic and benthic invertebrates was higher in the region of the Kanev Reservoir receiving hot effluent from the Tripol'ye Power Plant, U.S.S.R., thereby affecting the abundance and seasonal dynamics of the parasites. Markevich *et al.* (1976) stated that the results of observational studies by Strazhnik (1975, quoted from Markevich *et al.*, 1976) showed that warm water may speed up the reproduction of forms of the genera *Bothriocephalus* and *Ligula*, and to a lesser extent, those of *Triaenophorus*. However, Pojmańska (1976) and Pojmańska *et al.* (1978) reported lower numbers of *Triaenophorus nodulosus* in heated lakes in Poland, and indicated that this species prevailed in cooler conditions.

Newton (1932) reported *Triaenophorus tricuspidatus* (a synonym of *T. nodulosus*) from lakes in Western Canada. The pleroceroids, clearly those of

T. crassus, were observed throughout the year in coregonine fish muscles. Ekbaum (1937) has already disputed Newton's identification.

(c) *Family Amphicotylidae*

Eubothrium species

Eubothrium juveniles are found in a variety of freshwater fish hosts other than those in which they can develop to maturity. Frequently the incidence of the juveniles is low, so that no seasonal trends are apparent. Izyumova (1959) at the Rybinsk Reservoir, U.S.S.R., found a low incidence in *Gymnocephalus cernua* in winter (4·44%) and spring (8·33%), but none in this fish species during the summer and autumn.

2. *Order Diphyllidea*

The adult tapeworms of the species discussed below are found in avian and mammalian hosts (see McDonald, 1969 for lists of avian hosts).

(a) *Family Diphyllobothriidae.* Wardle *et al.* (1974) included the Ligulidae Dubinina, 1959 as part of their Family Diphyllobothriidae. Here the two families are treated separately.

The taxonomy of the Genus *Diphyllobothrium* remains troublesome, largely owing to the fact that it is vital to have very precise information about all stages of the life cycle before attempting to discriminate between species. As a result of recent studies the following species discussed here can be regarded as valid: *D. dendriticum*, *D. ditremum*, *D. latum* and *D. vogeli*. The others, *D. oblongatum*, *D. sebago* and *D. ursi* require further critical study to more closely define their morphological and biological characteristics. Numerous other species have been described, many of which are unlikely to be valid, but these are not relevant here either because their larvae are not found in fishes, or if they do occur, nothing is known of their patterns of seasonal occurrence. von Bonsdorff (1977) gave much recent information on the fish diphyllobothriids, as well as *D. latum*, and is recommended for a general account of the life cycle. The following authors have critically studied the taxonomy in relation to the biology of the plerocercoids of the species considered here: Andersen (1971, 1975), Bylund (1973, 1975a, b) Chizhova (1965), Chizhova and Gofman-Kadoshnikov (1959), Chizhova *et al.* (1962), Freze (1977), Freze *et al.* (1974), Gur'yanova *et al.* (1976), Kuhlow (1953a), Nyberg (1966), Vik *et al.* (1969), Wikgren (1964, 1966b) and Wikgren and Muroma (1956).

Diphyllobothrium dendriticum (Nitzsch, 1824)

Markowski (1949) regarded the following species as synonyms of *D. dendriticum: D. canadense*, *D. cordiceps*, *D. exile*, *D. fissiceps*, *D. laruei*, *D. oblongatum* and *D. strictum*. Chizhova (1958) has since confirmed this for *D. strictum*, but in depth studies of some of the North American forms are still awaited. Accordingly, *D. oblongatum* is considered later as a separate entity. *Diphyllobothrium medium* (Fahmy, 1954) was redescribed by Fraser (1960b), but is here considered a synonym of *D. dendriticum* (see Chubb, 1968). *Diphyllobothrium norvegicum* Vik, is also included as a synonym of

D. dendriticum following Andersen (1970, 1971), Bylund (1969, 1975a), Chubb (1968) and Halvorsen (1970). The adult worms of *D. dendriticum* occur naturally in the Laridae (gulls) (see Markowski, 1949), piscivorous mammals (see Vik, 1964a) and man (see Chizhova, 1958; Serdyukov, 1972).

Kuhlow (1953c) has examined the development of the eggs of *D. dendriticum* at different temperatures. At 0°C no development occurred, but eggs retained at this temperature for several months remained viable and hatched normally at room temperature. At warmer temperatures hatching was obtained as follows: 7°C, 48 days; 20°C, 10–11 days; 23°C, 8 days; at 37°C all eggs died by 6–8 days, owing to a lack of oxygen. Vik (1957) obtained similar results; at 1–2°C, no development during 5 months, but if raised to 20°C they hatched in the normal time; at 10°C no eggs hatched within less than 1 month; and at 20°C most hatched during a period of 9 to 14 days.

The procercoid of *D. dendriticum* developed at different rates in the copepod host, again determined by temperature. Thus Kuhlow (1953c) obtained mature procercoids in *Cyclops strenuus* at 18°C in 14–18 days and at 23°C after 9 days. Degenerate procercoids were seen mid-August to mid-September in some experimentally infected copepods. Halvorsen (1966) also studied growth of *D. dendriticum* procercoids in *C. strenuus*. At 20°C the procercoids were fully developed in 14 days, but at 6°C growth commenced between 42 and 52 days post-infection. In addition, Halvorsen found a seasonal variation in the incidence (frequency) and intensity in experimental invasions of female *Eudiaptomus gracilis* with procercoids of *D. dendriticum*. The incidence was minimal in March, higher in January and April, but maximal in June, July and October. The intensity of infection obtained was lowest in January, rising in March and April, and rising much higher in June and July to a peak in October. However, in *C. strenuus* no seasonal variation in experimental invasions was obtained, suggesting that susceptibility varied with season only in some copepod hosts. In natural waters it can be assumed that procercoids could occur in all months of the year, but that the incidence of the procercoids is likely to be minimal during the cold months and maximal during the warm months, owing to the interactions of environmental temperature with the rate of hatching of *D. dendriticum* eggs, the population dynamics of the copepod hosts, their susceptibility to invasion, and the rate of development of the procercoids to an invasive form within the copepods. Henricson (1978) has studied such seasonal dynamics of *D. dendriticum* in Lake Bjellojaure, Sweden, which are reported below.

Vik (1957) recognized a plerocercoid stage I in *Gasterosteus aculeatus* and a plerocercoid stage II in *Salmo trutta*, *Salvelinus alpinus* and *Thymallus thymallus*. If the terminology of Freeman (1973) is adopted these larvae should be named acaudate bothrio-plerocercoid (stage I) and strobilobothrio-plerocercoid (stage II). The pattern of transfer of the plerocercoids from *G. aculeatus* to *Salmo trutta* during the feeding activity of the latter fish was observed by Hickey and Harris (1947) at Poulaphouca Reservoir, County Wicklow, Ireland, Kuhlow (1953c) in Germany, and Vik (1957) in various Norwegian habitats. Experimental studies of Vik (1957), Bylund (1969) and Halvorsen (1970) have confirmed this, but suggest that relatively few

plerocercoids establish themselves in *S. trutta* via this route. However, Vik claimed that he only found plerocercoid stage II in *Salmo trutta*, *Salvelinus alpinus* and *Thymallus thymallus* in lakes where *G. aculeatus* also occurred. Chubb (1970) has found a low incidence of *D. dendriticum* plerocercoids in salmonid fishes in Llyn Tegid, Wales, where *G. aculeatus* is not present. It is most likely that the alternative pathways are facultative, so that fishes feeding on zooplankton will acquire an infection from this source, without the necessity for the plerocercoid stage I in *G. aculeatus*.

In experimentally infected *Gasterosteus aculeatus* Kuhlow (1953b) found that the pleroceroids reached a length of 1·5–2 cm in 3 months, but were invasive to a definitive host 38 days after infection of the fish. Banina and Isakov (1972) have studied plerocercoids in *Gasterosteus aculeatus* in the River Chernaya, not far from where the River Neva joins the Gulf of Finland. The incidence was low (2%) in February–March, rising to 6·7% in April, 15% in May and 21·2% in June: in July, incidence was 6·7%, in September 5% and in November 20%. In *Pungitius pungitius* at the same habitat, *D. dendriticum* plerocercoids were found in February–March (8·3%), the end of June (7·7%) and November (11·1%), but not in the other months. The incidences were low as copepods were not a significant item in the diet of these fishes in this habitat (Banina and Isakov, 1972).

Some seasonal information for the occurrence of mixed infections of *Diphyllobothrium dendriticum* and *D. ditremum* in *Salmo trutta* have been given by Duguid and Sheppard (1944), Hickey and Harris (1947) and Fraser (1951) from the British Isles. The habitats are shown in Table 1. These studies were stimulated by mortalities of *S. trutta* caused by epizootics of the *Diphyllobothrium* plerocercoids. The deaths of the fishes occurred in July (Duguid and Sheppard, 1944), May to September (Hickey and Harris, 1947) and May to October (Fraser, 1951). The death of the fishes was confined to the warmer months, and Hickey and Harris (1947) demonstrated that the activity of the plerocercoids was influenced by temperature. Migration of the plerocercoids through the vital organs of the fishes in conditions of high summer temperatures in the shallow reservoirs probably contributed to the deaths. Fraser (1960a) summarized much of this information.

Vik (1957), at the Ånøya Water System in Norway, examined *Salmo trutta* and *Salvelinus alpinus* from May to November. He was unable to establish any differences in incidence of the plerocercoids of *D. dendriticum* during these months. He caught very few fishes during the winter months, so no conclusions concerning differences in infection throughout the year were possible.

Henricson (1977, 1978) carried out a very extensive analysis of the dynamics of occurrence of *Diphyllobothrium dendriticum* in *Salvelinus alpinus* in Lake Bjellojaure in Swedish Lapland. No seasonal incidence of plerocercoids was seen, owing to the survival of the plerocercoids in their hosts for many years. However, the size range of the worms underwent a seasonal change, so that the structure of the parasite population varied with season. Henricson (1978) suggested the following dynamic pattern of infection. *Larus canus*, potential definitive hosts, arrived at their breeding sites in Lake Bjellojaure in early

June, and became invaded by *D. dendriticum* when feeding on fish weakened by heavy infections of plerocercoids, or, on entrails left by fishermen. The permanent plerocercoid population constituted a reservoir of invasion for the gulls. The worms matured and produced eggs in the avian hosts. At 15°C, the normal summer temperature in the littoral and upper waters of Lake Bjellojaure, the eggs would hatch in 2–3 weeks, so that from the end of July invasion of cyclopid intermediates was possible. The *Larus canus* left the area in September, therefore invasive coracidia larvae were available until mid-October. This corresponded with the peak abundance of *Cyclops scutifer*, which was probably the principal intermediate host.

Henricson (1978), quoting other work also reported above in this review, suggested that the procercoids in the copepods became invasive in 3 weeks, thus from the end of August they would be available to the fishes and would remain so until the copepods disappeared from the open water in the autumn. Henricson found that during September and October the *Salvelinus alpinus* were feeding heavily on planktonic crustaceans, so that the two most important factors controlling recruitment to the plerocercoid population were fulfilled. During the winter most of the *Cyclops scutifer* population was resting on the bottom mud and the fishes had reduced feeding activity. Accordingly, in winter, recruitment must have been negligible. Lake Bjellojaure was normally ice-covered from mid-November so no *D. dendriticum* eggs would hatch and no copepods would be invaded until spring.

In spring Henricson (1978) showed that the copepods returned to the open waters and *Salvelinus alpinus* increased its feeding activity. Thus, a further, but lower level of recruitment to the plerocercoid population in the fishes occurred from March to May.

The mortality of *Salvelinus alpinus* at Lake Bjellojaure was correlated with both number and site of occurrence of the plerocercoids in the fishes. Henricson (1978) discussed the probable mechanisms of regulation of the populations of plerocercoids of both *Diphyllobothrium dendriticum* and *D. ditremum*, which occurred together in the same hosts, and concluded that the plerocercoid populations in Lake Bjellojaure were limited through the success of transmission, but that changes in the ecosystem of biotic or climatic origin might favour transmission and make parasite numbers build up in the hosts until fish deaths occurred owing to lethal levels of plerocercoids, especially of *D. dendriticum*, being reached. Similar favourable transmission periods were probably involved in the sequences of events leading to the mortalities of *Salmo trutta* caused by epizootics of *Diphyllobothrium* plerocercoids noted from the British Isles (see Duguid and Sheppard, 1944; Hickey and Harris, 1947; Fraser, 1951, 1960a).

Henricson (1978) also provided some data about long-term fluctuations in infections of *Diphyllobothrium dendriticum* at Lake Bjellojaure. These fluctuations were primarily related to the success of transmission of the procercoids to the fishes during each year. Powell and Chubb (1966) also made some observations on the occurrence of *D. dendriticum* and *D. ditremum* plerocercoids in *Salmo trutta* and *Salvelinus alpinus* at Llyn Padarn, Wales, for the years 1963 to 1965. In this time there were no changes in the numbers

TABLE 1

Studies on seasonal occurrence of larvae of cestodes listed in the climate zones of the World (see map Fig. 1, Chubb, 1977). The species are in alphabetical order.

Climate zones	Cestode species	Host species	Locality	References
1. Tropical				
1a. RAINY (humid climate)		no seasonal studies	tropical forest	
1b. SAVANNA (humid climate)			tropical grassland	
	Ilisha parthenogenetica	*Hilsa ilisha*	River Hooghly, India	Pal (1963)
1c. HIGHLAND (humid climate)		no seasonal studies	tropical highland	
1d. SEMI-DESERT (dry climate)		no seasonal studies	hot semi-desert	
1e. DESERT (dry climate)		no seasonal studies	hot desert	
2. Sub-tropical				
2a. MEDITERRANEAN			scrub, woodland, olive	
	Ligula intestinalis	*Rutilus rutilus*	Lake Paleostomi, Georgia, U.S.S.R.	Cernova (1975)
		Gobio gobio lepidolaemus	Lake Skadar, Yugoslavia	Kažić *et al.* (1977)
	Triaenophorus nodulosus	*Esox lucius* *Tinca tinca*	Lake Piediluco, Italy	Aisa (1975)
2b. HUMID		no seasonal studies	deciduous forest	
3. Mid-latitude				
3ai. HUMID WARM SUMMERS			temperate grassland, mixed forest	
	Bothriocephalus sp.	*Lepomis macrochirus*	ponds, Albemarle County, Virginia, U.S.A.	Bogitsch (1958)

Dilepis unilateralis	*Cyprinus carpio*	Ivano-Frankov District, Ukraine, U.S.S.R.	Sapozhnikov (1975)
Diphyllobothrium latum	*Esox lucius* *Lota lota* *Perca fluviatilis*	Lausanne and Neuchâtel, Switzerland	Janicki and Rosen (1917)
Gryporhynchus sp.	*Cyprinus carpio*	Western districts of Ukraine, U.S.S.R.	Ivasik (1953)
Ligula intestinalis	*Alburnus bipunctatus* *Leuciscus cephalus*	Central and Eastern Balkan mountains, Bulgaria	Kakacheva-Avramova (1973)
	Scardinius erythrophthalmus	River Danube, Bulgaria	Kakacheva-Avramova (1977)
	Alburnus alburnus	Iskar Dam Lake, Bulgaria	Kakacheva-Avramova and Naidenov (1974)
	Abramis brama	Dubossary Reservoir, Moldavia, U.S.S.R.	Marits and Tomnatik (1971)
	Abramis brama *Rutilus rutilus*	near Warsaw Lake Wigry, Poland	Ruszkowski (1926)
	Rutilis rutilus	Old gravel pit, Košice, Czechoslovakia	Žitňan (1964)
	Alburnus alburnus *Leuciscus cephalus* *Rutilus rutilus*	Lake Leman (Geneva), Switzerland	Zschokke (1884)
Schistocephalus solidus	*Gasterosteus aculeatus*	Near Warsaw, Poland	Ruszkowski (1926)
Triaenophorus nodulosus	*Perca fluviatilis*	Lipno Reservoir, River Vlatava, Czechosolvakia	Ergens (1966)
	Salmo trutta	Germany	Scheuring (1923)
	Perca fluviatilis	River Svratka, Czechoslovakia	Vojtkova (1959)
	Perca fluviatilis *Esox lucius*	Lake Leman (Geneva), Switzerland	Zschokke (1884)

TABLE 1 (*continued*)

Climate zones	Cestode species	Host species	Locality	References
3aii. HUMID COOL SUMMERS			temperate grassland, mixed forest	
	Bothriocephalus sp.	*Perca flavescens*	Lake Opeongo, Ontario, Canada	Cannon (1973)
	Digramma interrupta	*Abramis brama*	Kutuluks Reservoir, U.S.S.R.	Dubinina (1953)
		Abramis brama	Kutuluks Reservoir, U.S.S.R.	Kosheva (1956)
		Carassius auratus gibelio *Carassius carassius*	Lake Bol'shoe, Omsk Region, U.S.S.R.	Lyubina (1970)
	Dilepididae species undetermined	*Lepomis gibbosus*	Ryan Lake, Ontario, Canada	Cone and Anderson (1977)
	Diphyllobothrium dendriticum	*Gasterosteus aculeatus* *Pungitius pungitius*	River Chernaya, near River Neva, U.S.S.R.	Banina and Isakov (1972)
	Diphyllobothrium latum	*Esox lucius*	River Volga, U.S.S.R.	Bogdanova (1958)
		Lucioperca lucioperca *Perca fluviatilis*	Rybinsk Reservoir, U.S.S.R.	Izyumova (1958)
		Gymnocephalus cernua	Rybinsk Reservoir, U.S.S.R.	Izyumova (1959)
		Esox lucius	Rybinsk Reservoir, U.S.S.R.	Izyumova (1960)
		Esox lucius *Lota lota* *Perca fluviatilis*	Lake Võrtsjärv, Estonia, U.S.S.R.	Tell (1971)
		Esox lucius *Lota lota*	River Kokemäenjoki, Finland	Wikgren (1963)
	Diphyllobothrium oblongatum	*Coregonus artedii*	Beaver Island archipelago, Lake Michigan, U.S.A.	Thomas (1949)

Diphyllobothrium species	*Salvelinus namaycush*	Lakes, Algonquin Park, Ontario, Canada	Freeman and Thompson (1969)
	Salvelinus namaycush	Lakes, Algonquin Park, Ontario, Canada	MacLulich (1943)
Eubothrium juveniles	*Gymnocephalus cernua*	Rybinsk Reservoir, U.S.S.R.	Izyumova (1959)
Ligula intestinalis	Cyprinidae	Central Ural and Transural Regions, U.S.S.R.	Deksbakh and Shchupakov (1954)
	Notropis cornutus frontalis	Wolf Lake, New York, U.S.A.	Dence (1958)
	Abramis brama	Kutuluks Reservoir, U.S.S.R.	Dubinina (1953)
	Abramis brama	Rybinsk Reservoir, U.S.S.R.	Izyumova (1958)
	Rutilus rutilus	Rybinsk Reservoir, U.S.S.R.	Izyumova (1959)
	Blicca bjoerkna	Rybinsk Reservoir, U.S.S.R.	Izyumova (1960)
	Abramis brama *Alburnus alburnus* *Blicca bjoerkna*	Estonian lakes, U.S.S.R.	Voore (1950)
Paradilepis scolecina	*Tinca tinca*	River Donets, U.S.S.R.	Komarova (1957)
Schistocephalus pungitii	*Pungitius pungitius*	River Chernaya, near River Neva, U.S.S.R.	Banina and Isakov (1972)
Schistocephalus solidus	*Gasterosteus aculeatus*	River Chernaya, near River Neva, U.S.S.R.	Banina and Isakov (1972)
Triaenophorus amurensis	Cyprinidae	River Amur, U.S.S.R.	Kuperman (1973b)
Triaenophorus crassus	Coregonidae	Lake Ladoga, Rybinsk Reservoir, U.S.S.R.	Kuperman (1973b)

TABLE 1 (*continued*)

Climate zones	Cestode species	Host species	Locality	References
3aii. (*continued*)				
	Triaenophorus nodulosus	*Esox lucius*	River Volga, U.S.S.R.	Bogdanova (1958)
		Lucioperca lucioperca *Perca fluviatilis*	Rybinsk Reservoir, U.S.S.R.	Izyumova (1958)
		Gymnocephalus cernua	Rybinsk Reservoir, U.S.S.R.	Izyumova (1959)
		Perca fluviatilis	Rybinsk Reservoir, U.S.S.R.	Kuperman (1973a)
		Perca fluviatilis	Lake Mälaren, Sweden	Lawler (1969a)
		Perca fluviatilis	Lake Dusia, Lithuania, U.S.S.R.	Rautskis (1970a)
		Esox lucius	Lake Dusia, Lithuania, U.S.S.R.	Rautskis (1970b)
		Perca fluviatilis	Lake Galstas, Obelija and Shlavantas, Lithuania, U.S.S.R.	Rautskis (1977)
		Esox lucius *Lota lota* *Perca fluviatilis*	Lake Võrtsjärv, Estonia, U.S.S.R.	Tell (1971)
	Triaenophorus orientalis	*Perccottus glehni*	River Amur, U.S.S.R.	Kuperman (1973b)
3aiii. EAST COAST			temperate grassland, mixed forest	
	Bothriocephalus sp.	*Perca flavescens*	Lake Oneida, New York, U.S.A.	Noble (1970)
	Digramma interrupta	*Carassius carassius*	Lake Ōnuma, Hokkaido, Japan	Awakura *et al.* (1976)
	Diphyllobothrium sebago	*Osmerus mordax*	Rangeley Lakes, Maine, U.S.A.	Meyer (1972) Meyer and Vik (1972)
	Ligula intestinalis	*Perca flavescens*	Lake Oneida, New York, U.S.A.	Noble (1970)

3b. MARINE WEST COAST			temperate grasslands, deciduous forest	
	Diphyllobothrium dendriticum	*Salmo trutta*	Reservoirs, South Wales	Duguid and Sheppard (1944)
		Salmo trutta	Yeo Reservoir, Blagdon, Somerset, England	Fraser (1951)
		Salmo gairdneri	British Isles	Fraser (1960a)
		Salmo trutta		
		Salmo trutta	Poulaphouca Reservoir, Eire	Hickey and Harris (1947)
		Gasterosteus aculeatus	Hamburg area, Germany	Kuhlow (1953c)
		Salmo trutta	Ånøya Water System, Trøndelag, Norway	Vik (1957)
		Salvelinus alpinus		
	Diphyllobothrium ditremum	*Salmo trutta*	Llyn Celyn, Wales	Aderounmu (personal communication)
		Salmo trutta	Llyn Tegid, Wales	Aderounmu (personal communication)
		Thymallus thymallus	Llyn Tegid, Wales	Chubb (1961)
		Salmo trutta	Poulaphouca Reservoir, Eire	Hickey and Harris (1947)
		Osmerus eperlanus	Hamburg area, Germany	Kuhlow (1953b)
		Salmo trutta	Llyn Padarn, Wales	Powell (1966)
		Salvelinus alpinus		
	Diphyllobothrium ursi	*Oncorhynchus nerka*	Karluk Lake area, Kodiak Island, Alaska, U.S.A.	Rausch (1954)
	Diphyllobothrium vogeli	*Gasterosteus aculeatus*	Wedeler Marsh and River Elbe, Germany	Kuhlow (1953b)
			River Elbe, Germany	Vik *et al.* (1969)

TABLE 1 (*continued*)

Climate zones	Cestode species	Host species	Locality	References
3b. (*continued*)				
	Diphyllobothrium species	*Perca fluviatilis*	Llyn Tegid, Wales	Andrews (1977b)
		Salmo gairdneri	Goodwin Lake, Washington State, U.S.A.	Becker (1967)
		Oncorhynchus kisutch *Salmo clarki* *Salmo gairdneri* *Salvelinus fontinalis*	Goodwin, Shoecraft and Silver Lakes, Washington State, U.S.A.	Becker and Brunson (1967)
		Salmo trutta	Loch Leven, Scotland	Campbell (1974)
		Salmo clarki *Salmo gairdneri*	Silver Lake, Washington State, U.S.A.	Mamer (1978)
		Salmo trutta	Dunalastair Reservoir, Scotland	Robertson (1953)
	Ligula intestinalis	*Rutilus rutilus* other Cyprinidae	Ravensthorpe Reservoir, Northampton Thrybergh Reservoir and Yeadon Tarn, Yorkshire, England	Arme and Owen (1968)
		Phoxinus phoxinus	Frongoch Lake, Wales	Bibby (1972)
		Alburnus alburnus	River Thames, England	Harris and Wheeler (1974)
		Scardinius erythrophthalmus	Ravensthorpe Reservoir, Northampton, England	Orr (1966)

	Phoxinus phoxinus *Rutilus rutilus*	Milngavie Reservoir, Hogganfield Loch, near Glasgow, Scotland	Orr (1967a)
	Gobio gobio *Leuciscus leuciscus* *Rutilus rutilus* *Scardinius erythrophthalmus*	Ravensthorpe Reservoir, etc., Northampton, England	Orr (1968)
	Rutilus rutilus	England	Owen and Arme (1965)
	Rutilus rutilus	gravel pit, Yateley, Hampshire, England	Sweeting (1976)
	Rutilus rutilus	Yeadon Tarn, near Leeds, Yorkshire, England	Sweeting (1977)
Schistocephalus solidus	*Gasterosteus aculeatus*	clay pit, near Farnley, Leeds, Yorkshire, England	Arme and Owen (1964, 1967)
	Gasterosteus aculeatus	Pond, Baildon Moor, Yorkshire, England	Chappell (1969)
	Gasterosteus aculeatus	Pond, Stourton, Leeds, England	Clarke (1954)
	Gasterosteus aculeatus	Flat Pond, Shoulder of Mutton Pond, England	Dartnall (1972)
	Gasterosteus aculeatus	Vartry Reservoir, County Wicklow, Eire	Hopkins (1950)
	Gasterosteus aculeatus	lake, Hunslet, Yorkshire, England	Hopkins and Smyth (1951)
	Gasterosteus aculeatus trachurus	Lake Alouette, Lake Haig-Gunflint, British Columbia, Canada	Lester (1971)

TABLE 1 (*continued*)

Climate zones	Cestode species	Host species	Locality	References
3b. (*continued*)				
		Gasterosteus aculeatus	Baltic Sea, Poland	Markowski (1933)
		Gasterosteus aculeatus	London area, England	Meakins (1974a)
		Gasterosteus aculeatus	Priddy Pool, Somerset, England	Pennycuick (1971a, b, c, d)
		Gasterosteus aculeatus	Ånøya Water System, Trøndelag, Norway	Vik (1954)
	Triaenophorus nodulosus	*Perca fluviatilis*	Llyn Tegid, Wales	Andrews (1977a, b)
		Perca fluviatilis	Bogstad Lake, near Oslo, Norway	Borgström (1970)
		Perca fluviatilis	Llyn Tegid, Wales	Chubb (1964)
		Gasterosteus aculeatus	Flat Pond, England	Dartnall (1972)
		Perca fluviatilis	Serpentine, London, England	Lee (1977)
		Perca fluviatilis	Bogstad Lake, near Oslo, Norway	Lien (1970)
		Perca fluviatilis	Shropshire Union Canal, Cheshire, England	Mishra (1966)
		Perca fluviatilis	Rostherne Mere, Cheshire, England	Rizvi (1964)
		Cottus gobio	River Avon, Hampshire, England	Rumpus (1975)
		Perca fluviatilis	Lake Dargin, Poland	Wierzbicki (1970, 1971)
	Valipora campylancristrota	*Cyprinus carpio*	Fish ponds, Chojnów Region, Poland	Jara and Olech (1964)
3c. SEMI-DESERT			prairie and steppe	
	Digramma interrupta	*Abramis brama*	Kakhovsk Reservoir, U.S.S.R.	Kamenskii (1962, 1964)
		Blicca bjoerkna		
		Abramis brama	Dnepr Delta, U.S.S.R.	Komarova (1964)
		Abramis brama	Mingechaur Reservoir, Azerbaidan, U.S.S.R.	Mikailov (1963)
		Varicorhinus capoëta		

	Ligula intestinalis	*Abramis brama* *Abramis brama* *Blicca bjoerkna* *Carassius carassius* *Rutilus rutilus*	Volga Delta, U.S.S.R. Kakhovsk Reservoir, U.S.S.R. Iriklin Reservoir, U.S.S.R.	Dubinina (1949) Kamenskii (1962, 1964) Kashkovski (1967)
		Abramis brama	Dnepr Delta, U.S.S.R.	Komarova (1964)
		Abramis brama *Varicorhinus capoëta*	Mingechaur Reservoir, Azerbaidan, U.S.S.R.	Mikailov (1963)
		Perca flavescens	Deer Creek Reservoir, Utah, U.S.A.	Pitt and Grundmann (1957)
	Ligulidae	*Cyprinus carpio* *Ctenopharyngodon idella* *Hypophthalmichthys molitrix*	Fish farms, Akhtarskii Estuary, Azov Sea, U.S.S.R.	Denisov and Vorob'ev (1974)
	Triaenophorus meridionalis	*Esox lucius* *Silurus glanis* *Mesogobius batrachocephalus* *Neogobius kessleri*	Rivers Dnepr, Kuban, U.S.S.R.	Kuperman (1973b)
3d. DESERT		no seasonal studies	cool desert	
3e. SUB-POLAR			coniferous forest	
	Diphyllobothrium dendriticum	*Coregonus migratorius*	Lake Baikal, Siberia, U.S.S.R.	Chizhova and Gofman-Kadoshnikov (1960)
		Salvelinus alpinus	Lake Bjellojaure, Sweden	Henricson (1977, 1978)
	Diphyllobothrium ditremum	*Salvelinus alpinus*	Lake Bjellojaure, Sweden	Henricson (1977, 1978)
	Diphyllobothrium latum	*Esox lucius*	Lake Winnipeg, Canada	Nicholson (1932)
		Esox lucius *Lota lota* *Perca fluviatilis*	Lake Konche, Karelia, U.S.S.R.	Malakhova (1961)
	Diphyllobothrium species	*Coregonus artedii*	Cold Lake, Alberta, Canada	Leong (1975)

TABLE 1 (*continued*)

Climate zones	Cestode species	Host species	Locality	References
3e. (*continued*)				
	Ligula intestinalis	*Catostomus commersoni* *Notropis hudsonius*	Heming Lake, Manitoba, Canada	Lawler (1964)
		Esox lucius *Perca fluviatilis* *Rutilus rutilus*	Lake Konche, Karelia, U.S.S.R.	Malakhova (1961)
		Abramis brama	Lake Ubinsk, Siberia, U.S.S.R.	Titova (1957)
	Triaenophorus crassus	Coregonidae	Lesser Slave Lake, Alberta, Canada	Miller (1943b, 1952)
	Triaenophorus crassus	Coregonidae	River Yenisei, Siberia, U.S.S.R.	Kuperman (1973b)
	Triaenophorus nodulosus	*Perca flavescens*	Heming Lake, Manitoba, Canada	Lawler (1969b)
		Lota lota *Perca fluviatilis*	Lake Konche, Karelia, U.S.S.R.	Malakhova (1961)
		Lota lota maculosa	Lesser Slave Lake, Alberta, Canada	Miller (1945a)
		Esox lucius	River Bol'shaya Rechka, U.S.S.R.	Pronin *et al.* (1976)
	Triaenophorus stizostedionis	*Percopsis omiscomaycus*	Lesser Slave Lake, Alberta, Canada	Miller (1945c)
4. Polar				
4a. POLAR			tundra	
	Triaenophorus crassus	Coregonidae	River Anadyr, Chukotka, U.S.S.R.	Kuperman (1973b)
4b. ICE-CAPS	no suitable habitats for freshwater cestodes		icefields and glaciers	
5. Mountain	no seasonal studies		heath, rocks and scree	

of plerocercoids in *S. alpinus*, but in *S. trutta* there was a fall in numbers of worms and the proportion of dead ones increased. These authors suggested that this might indicate an increasing immunity of these *S. trutta* to *D. dendriticum* and *ditremum*.

The growth of the plerocercoids in *Salmo trutta* has been studied experimentally by Halvorsen (1970). At 13–15°C there was a first and slow post-invasion phase of growth which lasted 30 to 50 days. During this period, major development of main nerve cords, osmoregulatory vessels and musculature occurred. In the next 200 days, there was little histological change, but growth in length was rapid. After this time, formation of genital primordia and segmentation of the plerocercoid took place and growth was slower again. Halvorsen concluded that the sizes and states of development which were reached within 1 year during his experiments at 15°C, indicated that the large plerocercoids found in *S. trutta* in natural infections were at least 2–3 years old.

The transfer of plerocercoid stage I from *Gasterosteus aculeatus* to *Salmo trutta* was noted earlier. Halvorsen and Wissler (1973) experimentally fed plerocercoids from *Salmo trutta* to *Salmo gairdneri* at 10°C. Of 100 plerocercoids, five were recovered from the body cavity and six from the stomach wall of *S. gairdneri*. The longer plerocercoids re-established most successfully. However, overall only 7·5% of *D. dendriticum* were successfully transferred, compared with a 50·1% transfer of *D. latum* plerocercoids. Such transfers are therefore unlikely to be of much significance in the life cycle of *D. dendriticum*.

Chizhova and Gofman-Kadoshnikov (1960) summarized the dynamics of a natural focus of *Diphyllobothrium dendriticum* at Lake Baikal, Siberia, U.S.S.R. The principal definitive hosts were Laridae (50% infection) but other hosts included man. In this instance *Coregonus migratorius* was the most important second intermediate host, and this fish provided the reservoir of infection through the year. It is noteworthy that in Lake Baikal only the shallow biotopes allowed the completion of the life cycle. In the deep open lake conditions were such that not all stages could be completed.

In colder areas where the definitive hosts have seasonal migrations (see also Henricson, 1978, above) it is clear that these hosts would only be infected, and would only excrete eggs into the ecosystem, on a restricted seasonal basis. However, in more temperate regions, especially in coastal areas, Laridae may be present all year. In the Hamburg area, Germany, Kuhlow (1953c) found 5·2% of *Larus ridibundus* infected in the winter months 1949–1952. However, from November to January, no diphyllobothrian adults were found, but infections occurred in February (6%), March and April (about 20% in each). The reason for the appearance of adult worms during these latter months was related to the presence of plerocercoids in *Gasterosteus aculeatus*, and the fact that these fishes became available as food for the gulls during the months from February onward. The adult *D. dendriticum* survived in the definitive host for less than 1 year. Kuhlow (1953c) reported his longest experimental infection as 4 months, with an immunity to further establishment of *D. dendriticum* in the same hosts which persisted for at least 6 months.

Buck *et al.* (1976) at the Bass Island region of Western Lake Erie, Ohio, U.S.A., examined 33 *Larus argentatus* from June to November. *Diphyllobothrium dendriticum* were found in late October to November, and they concluded that these infections were in migrant gulls, and not therefore, acquired in their study area.

In summary, it is clear from the evidence reported for occurrence of *Diphyllobothrium dendriticum* in the definitive hosts that there is significant variation in time of presence of adult worms. These differences will influence the period of availability of eggs in the ecosystem, thereby influencing presence of coracidia, procercoids and recruitment into the plerocercoid populations in the fish hosts. However, such variations will not affect the presence of a reservoir of potentially invasive plerocercoids which will occur through all seasons of the year in the intermediate fish hosts.

Diphyllobothrium ditremum (Creplin, 1825)

Diphyllobothrium osmeri (Linstow, 1878) is treated as a synonym of *D. ditremum* following Bylund (1971, 1973, 1975a), Chubb (1968), Halvorsen (1970) and Wikgren (1964). The adult worms occur naturally in diving birds, *Gavia arctica*, *Mergus merganser*, *Phalacrocorax aristotelis* and *P. carbo*, and *Ardea cinerea* (Markowski, 1949; Vik, 1964b; Halvorsen, 1970).

In many localities in north-western Europe *Salmo trutta* and *Salvelinus alpinus* carry heavy mixed infections of plerocercoids of both *Diphyllobothrium dendriticum* and *D. ditremum* (see for instances: British Isles, Duguid and Sheppard, 1944 and Powell and Chubb, 1966; Norway, Vik, 1957 and Halvorsen, 1970; Sweden, Henricson, 1977, 1978).

The life cycle of *Diphyllobothrium ditremum* has been demonstrated experimentally by Bylund (1973). In this study it was shown that procercoids from *Cyclops strenuus* were invasive to *Salmo trutta*. At 16–18°C by 18 days after infection some plerocercoids were enclosed in thin connective tissue capsules. After 45 days almost all of the plerocercoids were surrounded by capsules of a thickness found in natural infections and by 153 days the plerocercoids had reached a length of 5·5 mm in saline, which corresponded to about 10 mm length if relaxed in cold water. It is relevant to note that Kuhlow (1953b) could not infect *Gasterosteus aculeatus* with procercoids of *D. ditremum*, also from *C. strenuus*.

The plerocercoids of *Diphyllobothrium ditremum* survive in the fish hosts for a number of years. Accordingly, examination of fishes through the year in a habitat will show irregular fluctuations in incidence, these variations representing differences in the infection levels of individual fish and differences in the ages (lengths) of the fishes sampled. The overall trend is for increasing incidence and intensity of occurrence with increasing age (see Henricson, 1977). Such patterns of occurrence through the year have been reported in the following hosts: *Salmo trutta*, Llyn Celyn, Wales (Aderounmu, personal communication), Llyn Padarn, Wales (Powell, 1966) and Llyn Tegid, Wales (Aderounmu, personal communication); *Salvelinus alpinus*, Lake Bellojaure, Sweden (Henricson, 1978), Llyn Padarn, Wales (Powell, 1966); and *Thymallus thymallus*, Llyn Tegid, Wales (Chubb, 1961). Similar patterns of occurrence are almost certain to occur in coregonid fishes (Kozicka, 1958) and *Osmerus*

eperlanus (Kuhlow, 1963b; Voigt, 1975), but have not been studied through the year.

The dynamics of the population of *Diphyllobothrium ditremum* infecting *Salvelinus alpinus* in Lake Bellojaure, Sweden, have been studied by Henricson (1978). His work on this species was not as detailed as that on *D. dendriticum*, for instance the lengths of the plerocercoids of *D. ditremum* were not measured. Nonetheless, he was able to conclude that the seasonal pattern of intensity and overdispersion of infection seemed to approximate to that of *D. dendriticum* (described p. 16). The lowest level of infection occurred in spring, explained by the deaths of *S. alpinus* heavily infected by both species of *Diphyllobothrium*. As with *D. dendriticum*, Henricson suggested that the critical point in the life cycle of *D. ditremum* was the timing of the *Cyclops* population in relation to the availability of coracidia larvae, thereby determining success or failure of a generation at each period of invasion. In fish of 6+7+ ages there was an increase in infection level in 1969–70, and peak years were 1970 and 1971. After this there was a significant decline in infection in the age group 6+7+. In Lake Bellojaure, the potential definitive host was *Gavia arctica*, which arrived at ice break-up in June and left in September.

Fluctuations within populations of *Diphyllobothrium ditremum* plerocercoids were also reported by Powell and Chubb (1966) at Llyn Padarn, Wales. A census was carried out in the spring of each year, 1963 to 1965. In *Salvelinus alpinus*, the incidences (and intensities) were: 1963, 100% (66·2); 1964, 100% (24·3); 1965, 93% (36·5); whereas in *Salmo trutta* values were: 1963, 77% (84·6); 1964, 76% (38·3); 1975, 65% (3·1). Thus during these 3 years the populations in *S. alpinus* remained relatively stable, but those in *S. trutta* fell dramatically. Powell and Chubb suggested that an immunity had developed in *S. trutta*, as the proportion of dead plerocercoids had increased. However, Halvorsen (1970) favoured the view that such variations were likely to occur owing to fluctuations in the biotic and abiotic factors influencing all stages in the life cycle.

It is noteworthy that *Diphyllobothrium ditremum* plerocercoids occurred in *Salvelinus alpinus* in a water course at Finse, in arctic Norway (Halvorsen, 1970). According to Halvorsen, the water temperature in Finsevatn rarely exceeded 10°C and the lake was free of ice for only 3–4 months a year. He concluded that *D. ditremum* might be more successful than *D. dendriticum* at near arctic conditions, probably because its eggs, procercoids and plerocercoids were capable of developing at lower temperatures.

Wikgren (1966a, b) experimented with the growth of plerocercoids of *Diphyllobothrium ditremum* in Hanks' medium. The mitotic activity was very temperature-sensitive, and the generation times, which equalled the time needed by the cell population to double in number and hence was also approximately equal to the time needed by the plerocercoid to double in mass, were determined at a range of temperatures. The generation times were: 10°C, 960 h; 20°C, 252 h; 30°C, 91 h; 38°C, 33 h (Wikgren, 1966b). The calculated growth rates indicated that the development from procercoid to full-grown plerocercoid might last about 6–7 months at 10°C, but only 20 days at 20°C (Wikgren, 1966a).

Halvorsen and Wissler (1973) attempted to transfer plerocercoids of *Diphyllobothrium ditremum* by experimental feeding from *Salvelinus alpinus* to *Salmo gairdneri*. Only one larva (0·09%) re-established in *S. gairdneri*, therefore they concluded that *D. ditremum* could not use paratenic hosts to any significant extent. Variations of temperature, 4°C, 10°C and 15°C, did not affect this conclusion.

It was noted above that in northern conditions, as at Lake Bjellojaure, Sweden (Henricson, 1978), and in many parts of Norway (Halvorsen, 1970) the definitive hosts were present at habitats for only part of each year, from ice break-up in spring until ice formed again in the autumn. Such a seasonal presence of these birds, together with the low water temperatures of winter, will tend to produce a marked seasonal cycle of invasion and generations of worms. It is probable, although it has not been demonstrated, that in the milder conditions of, for example, the British Isles, in many years the life cycle of *Diphyllobothrium ditremum* could continue through the winter. Thus at Llyn Padarn and Llyn Tegid, Wales, *Phalacrocorax carbo* remain through the year, while water temperatures normally fall to only 4–5°C in shallow water and ice-cover is unusual (Chubb, personal observations). Although the rate of growth of all larval stages would be slower than at summer temperatures, nonetheless growth, and probably also transmission of *D. ditremum* from host to host, could occur.

Diphyllobothrium latum (Linnaeus, 1758)

The excellent book of von Bonsdorff (1977) provides much interesting information about this species. Criteria for the recognition of the species of *Diphyllobothrium* in man, including *D. latum*, are still in the process of evaluation, as demonstrated by the recent paper by Kamo (1978), and this is important as man can serve as a host for species other than *D. latum*.

According to von Bonsdorff (1977) the hatching season for the eggs of *Diphyllobothrium latum* was most intense from May to October, but it apparently persisted through the year. Embryonic development ceased at 0°C, and was inhibited at temperatures below 8°C. Optimum development occurred at about 18–20°C and death occurred at 35°C (Neu'min, 1952; Guttowa, 1961). It has been claimed that the eggs can remain viable for up to 3 years at a temperature somewhat below 0°C (see von Bonsdorff, 1977). Light was essential for hatching (Guttowa, 1961) and other factors including oxygen levels were important. Optimum temperatures for the coracidia were 18–20°C, full range for development 8–30°C (Guttowa, 1961).

The work of Janicki and Rosen (1917) demonstrated the necessity for a copepod intermediate host ocntaining the procercoid. Rosen (in Janicki and Rosen, 1917) infected *Cyclops strenuus* and *Diaptomus gracilis* with coracidia on 24 June and obtained fully developed procercoids in 2–3 weeks. Janicki (in Janicki and Rosen, 1917) found very young *D. latum* larvae in *Perca fluviatilis* stomach walls at the end of April to the end of May in Switzerland. Morozova (1955, quoted from Kuperman, 1973b) obtained invasive procercoids at 14–20°C in 14–16 days. Guttowa (1961) obtained similar results, and determined that Calanoida were preferred hosts rather than Cyclopoida. The copepod hosts fell into three categories, easily invaded, principal hosts,

less successfully invaded, auxiliary hosts and species that could not become infected. Guttowa found different copepod species served as first intermediate host in varying ecological conditions and geographical areas. von Bonsdorff (1977) provided lists of the copepod hosts.

The copepod fauna along the River Svir and its tributaries, U.S.S.R., were examined by Razumova and Gutkovskaya (1959). The copepods achieved maximum abundance during July and August, and were less numerous in the spring than the autumn. *Cyclops strenuus* were present during spring to autumn. Eggs of *Diphyllobothrium latum* were found only in small numbers, and were most numerous in the spring and beginning of the summer.

The presence of procercoids in *Cyclops strenuus* and *Thermocyclops oithonoides* in Lake Karperö, Finland, was studied between 25 May and 11 July by Guttowa (1963). In this habitat a high incidence of *Diphyllobothrium latum* procercoids in the copepods clearly revealed the patterns of occurrence. The highest incidence was in the littoral zone. The eggs accumulated during the autumn and winter. A rise in water temperature above 8°C in May caused a mass hatching of coracidia in the latter third of May, resulting in peak procercoid occurrence in mid-June. By the end of June the incidence fell, probably to achieve a stability thereafter through the summer until the autumn, when water temperatures fell below 8°C again and no further hatching of the eggs could occur. Thereafter the eggs passed into the waters of the lake would accumulate once again until the following spring.

The plerocercoids of *Diphyllobothrium latum* have been reported from a wide range of hosts. Experimentally procercoids have been used to infect *Esox lucius*, *Gasterosteus aculeatus*, *Gymnocephalus cernua* and *Perca fluviatilis* (Kuhlow, 1953b, 1955) and *Pungitius pungitius* (Gofman-Kadoshnikov *et al.*, 1963). Two types of plerocercoids occur, plerocercoid stage I (acaudate bothrio-pleroceroid I following the terminology of Freeman, 1973) which invade the fishes as a procercoid, and plerocercoid stage II (acaudate bothrio-plerocercoid II) which invade the fish hosts as a plerocercoid stage I (Vik, 1964a). In general, therefore, stage I plerocercoids occur in small fishes and others feeding on plankton, and stage II plerocercoids in piscivores especially *Esox lucius*, *Lota lota* and large *Perca fluviatilis*. Kuhlow (1955) administered plerocercoids orally to *Esox lucius*, *Gymnocephalus cernua*, *Perca fluviatilis*, *Rutilus rutilus* and *Salmo gairdneri* and all re-established. Halvorsen and Wissler (1973), at 4°C, obtained a 50·1% re-establishment of plerocercoids from *E. lucius* and *P. fluviatilis* which were fed to *Lota lota*. Borroni and Grimaldi (1974) ascribed the plerocercoids in the muscles of *P. fluviatilis* in Lake Maggiore, Italy, to the primary ingestion of procercoids, and the plerocercoids in the viscera to the piscivorous habits of the larger *P. fluviatilis* thereby leading to the secondary establishment of plerocercoids. In *Lota lota* plerocercoids occurred only in the viscera, and this was related to the piscivorous feeding of this species. Grimaldi (1974) also described this phenomenon, which was originally proposed by Hobmaier (1927).

Wikgren (1963), however, considered that there might be problems in this transfer of plerocercoids from plankton-eating to piscivorous fishes. He

examined the distribution ratio, number of plerocercoids in viscera to number in muscle, in *Esox lucius* and *Lota lota* in 23 samples taken during a 12 month period. These ratios showed a difference between spring and autumn: *E. lucius*, spring 2 : 6, autumn 1 : 9; and *L. lota*, spring 1 : 5, autumn 0 : 5. He suggested that these differences might be due to seasonal variations in the feeding habits of the fishes. Thus *L. lota* feed more actively during the winter than during the summer. Or, Wikgren suggested, it was also possible that low temperatures retarded the movements of the plerocercoids and that they accumulated in the organs of the body cavity during the winter. Wikgren stated that further investigations were needed.

Kuhlow (1953b) found a slow development of the plerocercoids of *Diphyllobothrium latum*. At 6 months of age they were scarcely 1 cm long. Invasiveness on dogs and human volunteers was tested at 70 days, 3, 5½ and 7 months, but adult worms were not obtained. The plerocercoids were histologically and morphologically completely developed at 2–7 months. However, Kuhlow (1955) fed a 3½-month-old plerocercoid from *Gymnocephalus cernua* to a man and obtained an adult worm.

Nicholson (1932), at Lake Winnipeg, Canada, found that the plerocercoids in *Esox lucius* in summer were numerous, active and crawled vigorously. However, the larvae from winter fish, collected March, April and May, were very small, degenerated and broke-up on moving with a probe. He suggested that invasion of the fishes occurred in summer, but that the worms died after 5–6 months. According to Nicholson, further evidence that the worms died was the fact that 25–45 cm *E. lucius* harboured plerocercoids, but fish longer than 60 cm contained few plerocercoids, with no trace of past infections. Experimental invasions of plerocercoids fed to dogs showed that in January–February only 27% of the motile larvae developed to adult worms, whereas in August 60% were successfully established.

The occurrence of the plerocercoids of *Diphyllobothrium latum* through the year has been shown in a variety of fish species and localities. Even though Nicholson (1932) claimed a life of only months for the plerocercoids in *Esox lucius*, it is generally accepted that they accumulate with increasing host age (von Bonsdorff, 1977). One example is quoted: Tallqvist (1965), at Lake Iisvesi, Finland, found these incidences in relation to age of *Perca fluviatilis:* 2–3, 32·2%; 4–5, 42·7%; and 5 years plus 62·2% incidence. Therefore some of the incidences quoted below vary somewhat through the year, as the samples of fishes contained a number of different age groups. Thus Tell (1971), at Lake Võrtsjärv, Estonia, U.S.S.R., found a uniform incidence and intensity the whole year round in *Esox lucius* (45%), *Lota lota* (72·5%) and *Perca fluviatilis* (8%). Izyumova (1958, 1959, 1960) at the Rybinsk Reservoir, U.S.S.R., gave the following incidences (and intensities): *E. lucius*, winter 36·5% (1–5), spring 66·6% (1–3), summer 33·3% (1–4) and autumn 25% (2–9); *Gymnocephalus cernua*, winter 2·2% (1), spring and summer zero incidences, and autumn 6·7% (1); *Lucioperca lucioperca*, January–April 3·7% (1), May–August 3·7% (1), October–November 0; *P. fluviatilis*, January–April 11·5% (2); May–July 13·5% (2), October–November 10·0% (7). Much higher incidences and intensities (average, range) were reported by Malakhova (1961)

from Lake Konche, Karelia, U.S.S.R.: *E. lucius*, autumn 81·8% (8·3, 1–57), winter 67·5% (10·8, 1–33), spring 87% (15·6, 1–141) and summer 89% (10·7, 1–34); *L. lota*, autumn 51·2% (8·6, 1–43), winter 54·6% (9, 1–63), spring 70·2% (83, 1–138) and summer 24% (7·2, 2–19); *P. fluviatilis*, autumn 12·2% (1·4, 1–4), winter 30·5% (1·3, 1–3), spring 33·3% (1·2, 1–3) and summer 23·3% (1·4, 1–3). The above figures also indicate that in any one habitat there is considerable variation, maintained through the seasons, in the level of incidence and intensity of infection of the various host species.

Kuperman (1977), at the Rybinsk Reservoir, U.S.S.R., reported that between 1966 and 1976 the level of infections of *Esox lucius*, *Gymnocephalus cernua*, *Lota lota*, *Lucioperca lucioperca* and *Perca fluviatilis* remained the same. However, in the Gorkov region of the U.S.S.R., *Diphyllobothrium latum* was rare until after the opening of the Gorkov Reservoir, when it spread rapidly through the human and fish populations (Romanov, 1964).

Diphyllobothrium oblongatum Thomas, 1946

A synonym of *Diphyllobothrium dendriticum* according to Markowski (1949) but not according to Kuhlow (1953b). It is considered as a separate entity here until critical studies have resolved its status. The plerocercoids occurred in *Coregonus artedii* and the adult tapeworms in *Larus argentatus* at Lake Michigan, U.S.A. Natural infections in *L. argentatus* were recovered in July (Thomas, 1946).

Eggs collected from fledgling *Larus argentatus* hatched in 8 days at 22–29°C, and both cooled and frozen eggs hatched at the appropriate rate when warmed. Procercoids were obtained in *Diaptomus oregonensis*; after 2 weeks they were invasive, depending on temperature. Typical plerocercoids were developed in *Poecilia reticulata* 135 days post-infection. Adult worms were reared in *L. argentatus* and *L. delawarensis* (Thomas, 1947).

The following seasonal relationships were proposed by Thomas (1949) for the circulation of *Diphyllobothrium oblongatum* in the Beaver Island archipelago at Lake Michigan. Ephemeropteran and trichopteran emergents during June and July coincided with the appearance of *Coregonus artedii*, near the surface but over deep water, and the development of young Laridae, which were fed coregonids by their parents. *D. oblongatum* was shown experimentally to mature in young laboratory hatched and reared gulls and *Hydroprogne caspia* in 3½–4 weeks, after which time they were usually eliminated. *D. oblongatum* developed in birds on their nesting sites and were shed in the waters around the islands in late July and August. Water currents tended to confine the proglottids to that area. During August and September, the coregonids retired into deep water and both young and old birds dispersed in numbers to inland lakes. The coregonids spawned in shoals about the islands in October and November and their spawn hatched in spring. Experiments with *D. oblongatum* eggs indicated that they could withstand freezing for at least 1 month and remain viable at 2°C for 5 years. According to Thomas (1949), a continual source of invasion was therefore present for *Diaptomus oregonensis*, which was one of the chief foods of the coregonids. The procercoid from the copepod became an encapsulated plerocercoid in about 1–2 months in the stomach and mesenteries of the fishes.

Diphyllobothrium sebago (Ward, 1910)

This species is also treated as a separate entity here, but it is probable that future critical studies will reduce it to a synonym of *Diphyllobothrium dendriticum*. However, Meyer and Robinson (1963), who provided details of the anatomy of both adults and plerocercoids of *D. sebago*, considered it close to but different from *D. dendriticum*, chiefly on the basis of comparison with Kuhlow's material (1953a, b, c).

Meyer and Vik (1963) completed all stages in the life cycle of *Diphyllobothrium sebago* commencing with eggs obtained from experimentally infected *Mesocricetus auratus*. Eggs incubated at 20–22°C hatched in 12–17 days, but only 10% viability was obtained compared with 94% in eggs from Laridae. At 25–28°C, hatching started in 9 days. *Cyclops bicuspidatus thomasi*, *C. scutifer* and *C. vernalis* infected by coracidia and procercoids which developed at room temperature, had cercomers in 13 days. *Salvelinus alpinus* was used as the fish host. Plerocercoid growth was slow during the first 113 days, at a water temperature near 1°C, but increased rapidly during the next 60 days when the water temperature rose to about 9°C. The plerocercoid worms gave F1 adults in *M. auratus*, *Larus argentatus* and a kitten.

The development of the eggs of *Diphyllobothrium sebago* was studied in natural conditions by placing them in covered jars filled with lake water, which were then suspended at various depths down to 27·5 metres in Branch Lake, Maine, U.S.A. (Meyer, 1967). Within a range of temperature of 9 to 20°C and down to the depth of 27·5 m the percentage of eggs hatching increased with time, temperature being the most important factor. Fig. 1 summarizes the results obtained. Meyer concluded that regardless of where the eggs were deposited, within the temperature and depth ranges he investigated, it appeared that there was juxtaposition in habitat between the coracidia and the required copepods.

Johnson (1975) searched incoming water at a fish hatchery in Maine, U.S.A., for the natural infections of the copepod hosts of *Diphyllobothrium sebago*. Some 17 000 copepods of 11 species were examined. Of almost 200 *Cyclops scutifer*, 1·7% had procercoids in July. Some fry of *Salmo salar* (landlocked populations) first became invaded by procercoids during the same month.

It is evident from the experiments of Meyer and Vik (1963) that much of the plerocercoid growth will occur in summer at higher water temperatures. Meyer and Vik (1968) showed that at Rangeley Lake, Maine, U.S.A., the incidence and mean intensity of occurrence of the plerocercoids of *Diphyllobothrium sebago* increased with host size in *Salmo salar*, suggesting that the plerocercoids probably had a longevity greater than 1 year. They would thus be potentially available to infect the definitive hosts at all seasons.

Vik and Meyer (1962) examined more than 250 *Larus argentatus* for the occurrence of adult *Diphyllobothrium sebago*. In data collected between April and December, they found a seasonal incidence of adult worms, independent of host age or the seasonal condition of the plerocercoids. Meyer and Vik (1972) provided more details. At the Rangeley Lakes, Maine, U.S.A., the overall incidence of adult worms in *L. argentatus* from April

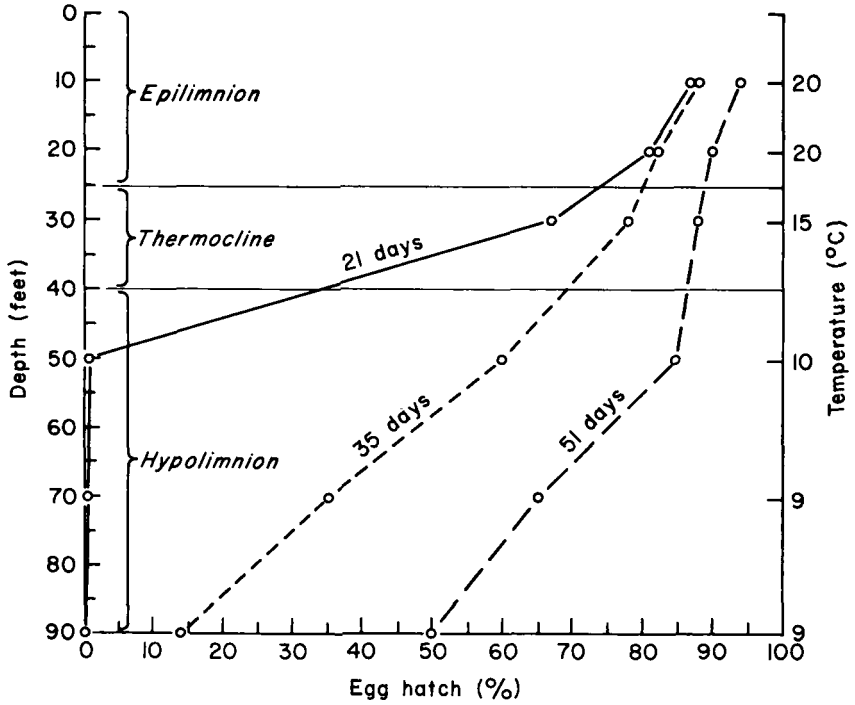

FIG. 1. Percentage of *Diphyllobothrium sebago* eggs hatching in jars in Branch Lake, Maine, U.S.A., versus temperature, depth and time. [The experiment is described fully in the text and the figure was reproduced from Meyer (1967), Fig. 1, p. 241.]

to November was 39%, but the highest was in June (77%) and through the summer months (67%). These seasonal changes were attributed to the availability of *Osmerus mordax* containing plerocercoids and the short life-span of adult *D. sebago* in the *L. argentatus*. At the Rangeley Lakes *L. argentatus* arrived early in April, following the departure of winter, and remained until ice formed again about mid-November. The peak population was nearly 100 birds, in June and July. They were seen to feed by scavenging. *O. mordax* was probably the most important host for the plerocercoids to achieve completion of the life cycle, and were an available food for the birds in spring and early summer. These fishes normally inhabited deeper parts of the lakes, but spawned in the tributaries, at night, in late April and May. A heavy post-spawning mortality on their return to the lake provided the chief source of plerocercoids for *L. argentatus*, hence the maximal occurrence of adult *D. sebago* in June. Meyer (1972) has summarized the seasonal circulation pattern of *D. sebago* in the conditions of western Maine.

Diphyllobothrium ursi Rausch, 1954

Rausch (1954) described *D. ursi* from *Ursus arctos middendorfii* and *U. americanus*. In the Karluk Lake area, Kodiak Island, Alaska, U.S.A., the plerocercoids of *D. ursi* occurred in *Oncorhynchus nerka*. These fishes were an

important item in the diet of *U. arctos middendorfii* in July, August and to a lesser extent September. Large numbers of plerocercoids were ingested by the bears at this time, and the adult worms rapidly attained sexual maturity as depleted pieces of strobila appeared in the faeces by 1 August, and in increasing quantities as the season progressed. At about 1 September, when berries became available, the bears left the streams to feed on vegetation; however, some *O. nerka* were eaten until December. In autumn, large numbers of *D. ursi* were seen in bear faeces, with vegetation. It was not clear whether or not the bears lost all the adult worms before winter, but bears examined in June (from a different watershed) were not infected.

During the period that the bears were feeding on *Oncorhynchus nerka* their faeces fell into the streams and Rausch (1954) considered that the life cycle was probably completed in Karluk Lake, which had an abundant copepod fauna. *O. nerka* spent 3 years in this lake, largely feeding on zooplankton, before 2–3 years at sea. The plerocercoids must have a longevity such as to allow them to invade the bears that eat the adult fishes after their return from the sea.

Diphyllobothrium vogeli Kuhlow, 1953

Kuhlow (1953b) found the plerocercoids of this species in the liver of *Gasterosteus aculeatus* at the Wedeler Marsh, River Elbe, Germany. Adult worms were obtained experimentally in *Larus ridibundus*. At laboratory temperatures, the first eggs hatched on day 9, and *Cyclops strenuus* were infected. The plerocercoids in *G. aculeatus* were found in winter (River Elbe) and spring (Wedeler Marsh), but not in summer (River Elbe) (Kuhlow, 1953b) and spring (River Elbe) (Vik *et al.*, 1969).

Chubb (1968) suggested that the adult worms described as *D. vogeli* by Kuhlow (1953b) were primary strobilas of *D. ditremum*. Halvorsen (1970) also tentatively considered that *D. vogeli* was a synonym of *D. ditremum*. However, Bylund (1975a, b, c) validated *D. vogeli* as a distinct species and carried out further experimental studies. Subsequently other work has confirmed this view (Freze *et al.*, 1974; Gur'yanova *et al.*, 1976; Bylund and Djupsund, 1977; Freze, 1977; Bylund, 1978).

Diphyllobothrium species

Seasonal data on the occurrence of unidentified or mixed populations of *Diphyllobothrium* plerocercoids have been reported as follows (the hosts and localities are shown in Table 1): Andrews (1977b), probably *D. dendriticum*; Becker (1967); Becker and Brunson (1967); Campbell (1974), probably both *D. dendriticum* and *D. ditremum*; Freeman and Thompson (1969) *D. dendriticum*, *D. ditremum* and a third species; Leong (1975); MacLulich (1943); Mamer (1978); and Robertson (1953), probably both *D. dendriticum* and *D. ditremum*. These studies are not discussed further here.

Rausch *et al.* (1967) examined the occurrence of *Diphyllobothrium* sp. in Eskimos of the lower Kuskokwim River region of western Alaska, U.S.A. Incidence was highest in the Eskimos in winter and early spring, after the period of greatest consumption of the fishes *Dallia pectoralis* and *Pungitius pungitius*. Later, in May and early June, the Eskimo diet was diversified and these fishes were no longer eaten. The observed rate of elimination of

Diphyllobothrium was lowest during August, possibly indicating that the worms were lost gradually after the period in which uncooked fishes were eaten.

(b) *Family Ligulidae*. The plerocercoids of the tapeworms included in this family are large and easily recognized; however, in many instances the species may not have been determined with accuracy. Dubinina has contributed a great deal of information about the biology of the family which is summarized in her book (Dubinina, 1966). The adult worms occur in avian hosts; lists of these, with brief outlines of the life cycles and lists of intermediate hosts can be conveniently found in McDonald (1969).

The plerocercoids can live for many years in the fish host. The plerocercoids are relatively narrowly specific parasites which occur in particular families or even genera of fishes (Dubinina, 1964a).

Ligula colymbi Zeder, 1803

The plerocercoids of this species occur primarily in fishes of the family Cobitidae (Dubinina, 1966). The development of the plerocercoid required more than 1 year (Dubinina, 1964b), so that they become invasive only in their second summer of life in the fish (Bauer, 1959).

Ligula intestinalis (Linnaeus, 1758)

The life cycle of *L. intestinalis* was first shown by Rosen (1919b).

Numerous publications refer to *Ligula intestinalis*, but it should be noted that authors do not always separate *L. colymbi*, *L. intestinalis* and *L. pavlovskii* and that in addition there are indications of other species as well (Dubinina, 1966). Further, the *Digramma* species may be reported as, or included with, *L. intestinalis*. The plerocercoids are very host specific, to fishes of the family Cyprinidae, although some exceptions are noted below.

In general *L. intestinalis*, and other Ligulidae, have a high optimum temperature for development. Normal development can occur between 10 and 32°C (Dubinina, 1965, quoted from Kuperman, 1973b; Dubinina, 1966; Bauer *et al.*, 1969) with an optimum range about 23–25°C. At 24–28°C hatching of the coracidium from the eggs occurred in 5–6 days, and at 15–18°C in 8–9 days. The emergence of the coracidia at high temperatures was more or less simultaneous, but at lower temperatures occurred over an extended period (Dubinina, 1953). The development of ligulid embryos was strongly retarded at lower temperatures, and may cease altogether, but development was resumed when temperatures rose again (Dubinina, 1965, quoted from Kuperman, 1973b).

The necessity for higher temperatures for successful completion of the life cycle of *Ligula intestinalis* is shown in several ways. Dubinina (1959a) stated that the climatic conditions of the southern latitudes of the U.S.S.R. were extremely favourable to the species, leading to epizootics among cyprinid fishes in reservoirs. More recently, Kiseliene *et al.* (1978) have suggested several factors that favour the development of foci of Ligulidae, including a water temperature above 10°C for 9½ months in the year. Studies on helminths in lakes and reservoirs with thermal pollution from power stations have also shown that in the heated areas there were increases in abundance of *L. intestinalis* (Pojmańska, 1976; Pojmańska *et al.*, 1978), due

to, at least in part, the speeding up of the reproductive processes (Markevich *et al.*, 1976). Bauer and Solomatova (1978) noted that in heated reservoirs during the winter, fishes infected with plerocercoids of Ligulidae were crowded into the heated zone.

Reshetnikova (1969) has examined the effects of wind, temperature and water level over a period of years on the incidence of infection of Ligulidae in *Abramis brama* in a reservoir.

Sedinkin (1975) examined potential copepod intermediate hosts for *Ligula* and *Digramma* in Lake Batush, U.S.S.R., during the summer of 1969. He found no infected crustaceans, and he attributed this absence to the coolness of the summer during that year. It is clear, however, that invasion of fishes by procercoids will occur during the warmer months, probably mainly during summer. Young of the year fishes became infected at this time (see for instance, Arme and Owen, 1968; Denisov and Vorob'ev, 1974; Sweeting, 1976). At Thrybergh Reservoir, Yorkshire, England, Arme and Owen (1968) found that invasions became established in many fishes before the age of 3 months. In some years, by September, more than 50% of the fry of the current season were infected. Most fishes were invaded during their first year of life, a smaller number during their second, and relatively few thereafter, although the plerocercoids will then persist for the remaining life span of the fish (Arme and Owen, 1968; Harris and Wheeler, 1974; Owen and Arme, 1965; Sweeting, 1976). It is of interest to note that Arme and Owen (1968) found that no fish exceeding 7 cm in length harboured small parasites (less than 10 mg) unaccompanied by much larger worms. This suggested that the presence of long-established infections rendered the adult fish more susceptible to invasion than similar non-infected individuals.

The diet of the fish hosts is important to allow the invasion by procercoids from copepods. High incidences in young fishes were related to their predominantly copepod diet (Arme and Owen, 1968; Harris and Wheeler, 1974; Sweeting, 1976).

Changes in behaviour of fishes infected by *Ligula intestinalis* have been noted. Differences in the host pituitary were shown and spawning was prevented (Kerr, 1948). Thus samples of spawning fishes contained only a low incidence of *L. intestinalis*, whereas samples of the general population had a high incidence (Dence, 1958; Orr, 1966). The behaviour was also different in non-spawning host fishes. Dence (1958), in infected *Notropis cornutus frontalis*, observed that heavily infected fishes migrated shoreward throughout summer and especially in calm weather. The infected fishes left the uninfected shoals when they became too cumbersome and became solitary, or remained in small groups of about 12. Their movements were sluggish and capture was easily achieved. Similar changes were seen by Harris and Wheeler (1974) in *Alburnus alburnus*, and a deformation of the vertebral column was noted in this instance. These infected fishes were more readily available for predation by prospective avian definitive hosts.

Many authors have provided seasonal data for occurrence of plerocercoids in fishes. These include the following (for hosts and localities see Table 1): Arme and Owen (1968), Bibby (1972), Cernova (1975), Deksbakh and

Shchupakov (1954), Dence (1958), Dubinina (1949), Harris and Wheeler (1974), Izyumova (1958, 1959, 1960), Kakacheva-Avramova (1973, 1977), Kakacheva-Avramova and Naidenov (1974), Kamenskii (1962, 1964), Kashkovski (1967), Kažić *et al.* (1977), Komarova (1964), Lawler (1964), Malakhova (1961), Marits and Tomnatik (1971), Mikailov (1963), Sweeting (1976), Titova (1957), Voore (1950), Zschokke (1884). However, in many instances it is difficult to determine the exact significance of seasonal variations unless information related to age-classes of fishes was also provided. Nevertheless, in general, it can be concluded that *Ligula intestinalis* plerocercoids are potentially available for invasion of definitive hosts at all times of the year. Ice, or migrations of these hosts, will impose seasonal limitations, which will vary from place to place according to geographic location.

It was noted earlier that the plerocercoids can survive for the life of the host individual. However, some authors have implied a shorter life of some 2–3 years (Deksbakh and Shchupakov, 1954; Kosheva, 1956) for the plerocercoid. It seems probable, in most instances, that the life of the plerocercoid is determined by the length of survival of the host. This period of survival may be short, owing to mortality of the host caused by stress, or to predation by piscivorous fishes or birds. Harris and Wheeler (1974) suggested that with *Ligula intestinalis* infections in *Alburnus alburnus* in the River Thames, England, the fall in incidence and intensity they observed between July and September was due to a substantial proportion of the young fishes succumbing to predators or dying from other causes during the autumn. They also considered that many of the I and II year parasitized *A. alburnus* died during the winter following ingestion of the procercoids. Sweeting (1976) also observed a similar decline in 0+ *Rutilus rutilus* in a gravel pit at Yateley, Hampshire, England. Incidence fell from 92% in April to 25% by January of the following year, and this fall was accompanied by a reduction in worm burden. If, as Dubinina (1953, 1964a, 1966) has found, the plerocercoids required 12 to 14 months to become invasive, then many of the plerocercoids must be lost before they have reached their invasive condition. In a tropical fish, *Danio malabaricus*, maintained at 25°C, plerocercoids were obtained with genital primordia after 60 days (Orr and Hopkins, 1969b), so that it is probable that the potential to invade the definitive host will be reached after different lengths of time, determined by host factors, season of invasion and temperature regimes in the particular water-body and year.

Annual fluctuations and also marked regional differences in incidence of *Ligula intestinalis* were found by Lawler (1964) in Heming Lake, Manitoba, Canada. The plerocercoids were most common from a creek flowing into the lake where a pair of *Mergus merganser* (definitive hosts) nested. The more heavily infected fishes were obtained from an area of the lake that was highly productive in yield of both plankton and fish. Other irregular fluctuations of occurrence of *L. intestinalis* are reported. Sweeting (1977) found no fry of *Rutilus rutilus* during 2 years of study at Yeadon Tarn, near Leeds, Yorkshire, England, and this was attributed to the high incidence of plerocercoids, which prevented the fishes breeding. The population of *R. rutilus* fell dramatically in spring 1969, so much so as to cause suspension of further

sampling. Orr (1967a) noted that in Hoggenfield Loch, near Glasgow, Scotland, only older fishes were infected which indicated that the life cycle of the parasite was no longer being completed, whereas in Milngavie Reservoir in the same area, fry were infected so that he concluded transmission to the fishes was occurring. Orr (1968) emphasized this point again by stating that, owing to the longevity of the plerocercoids, infected fishes would be found for some years after cycling transmission had ceased.

It was indicated earlier that *Ligula intestinalis* was specific to Cyprinidae. However, *L. intestinalis* is reported in *Perca flavescens* from some North American habitats (see Noble, 1970; Pitt and Grundmann, 1957). High incidences were observed in both localities (see Table 1). Orr (1967b) reviewed the geographical distribution of *L. intestinalis* and suggested that the plerocercoids from *P. flavescens* were distinct physiologically, if not morphologically. Dubinina (1966) certainly considered the possibility of further species of *Ligula*, currently included within the species *L. intestinalis*.

Transplants of plerocercoids from one host species to another can help demonstrate physiological specificity. Sweeting (1977) successfully transplanted *Ligula intestinalis* plerocercoids from *Rutilus rutilus* to *R. rutilus*, *Carassius auratus* and *Gobio goibo* (all Cyprinidae), but not into *Leuciscus cephalus* (Cyprinidae), *Esox lucius* (Esocidae) or *Perca fluviatilis* (Percidae).

Parasite specific antibodies were demonstrated for *Ligula intestinalis* and *Digramma interrupta* plerocercoids in the blood of *Abramis brama* by an agar-gel-diffusion test by Molnár and Berczi (1965). Although *A. brama* was capable of developing these specific antibodies against Ligulidae, they appear to play no part in the seasonal dynamics of these worms. Sweeting (1977), using *L. intestinalis*-infected *Rutilus rutilus*, was unable to demonstrate precipitating antibodies in the serum using Ouchterlony double-diffusion techniques. Polyacrylamide-gel electrophoresis showed an increase in the quantity of gammaglobulin fraction in infected compared with uninfected *R. rutilus*.

Jones (1953) studied the scales of *Rutilus rutilus*. A narrow ring band was formed which was not a spawning mark. It was found in the process of formation on the scales of fishes that had immature gonads and were not going to spawn that year, and also on scales of fishes parasitized by *Ligula intestinalis*, which would not spawn. Scale erosion was seen on spawning *R. rutilus*, but was absent from the scales of immature fishes taken during the spawning season and afterwards, and also from the scales of fishes parasitized by *L. intestinalis*.

There is virtually no specificity at the definitive host phase in the life cycle of *Ligula intestinalis* (Dubinina, 1966). In avian hosts maturity was reached in 45–50 h, and the worms were eliminated after 95–100 h (Dubinina, 1953). The eggs passed from definitive hosts during the cold months of the year will normally survive to hatch after the return of water temperatures of 10°C or above (Dubinina, 1966).

Digramma interrupta (Rudolphi, 1810)

The species of *Digramma* are frequently included with *Ligula* in survey studies (see, for instance, plerocercoids, Kamenskii, 1962, 1964; adult worms,

Zhatkanbaeva, 1969). The life cycle is similar to that of other Ligulidae (Dubinina, 1966). The plerocercoids reached an invasive condition in 12–14 months (Dubinina, 1953). The plerocercoids probably live for the life span of the host. In the Tsumlyansk Reservoir, U.S.S.R., the incidence increased with host age up to 5 years, but gradually decreased thereafter, until *Abramis brama* aged 8–9 years were uninfected (Reshetnikova, 1965). Dubinina (1964b) has suggested that *D. interrupta* may comprise two species, one in *Carassius* and *Cyprinus* (subfamily Cyprinini) and one in *A. brama* and other species (subfamily Leuciscini).

Sexual maturity of a plerocercoid of *Digramma interrupta* in *Carassius auratus gibelio* was achieved experimentally by Dubinina (1960). The water temperature in an aquarium was gradually raised to above 35°C, to approach 37°C, the temperature of the definitive host, during the last 4 days. The fish died of peritonitis, but the worm contained normal eggs in its middle section.

Almost all seasonal studies of the plerocercoids are from the U.S.S.R. As is expected with a larval parasite with a life span of more than 1 year, seasonal variations in incidence were not marked, for example, at the Kakhovsk Reservoir, in *Abramis brama*, April–June 8·9%, July–August 8·6% and September–October 4·2%, and in *Blicca bjoerkna*, April–June 5·0%, July–August 4·5% and September–October 3·2% (Kamenskii, 1964). Other seasonal records can be found in Komarova (1964), Kosheva (1956), Lyubina (1970) and Mikailov (1963) (hosts and localities, see Table 1).

In Japan, Awakura *et al.* (1976) (as *Digramma alternans*) have reported that *Carassius carassius* in Lake Ōnuma, Japan, had a low incidence of plerocercoids in spring (May) and a high incidence by autumn (November). Young plerocercoids, less than 1 g in weight, were observed mid-July and late November. They suggested that invasion of the fishes by procercoids occurred from early summer to late autumn.

Schistocephalus pungitii Dubinina, 1959

The plerocercoids were specific to *Pungitius pungitius* and reached an invasive condition after 2 months development (Dubinina, 1959b, 1964b). Kafanov and Yazykova (1971) reported an epizootic focus of *S. pungitii* in Lake Manych-Gudilo, southern U.S.S.R. Adult worms occurred in *Gelochelida nilotica*, *Larus argentatus cachinnans* and *Sterna hirundo*, plerocercoids in *Pungitius platygaster* and procercoids in *Diaptomus salinus*. The seasonal dynamics of *S. pungitii* are likely to be similar to *S. solidus*. Banina and Isakov (1972) studied *Gasterosteus aculeatus* and *Pungitius pungitius* parasites in the area of the delta of the River Neva, U.S.S.R., *S. pungitii* was found once in a *P. pungitius* in October.

Schistocephalus solidus (Müller, 1776)

The taxonomy of the genus was clarified and discussed by Dubinina (1959b, 1966). The life cycle was considered in detail by Clarke (1954) and Dubinina (1957).

Clarke (1954) collected his material from a pond at Stourton, Leeds, Yorkshire, England. He found the procercoids present in *Cyclops* species from spring to autumn, but was of the opinion that the procercoids did not act as a reservoir of invasion for *Gasterosteus aculeatus* throughout the

winter. However, Pennycuick (1971b), at Priddy Pool, Somerset, England, suggested that new invasions occurred in December, January, February, March and April, although the rapid and large rise in level of infection, representing heavy invasion, occurred from the end of July to September. This peak period of invasion corresponded to peak feeding by *G. aculeatus*, perhaps also with maximum levels of abundance of *Cyclops* infected with procercoids. Pennycuick (1971b) commonly found *Cyclops* in the fish stomachs, but Clarke (1954) observed that *Cyclops* was almost a casual item of diet in his population of *G. aculeatus*. Hopkins and Smyth (1951) also demonstrated that the peak period of invasion occurred during the summer months in a small lake, Hunslet, Yorkshire, England, whereas Meakins and Walkey (1973) suggested that in their material from the London area the majority of invasions occurred in the spring. It is not unexpected to find peak invasions during the warmer months of the year, as both host activity and parasite growth are near optimum at higher temperatures. In the relatively mild winter conditions of the British Isles invasion of *G. aculeatus* can clearly occur through some winters, although this is unlikely to occur in those regions that have much colder winters.

As is expected for a larval parasite that survives for the life span of its host, the plerocercoids occur in *Gasterosteus aculeatus* all year. Hopkins (1950), at Vartry Reservoir, County Wicklow, Eire, found that the incidence of *Schistocephalus solidus* fell from November 1948 onward to a low level,to increase again the following spring. Small plerocercoids were collected January–April, fresh weight rarely exceeding 120 mg, whereas in the summer and autumn of 1948 they were larger, often 200 mg or more. Clarke (1954) found the mean weight of the plerocercoids was high in August, fell to January, increased from January to October, to decrease again from October to November. Arme and Owen (1964, 1967) at a small pond near Leeds, Yorkshire, England, found that all mature *G. aculeatus* were infected by *S. solidus* in June 1962, but that both incidence and intensity of infection declined during 1963, so that by the last quarter of 1964 only 35% were infected. Chappell (1969) found no obvious seasonal incidence in a pond at Baildon Moor, also in Yorkshire, except that in September incidence was significantly lower. This represented young fishes recently acquired into the population, with many uninvaded at that time. Pennycuick (1971b), in Somerset, also found no very marked seasonal patterns of incidence or intensity of *S. solidus* infections. She observed some evidence of longer term cyclical fluctuations in *G. aculeatus* and *S. solidus* populations at Priddy Pool, similar to those of Arme and Owen (1967).

Pennycuick (1971b) explained the cyclical fluctuations in the following manner. The infection tended to build up in *Gasterosteus aculeatus* until many fishes died or were eaten by predators, or were prevented from breeding. This caused a gradual decline in the numbers of fishes in the population, and hence in the size of the population of plerocercoids of *Schistocephalus solidus*. As the number of fishes decreased and the infection became lighter, breeding was more successful and the numbers of fishes increased, which in turn, enabled the *S. solidus* infection to build up once more. These fluctuations

would be modified by other external factors, such as food supply, winter temperature, abundance of the other host species in the life cycle, etc. (Pennycuick, 1971b).

Other reports of seasonal occurrence of plerocercoids of *Schistocephalus solidus* are listed in Table 1.

Infections were found to be similar in male and female *Gasterosteus aculeatus*, although the weights of the *Schistocephalus solidus* plerocercoids were greater in female fishes. Overall, infection increased with host length (Pennycuick, 1971d).

Vik (1954) observed an epizootic of *Gasterosteus aculeatus* caused by *Schistocephalus solidus* in August at a water temperature of 25°C in the Ånøya Water System, Trøndelag, Norway. Pennycuick (1971d), at Priddy Pool, noted that between November 1967 and April 1968 *G. aculeatus* 3 and 4 years old with large numbers and weights of plerocercoids were dying. Pennycuick (1971a) stated that plerocercoids did not die inside *G. aculeatus*, but Vik (1954) found dead *S. solidus* in the abdominal cavities of these fishes in the Ånøya Water System. Vik postulated the existence of an immune reaction in *G. aculeatus*.

No seasonal variations in the glycogen reserves of the plerocercoids were found by Hopkins (1950). Glycogen was fairly constant through the year at about 16% of the fresh weight of the worms.

The presence of plerocercoids of *Schistocephalus solidus* in adult *Gasterosteus aculeatus* delayed the onset of maturation of the host gonads and influenced reproductive behaviour (Arme and Owen, 1964, 1967; Pennycuick, 1971a; Meakins, 1974a). Gametogenesis might be delayed or prevented (Pennycuick, 1971a) and host courtship altered so that spawning was unlikely (Meakins, 1974a). Uninfected fishes bred in April–May, those infested in May–July (Pennycuick, 1971a).

Clarke (1954), Arme and Owen (1967), Meakins and Walkey (1975) and Lester (1971) observed that *Gasterosteus aculeatus* infected by *Schistocephalus solidus* behaved differently from uninfected individuals. All these workers noted that the infected fishes swam more slowly and tended to be nearer the water surface. At Lake Aloutte and other localities in British Columbia, Canada, Lester (1971) caught the infected fishes in shallow water at creek mouths. On hot days, their sluggish movement at the surface led him to infer that this behaviour related to changed respiratory requirements. He tested this hypothesis and found that the respiration rate of infected fishes was higher than an estimated value based on fish tissue alone, and the difference was presumably due to the parasites. Lester (1971) also observed evidence that the infected and uninfected populations became segregated within the habitats he investigated.

Meakins (1974b) studied the bioenergetics of the host–parasite system, *Gasterosteus aculeatus*–plerocercoid *Schistocephalus solidus*, at 15°C. Meakins and Walkey (1975) found that in winter (February) at routine and maximum levels of activity *G. aculeatus* infected with *S. solidus* consumed more oxygen than did uninfected fishes, whereas in summer (August) the level of respiration was higher in unparasitized fishes. Walkey and Davies (1964) had shown the

same phenomenon for oxygen consumption of plerocercoids removed from winter *G. aculeatus* compared with those from summer fishes. These differences were explained by thermal acclimatization, those worms acclimatized to the low temperature respiring more rapidly than plerocercoids adapted to the high temperature when both were maintained at an intermediate temperature.

Dubinina (1957) showed experimentally that the plerocercoids were invasive to the definitive host only when they had a fully formed genital system. Details of the morphology of the plerocercoids after 72, 112, 145 and 177 days of development were given by Dubinina (1959b). At 112–114 days, although the plerocercoids were fully segmented, they were not invasive to experimental definitive hosts, but from 177 days onwards, they were capable of successful invasion. Dubinina carried out her study at laboratory temperatures.

Hopkins and McCaig (1963) proposed that the changes in weights of the plerocercoids during their development in the fish host showed two phases of growth (see Fig. 2). On entry to the fish they estimated that the procercoid weighed about 0·3 μg. A synthesis of both somatic and genital tissues

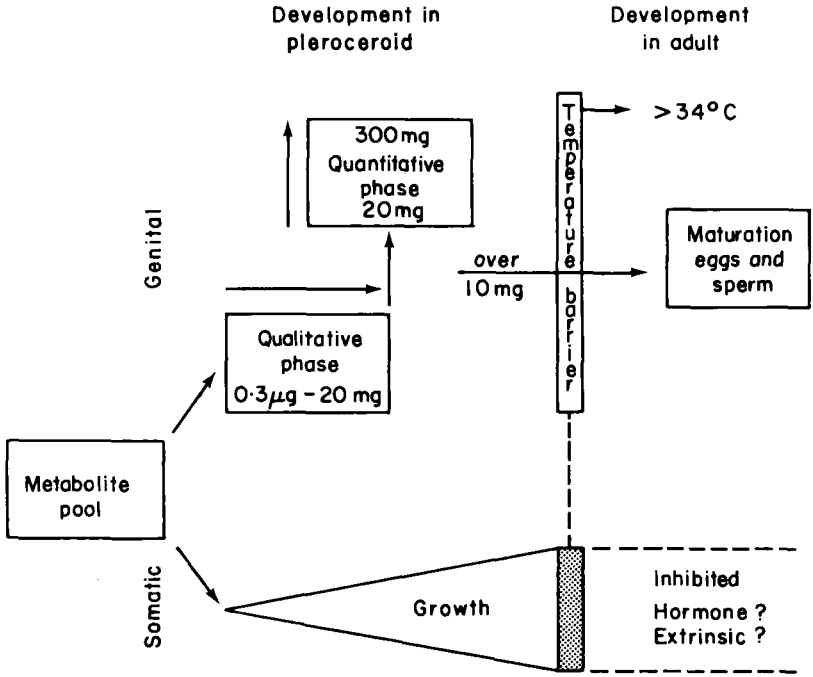

FIG. 2. The pattern of development in *Schistocephalus solidus* during the plerocercoid and adult phases. The course of development and suggested control mechanisms are detailed in Hopkins and McCaig (1963). [Reproduced from Hopkins and McCaig (1963), Fig. 1, p. 242.]

occurred in the plerocercoid. Genital development involved two phases, a qualitative one during which the various rudiments were differentiated, and a quantitative one, during which these rudiments increased in size but showed little further progress towards maturation. The final phases, oogenesis, vitellogenesis and spermatogenesis were inhibited by the low temperatures of the fish host, but occurred at the high temperatures of the definitive host. At the onset of maturation growth ceased, perhaps due to an internal hormone mechanism acting as feed-back from the genitalia, or extrinsic factors such as absence of suitable metabolites, or some other unknown reason.

Sinha and Hopkins (1967) measured the growth rate of plerocercoids *in vitro* at a range of temperatures between 4 and 40°C. Between 7 and 23°C the rate of growth increased nearly exponentially with a Q_{10} of 3·6, and between 23 and 27°C the growth rate was maximal. At above 27°C, and after 8 days in culture, the weight increase was shown only by worms too small to mature. A temperature of 35°C for 4 days induced function of genitalia and suppressed somatic growth; however, if the worm was returned to 23°C the somatic growth was resumed. Sinha and Hopkins (1967) proposed an hypothesis for a two-enzyme system of control, one with peak efficiency of about 23°C controlling somatic growth and the other with peak efficiency near 40°C controlling genital maturation.

Orr and Hopkins (1969a) also described the laboratory maintenance of the life cycle of *Schistocephalus solidus*. At 19°C, the plerocercoids formed genital rudiments at 6–7 weeks after invasion of *Gasterosteus aculeatus*, and in exceptional instances, were capable of maturing in the definitive host after 8 weeks, but normally full invasive potential was not reached until about 12 weeks. Earlier, Hopkins and McCaig (1963) had shown that success of establishment of plerocercoids increased with weight, which reflected their increasing genital development. The establishment successes (as percentages) were: below 10 mg fresh weight, 3%; 10–30 mg, 40%; above 30 mg, 80%.

Bråten (1966) demonstrated by surgical-transfer techniques that *Schistocephalus solidus* was specific to *Gasterosteus aculeatus*. In all other fish species he used normal growth of the plerocercoid did not occur, and death resulted in 2–10 days.

Orr *et al.* (1969) used procercoids of *Schistocephalus solidus* to carry out experimental invasions of *Barbus* 'Schuberti', *Gasterosteus aculeatus* (freshwater and marine forms) and *Pungitius pungitius*. No infections were obtained in *Barbus* 'Schuberti'. In *P. pungitius* growth occurred at first, slower than in *G. aculeatus*, but this ceased after 6–8 days and the plerocercoids were dead by day 11 at 19·5°C and by day 14 at 10°C. A challenge invasion, following two previous invasions given 14 and 28 days before, became established in *G. aculeatus* but in *P. pungitius* rejection of the plerocercoids occurred in 3 to 5 days. An immune response was proposed, with the formation of antibodies against *S. solidus* by *P. pungitius*.

B. CLASS EUCESTODA

1. *Order Trypanorhyncha*

(a) *Family Pterobothriidae*

Pterobothrium filicolle (Linton, 1889)

Pal (1963) reported this species, as *Syndesmobothrium filicolle*, from *Hilsa ilisha* in the estuary of the River Hooghly, India. Capsules containing the plerocercoids were found in all fishes examined during September 1962. The monsoon month of August, and the late winter months, February–March, also showed more or less heavy infections by these capsules. The adult worms are in selachians.

2. *Order Dilepididea*

(a) *Family Dilepididae*

Dilepis unilateralis (Rudolphi, 1819)

The adult worms occur in herons (Ardeidae). Sapozhnikov (1975) found infections of the cysticercoid stage all year in *Cyprinus carpio* in the Ivano-Frankov District of the Ukraine, U.S.S.R. A 100% incidence, with an intensity of 1–270 cysticercoids per host, was present in September and October. Group 0 fishes became invaded mainly in April and May when infected *Cyclops* and *Diaptomus* were present in large numbers. In small fry the incidence and intensity of infection was generally low. The cysticercoids often overwintered in the fishes.

Gryporhynchus sp.

Presumably a species of *Neogryporhynchus* Baer and Bona, 1960. The larva of *Gryporhynchus pusillus* Nordmann, 1832 from *Tinca tinca* corresponds to *Neogryporhynchus cheilancristrotus* (Wedl, 1855) according to Baer and Bona (1960). The life cycle of *N. cheilancristrotus* was studied by Jarecka (1970b) who was unable to prove whether or not the fish was an obligatory host in the life cycle.

Ivasik (1953) investigated the occurrence of *Gryporhynchus* larvae in *Cyprinus carpio* in fish farms in western districts of the Ukraine, U.S.S.R. Low incidences (and average intensities) were found in 0+ fishes: spring, 0·09% (7); summer, 0; autumn, 0·2% (5·5); winter, 0·9% (5); higher levels of infection in 1+ fishes: spring, 1·3% (2); summer, 3·6% (10·4); autumn, 0·4% (10); winter, 0; but no infections at all in 2+ or 4–5+ *C. carpio*.

Paradilepis scolecina (Rudolphi, 1819)

Cysticercus dilepidis was regarded as the larva of *P. scolecina* by Dubinina (in Bykhovskaya-Pavlovskaya *et al.*, 1962). *P. scolecina* occurs as an adult worm in Pelecaniformes (see McDonald, 1969). The life cycle of *P. scolecina* was examined by Jarecka (1970b). She was unable to prove that fishes were obligatory hosts in the life cycle.

Komarova (1957) found low incidences of *Cysticercus dilepidis* in *Tinca tinca* in the River Donets, U.S.S.R., from 1·6 to 3·3%. Fishes were examined

in April, July, October and December of 1952 and 1953, and *C. dilepidis* was found in each of these months, except for October 1952.

Valipora campylancristrota (Wedl, 1855)

Jarecka (1970a) showed that the life cycle of this species required two intermediate hosts, the first a copepod *Eudiaptomus graciloides*, and the second cyprinid fishes. The larva in the fishes was termed a plerocercus, and it occurred in the gall bladder.

Jara and Olech (1964) studied the dynamics of *Cysticus dilepidis campylancristrotae* in *Cyprinus carpio* in fish ponds in the Chojnów Region, Poland. The small-sized worms in gall bladders from July suggested that the fishes were invaded during their early months of life. The site of part of the infection shifted from the gall bladder to the intestine during the period summer to autumn, which indicated that the larvae might transfer from one organ to the other. The major location of the worms remained the gall bladder, however.

Dilepididae species undetermined

Cone and Anderson (1977) found these larvae in the liver or attached to the mesentery near the liver of *Lepomis gibbosus* in Ryan Lake, Ontario, Canada. Larvae in age-group I fishes moved actively within the capsules. 63% and 86% of larvae in fishes of age groups II and III respectively appeared degenerated, and all larvae in age group IV were degenerated. Young of the year *L. gibbosus* were not infected, but larvae were common in fishes of age-group I and older. Incidence and intensity in age group I was significantly higher than in all other age groups. Most of the invasion occurred during the second summer of life of the *L. gibbosus*. Thereafter a gradual degeneration of larvae occurred, reflected in the decrease in incidence and intensity of infection with increasing fish age. Some evidence that heavy invasions caused fish mortality was given.

C. CESTODE LARVA OF UNCERTAIN AFFINITY

Ilisha parthenogenetica Southwell and Prashad, 1918

Southwell and Prashad (1919) and Southwell (1930) have described the budding process shown by this larval form. Pal (1963) found *I. parthenogenetica* encapsulated in the intestines of *Hilsa ilisha* in the Hooghly Estuary, India. It occurred almost throughout the whole period of the investigation, July 1961 to December 1962. Incidences of 100% were seen during the monsoon months of July and August in both years and in September of 1962. A lower incidence prevailed during early winter, but other times of heavy infection were December 1961 and January–March 1962. These larvae predominated in the freshwater zone of the area of river investigated by Pal (1963).

D. CLASS NEMATODA, SUBCLASS ADENOPHOREA

1. *Order Enoplida*

(a) *Family Dioctophymidae.* The adult worms of the genera of the subfamily Eustrongylinae occur in fish-eating birds (Baruš *et al.*, 1978).

Eustrongylides excisus Jägerskiöld, 1909

The hosts for the adult worms are *Pelecanus crispus*, *P. onocrotalus*, *Phalacrocorax carbo*, *Ph. pygmeus*, *Ardeola bacchus* and *Nycticorax nycticorax* (Baruš *et al.*, 1978). Karmanova (1965) experimentally infected *Lumbriculus variegatus*, *Tubifex tubifex* and *Limnodrilus* sp. According to Baruš *et al.* (1978), Karmanova (1968) showed that the development of the egg of *E. excisus* to the first-stage larva occurred in water and took 21 to 30 days during summer in the Volga Delta, U.S.S.R. When the eggs containing the larvae were swallowed by the oligochaete intermediate host, development continued for 60–70 days, with two moults, by which time they were invasive for the second intermediate hosts, benthophagous fishes. In these fishes the larvae were localized in the body cavity and further growth and moulting occurred. Paratenic hosts may be found at this stage, including predatory fishes. In the avian definitive host a final moult occurred before maturity was achieved (Karmanova, 1968).

Some seasonal information about occurrence in fishes is available, but not a great deal. Molnár (1968) reported an occurrence in *Phoxinus phoxinus* from Teich (pond) T, Hungary, in January, but not again from February to December. In *Rutilus rutilus* from Lake Paleostomi, Georgia, U.S.S.R., Cernova (1975) found *E. excisus* in spring (9·1%, 1), not summer or autumn, and again in winter (6·7%, 2). It is perhaps of interest to note that Lee (1977) found *E. excisus* in *Perca fluviatilis* in the Serpentine, London, England, in all age groups of fishes, but only from October to March.

In predatory fishes, Bogdanova (1958) reported *Eustrongylides excisus* from *Esox lucius* in the River Volga, U.S.S.R., in February–March and May 1957 (6·6% in each), but not in August 1956. Dubinina (1949), in the Volga Delta, noted *E. excisus* in *Lucioperca lucioperca* in spring 1941 (6·7%), but not summer 1940 or winter 1941, and in *Silurus glanis* in summer 1940 (53·3%), winter 1941 (20%) and spring 1941 (46·7%).

Eustrongylides ignotus Jägerskiöld, 1909

Von Brand (1944) experimentally demonstrated that larval *E. ignotus* could transfer from *Fundulus heteroclitus* to other fishes; some larvae died, but many penetrated the tissues and became re-encapsulated. This demonstrated that fishes need not eat the first intermediate host, but could acquire an infection by predation on smaller fishes (see *E. excisus*, above). Winterfield and Kazacos (1977) have attributed mortality of *Ardea herodias* to the presence of numbers of adult *E. ignotus*.

Eustrongylides tubifex (Nitzsch, 1819)

Bangham (1972) noted the presence of this species as a seasonal economic factor for fishermen in Lake Erie, U.S.A. Its occurrence in the flesh of *Perca flavescens* caused fillets to be rejected. However, the worms are present in the fishes at all times of the year.

The population biology and behaviour of larval *Eustrongylides tubifex* at Lake Erie, U.S.A., have been investigated by Cooper *et al.* (1978). *Perca flavescens* were most frequently infected. Young of the year and 1+ *P. flavescens* showed significantly increasing monthly larval numbers during parts of the sampling period (July to October), but no increases were seen

in older fishes. The accumulation of worms was related to feeding habits. Infections in *Aplodinotus grunniens* and *P. flavescens* were thought to be acquired primarily by eating oligochaetes, whereas infections in *Ictalurus punctatus* and *Micropterus dolomieui* were obtained primarily by predation on smaller fishes. Transfer experiments attempted by Cooper *et al.* (1978) were invariably successful. It was suggested that *P. flavescens* obtained the third stage larvae during their first 2 years of life from oligochaetes, and that older individuals with fourth stage larvae had probably been infected for more than 1 year. The larvae were free in the mesenteries during the third stage, but encapsulated at the fourth stage. Longevity of larvae was some years. Larvae in hardened capsules were dead (for example, in *A. grunniens*). If the freshly trawled fishes were warmed to 40°C, the body temperature of the avian definitive hosts, active larval emergence from capsules resulted.

Eustrongylides species undetermined

The records given below are those in which the authors did not identify their material to specific level.

In the British Isles *Eustrongylides* sp. has been studied in *Salmo trutta*. Robertson (1953) at Dunalastair Reservoir, Scotland, found the number of worms per infected fish high in March and again in late-autumn, but stated that her figures did not suggest any seasonal cycle of infestation. At Loch Leven, Scotland, Campbell (1974) examined occurrence of *Eustrongylides* sp. from April 1967 to March 1972. Incidence did not exceed 16% until December 1967, when it increased to about 20%, with a maximum of 40% in May 1968. It continued at about 20% until January 1970, and from then was reduced, so that until the end of the survey in March 1972 it only once again exceeded the 10% level. Kennedy and Lie (1976) found *Eustrongylides* sp. in *S. trutta* in Fernworthy Reservoir, Devon, England. As elsewhere in Britain, the worm was acquired only in the lake itself, and not in the feeder streams. The larvae were found to occur especially in female *S. trutta* of length greater than 20 cm. No seasonal changes in incidence or intensity were seen over 2 years. The worms did not accumulate in older fishes, owing to the fact that in Fernworthy Reservoir the *S. trutta* were already large and near the end of their life span because of angling or natural mortality. However, a greater frequency of smaller larvae was noted in the winter months. In *Gasterosteus aculeatus* at Shoulder of Mutton Pond, England, Dartnall (1972) found incidences (and intensities) as follows: January–March, 1·4% (1); April to June, 6·4% (1); July, 9·1% (1); December, 4·9% (1).

In Europe, a few seasonal surveys provide data for *Eustrongylides* sp. larvae. Molnár (1966), at Lake Balaton, Hungary, found larvae twice in *Gymnocephalus cernua*, in July and August, but not otherwise from June 1960 to October 1961. Kažić *et al.* (1977), at Lake Skadar, Yugoslavia, found *Eustrongylides* sp. larvae in *Gobio gobio lepidolaemus* from April to October, but not in January to March, or November and December. Only a few fishes were infected with a few larvae. Similar sporadic occurrences have been noted in western Russia. Dubinina (1949) found infections in *Lucioperca lucioperca* and *Silurus glanis* in the Volga Delta, summer 1940 (6·7% and 26·7% respectively) and spring 1941 (46·7% and 20% respectively), but not during the

winter of 1941 in either host. At the Dnepr Delta, Komarova (1964) found incidences in the following hosts and months, but not otherwise: *Esox lucius*, March, 6·6%; *Lucioperca lucioperca*, June, 6·6%, July–August, 40% and October, 13·2%; *Pelecus cultratus* April–May, 5·5% and October, 13·2%; and in *Rutilus rutilus heckeli* February, 6·6% and May, 6·6%.

In Canada, at Ryan Lake, Ontario, Cone and Anderson (1977) found that *Lepomis gibbosus* of age groups III and IV were infected. Incidence remained similar before and after winter, but increased in summer. Its rarity in 0 to II age groups of fishes suggested that oligochaetes, the potential intermediate host, were not a significant item of diet for these younger fishes. The presence of larvae in the older *L. gibbosus* suggested that the worms were acquired from other fish hosts taken as prey. The *Eustrongylides* sp. larvae were acquired in summer, as the incidence in fishes of age group III was significantly higher in autumn 1975 than in those fishes of age group III the previous spring. There was no evidence of invasion during winter; food consumption was minimal, therefore the possibility of transmission greatly reduced.

A mortality of *Mergus serrator* in December 1962 at Lake Holly, Virginia, U.S.A., was attributed to adult worms of *Eustrongylides* sp. by Locke *et al.* (1964). Fifty birds died and 70 were sick. Fishes *Gambusia affinis* and *Menidia beryllina* were dead, or weakened by ice conditions, and some contained *Eustrongylides* sp. larvae. Locke *et al.* (1964) suggested that the mergansers had engorged themselves on the dead or weakened fishes. On ingestion the worms had freed themselves from the digested fishes and migrated through the vital organs, thereby causing the death of many of the birds.

Hystrichis tricolor Dujardin, 1845

The larvae occur in fishes in an unencapsulated condition, and the adult worms are found in aquatic birds. The eggs are swallowed by fresh-water oligochaetes, and in these hosts the larvae reach an invasive condition in 180–200 days. In aquatic birds the worms become adult in 25–30 days (Baruš *et al.*, 1978, quoting Karmanova, 1968, etc.). The fishes are probably accidental hosts.

Marits and Vladimirov (1969) found *Hystrichis tricolor* in *Vimba vimba vimba* natio *carinata* at the Dubossary Reservoir, Moldavia, U.S.S.R., in spring (4·5%, 1–2), but not in summer or autumn.

E. CLASS NEMATODA, SUBCLASS SECERNENTEA

1. *Order Ascaridida*

(a) *Family Anisakidae*

Anisakis species undetermined

Dubinina (1949) found an undetermined *Anisakis* sp. in *Silurus glanis* at the Volga Delta, U.S.S.R., in the spring of 1941 (6·7%), but not in the spring or summer of 1940 or winter of 1941. Bogdanova (1958) reported an occurrence of *Anisakis* sp. in *Abramis brama* from the River Volga, U.S.S.R., in February/March 1957 (6·6%), but not in July/August 1956 or in May 1957.

Contracaecum aduncum (Rudolphi, 1802)

The adult and larval stages occur in marine fishes. The larvae are found in the liver, mesenteries and muscles. Dartnall (1972) observed larvae of *Contracaecum aduncum* in *Gasterosteus aculeatus* in brackish conditions at Hadleigh Marsh, England, in May (9·1%) but not in March, October or November. In the River Danube, Bulgaria, Kakacheva-Avramova (1977) reported *C. aduncum* from the migratory *Alosa pontica pontica* in the spring and summer (30·4%, 2 to 878 larvae).

Contracaecum microcephalum (Rudolphi, 1809)

The adult worms occur in a wide range of fish-eating birds. The life cycle involves a free-living second larval stage, an obligatory period in cyclops species, followed by a second intermediate host, dragonflies, chironomids or fry of cyprinid fishes (see Baruš *et al.*, 1978).

The larvae in fishes were recorded as *Contracaecum squalii* by Bogdanova (1958), Dubinina (1949), Komarova (1957) and Mikailov (1963). Bogdanova (1958), at the River Volga, U.S.S.R., found the larvae in *Abramis brama* in February/March (6·6%, 2) and May 1957 (6·6%, 3) but not in July or August 1956. At the Mingechaur Reservoir, Azerbaidan, U.S.S.R., Mikailov (1963) found *C. microcephalum* rarely during the autumn and winter. At the Volga Delta, U.S.S.R., Dubinina (1949) did not find larvae in *Cyprinus carpio* during spring and summer 1940, nor winter 1941, but in the spring of 1941 there was a high incidence (46·7%) of larvae. The most complete data appear to be those of Komarova (1957) from *Tinca tinca* in the River Donets, U.S.S.R. The incidences were studied in 1952 and 1953. The larvae were present at times, at levels between a minimum of 20% (winter 1952) to a maximum of 66·6% (summer 1952). The maximum incidences in both years were in summer (1952, 66·6%; 1953, 60%), which probably represents the season of invasion of the fishes.

Contracaecum rudolphii Hartwich, 1964

Includes *Contracaecum spiculigerum* (Rudolphi, 1809) according to Hartwich (1964). The material discussed here was originally reported as *C. spiculigerum*, except that of Dubinina (1949), which was named *C. siluri-glanis*.

The adults of *Contracaecum rudolphii* occur in a wide range of fish eating birds. The life cycle utilizes copepods as first intermediate (or transport) hosts and the second intermediate (or first) hosts are fishes and Odonata (see Baruš *et al.*, 1978).

Dubinina (1949) found larvae in *Silurus glanis* in the Volga Delta, U.S.S.R., summer 1940, 33·3%, winter 1941, 6·7% and spring 1941, 6·7%. At the Bay of Quinte, Lake Ontario, Canada, Tedla and Fernando (1969) observed larvae in *Perca flavescens* during every month of the year. There were no distinct seasonal changes in incidence or intensity during their period of study. The worms occurred mostly in the liver. Thin-walled capsules contained the most active worms, hard-walled capsules contained non-motile worms and scars in the liver may have represented totally degenerated capsules. Tedla and Fernando found both active and degenerate nematodes simultaneously in most fishes. They concluded that these indicated either successive invasions of the fishes throughout the year, or two seasons of invasion from successive

years. Spall and Summerfelt (1969), who studied *Ictalurus punctatus*, *Pomoxis annularis* and other fishes noted similar active and senile worms in Lake Carl Blackwell, Oklahoma, U.S.A. Capsules containing lifeless worms were especially evident in older fishes. The occurrence of *C. rudolphii* larvae in *I. punctatus* and *P. annularis* was established from about 4 or 5 years of life, at which time they began to eat smaller fishes. However, in *Micropterus salmoides* and *Pylodictis olivaris* the incidences of *C. rudolphii* larvae were high for all age classes, which reflected a change to piscivorous habits by these hosts at a very young age. The higher incidence of larvae in large piscivorous fishes appeared to reflect the transfer of the invasive larvae through the food chain of these predatory fishes, according to Spall and Summerfelt (1969). The accumulation of high numbers of this nematode larva in older fishes indicated that it was long-lived in the fish host.

Cloutman (1975) at Lake Fort Smith, Arkansas, U.S.A., also noted incidences of *Contracaecum rudolphii* in *Micropterus salmoides* during all months except February and March. In *Lepomis gulosus* and *L. macrochirus* occurrences were more irregular, May, June and September in *L. gulosus*, and January, April, May, September, November and December in *L. macrochirus*.

Malvestuto and Ogambo-Ongoma (1978) investigated a species of *Contracaecum*, probably *C. spiculigerum* (=*C. rudolphi*), in *Tilapia leucosticta* from Lake Naivasha, Kenya. They sampled monthly from September 1972 to February 1974, and 85% of the fishes were infected, with a mean intensity of nine larvae per fish. No seasonal variations in occurrence were reported. The larvae were always unencapsulated in the pericardial cavity of *T. leucosticta* rather than encapsulated as in temperate regions. Paperna (1974) also observed unencapsulated *Contracaecum* sp. in the pericardial cavity of *Tilapia nilotica*, *Bagrus docmac* and *Haplochromis* spp. in Lake George and Lake Victoria, Uganda.

Contracaecum species undetermined

Some seasonal data for undetermined species of *Contracaecum* can be found in papers by Edwards *et al.* (1977) and Ponyi *et al.* (1972). The host fishes and localities are given in Table 2.

Eure and Esch (1974) examined the effects of thermal effluent on the population dynamics of helminths in *Micropterus salmoides* at Par Pond, Savannah River Plant, Aiken, South Carolina, U.S.A. *Contracaecum* sp. was more abundant in the unheated areas, and the numbers reached a peak during the summer and early autumn.

At several habitats near San Marcos, Texas, U.S.A., Davis and Huffman (1978) found larvae of *Contracaecum* sp. in *Gambusia affinis*. First- (or second-) stage larvae were found in tangled masses in the mesenteries near the livers of some fishes. However, peak abundance of fourth-stage larvae occurred 7 to 12 months after the peak for the earlier stage. Davis and Huffman (1978) suggested that owing to the absence of a growth series the earlier stage did not develop into the later stage in the one host fish.

A *Contracaecum* sp. was found in eight species of fishes originating from the rivers Tigris and Euphrates, Iraq, by Shamsuddin *et al.* (1971). The

fishes were obtained from Baghdad Market. During the winter these nematodes were usually restricted to *Mugil abu* and *Silurus triostegus* (maximum numbers of worms 75, range 5–75). In the late spring and summer, almost all the fish species examined had the worms (maximum 123, range 7–123). It appeared, therefore, that the larvae were more common during summer than winter, although Shamsuddin *et al.* did add that the areas of collection of the fishes might influence the greater intensity of infection.

Raphidascaris acus (Bloch, 1779)

The adult worms of this species infect fishes (see Part IV of review). The larval worms are encapsulated in the liver, mesenteries, intestine wall and other viscera. Overall, the larvae occur throughout the year, chiefly in cyprinid fishes, but in other species as well. The following authors have provided seasonal data, hosts and localities are given in Table 2: Davies (1967), Dubinina (1949), Izyumova (1958, 1959, 1960), Kashkovski (1967), Kažič *et al.* (1977), Komarova (1964), Lyubarskaya (1970), Rautskis (1970a), Shul'man *et al.* (1974), Sous (1968) and Zschokke (1884).

The data of Malakhova (1961) from Lake Konche, Karelia, U.S.S.R., show the presence of larvae at all seasons: in *Rutilus rutilus*, autumn 32·2%, range 1–37; winter, 35·5%, 1–25; spring, 32·2%, 1–19; and summer, 31·1%, 1–18. In *Perca fluviatilis* the incidences and ranges at Lake Konche were: autumn, 2·2%, 1–6; winter, 1·5%, 1; spring, 1·1%, 1; and summer, 5·6%, 1–3.

The infections are cumulative through the life of the host, although degenerating and dead larvae are found suggesting that the life span of the larvae is less than that of the host. In the Aral Sea, U.S.S.R., Dergaleva and Markevich (1976) found that the infection of underyearling (0+) *Atherina mochon pontica* was 9% (maximum worms 7) in July, 18% (7) in September and 14% (16) by October. Žitňan (1973), at Dobsina Dam, Czechoslovakia, followed the seasonal incidence and intensity of infection of *Phoxinus phoxinus* by *Raphidascaris acus* larvae. Within year class I, the incidence of infection was 5% in April, rising to 10% by October. The incidences and intensities, average and maximum, for each year class in April were: I, 5%, 2·0 and 2; II, 30%, 5·1 and 16; and III, 60%, 5·5 and 18. In Lake Ubinsk, Siberia, Titova (1957) found that *Abramis brama* 5 years old, in the autumn, had the highest infections of that population. Older *A. brama* had fewer larvae. It is likely, therefore, that the main period of invasion of the fishes is during the summer months.

(b) *Family Ascarididae*

Porrocaecum reticulatum (von Linstow, 1899)

Kakacheva-Avramova (1977) noted this species in *Benthophilus stellatus* from the River Danube, Bulgaria, in the autumn. Sous (1968) also made mention of the species in relation to the effect of winter mass mortalities on the parasite fauna of fishes.

Porrocaecum species undetermined

Dubinina (1949) recorded *Porrocaecum* sp. larvae in *Lucioperca lucioperca* in the Volga Delta, U.S.S.R., as: summer 1940, 6·7%; winter 1941, 25%; and spring 1941, 6·7%.

TABLE 2

Studies on seasonal occurrence of larvae of nematodes listed in the climate zones of the World (see map Fig. 1, Chubb, 1977). The species are in alphabetical order

Climate zones	Nematode species	Host species	Locality	References
1. Tropical				
1a. RAINY (humid climate)	no seasonal studies		tropical forest	
1b. SAVANNA (humid climate)	no seasonal studies		tropical grassland	
1c. HIGHLAND (humid climate)			tropical highland	
	Contracaecum? *rudolphii*	*Tilapia leucosticta*	Lake Naivasha, Kenya	Malvestuto and Ogambo-Ongoma (1978)
1d. SEMI-DESERT (dry climate)			hot semi-desert	
	Contracaecum sp.	*Mugil abu* *Silurus triostegus* and 6 others	Rivers Tigris and Euphrates, Iraq	Shamsuddin *et al.* (1971)
1e. DESERT (dry climate)	no seasonal studies		hot desert	
2. Subtropical				
2a. MEDITERRANEAN			scrub, woodland, olive	
	Eustrongylides excisus	*Rutilus rutilus*	Lake Paleostomi, Georgia, U.S.S.R.	Cernova (1975)
	Eustrongylides sp.	*Gobio gobio lepidolaemus*	Lake Skadar, Yugoslavia	Kažić *et al.* (1977)
	Raphidascaris sp.	*Gobio gobio lepidolaemus*	Lake Skadar, Yugoslavia	Kažić *et al.* (1977)
	Streptocara sp.	*Gobio gobio lepidolaemus*	Lake Skadar, Yugoslavia	Kažić *et al.* (1977)

2b. HUMID			deciduous forest	
	Contracaecum sp.	*Gambusia affinis*	near San Marcos, Texas, U.S.A.	Davis and Huffman (1978)
		Micropterus salmoides	Par Pond, Savannah River Plant, Aiken, South Carolina, U.S.A.	Eure and Esch (1974)
	Spiroxys sp.	*Gambusia affinis*	near San Marcos, Texas, U.S.A.	Davis and Huffman (1978)
3. Mid-latitude				
3ai. HUMID WARM SUMMERS			temperate grassland, mixed forset	
	Contracaecum aduncum	*Alosa pontica pontica*	River Danube, Bulgaria	Kakacheva-Avramova (1977)
	Contracaecum rudolphii	*Lepomis gulosus* *Lepomis macrochirus* *Micropterus salmoides*	Lake Fort Smith, Arkansas, U.S.A.	Cloutman (1975)
		Ictalurus punctatus *Micropterus salmoides* *Pomoxis annularis* *Pylodictis olivaris*	Lake Carl Blackwell, Oklahoma, U.S.A.	Spall and Summerfelt (1969)
	Contracaecum sp.	*Ictalurus punctatus*	Kentucky River, Kentucky, U.S.A.	Edwards *et al.* (1977)
		Gymnocephalus cernua	Lake Balaton, Hungary	Ponyi *et al.* (1972)
	Eustrongylides excisus	*Phoxinus phoxinus*	Teich T, Hungary	Molnár (1968)
	Eustrongylides tubifex	*Aplodinotus grunniens* *Ictalurus punctatus* *Micropterus dolomieui* *Perca flavescens*	Lake Erie, U.S.A.	Cooper *et al.* (1978)
	Eustrongylides sp.	*Gymnocephalus cernua*	Lake Balaton, Hungary	Molnár (1966)
	Hystrichis tricolor	*Vimba vimba vimba* natio *carinata*	Dubossary Reservoir, Moldavia, U.S.S.R.	Marits and Vladimirov (1969)
	Porrocaecum reticulatum	*Benthophilus stellatus*	River Danube, Bulgaria	Kakacheva-Avramova (1977)

TABLE 2 (*continued*)

Climate zones	Nematode species	Host species	Locality	References
3ai. (*continued*)				
	Raphidascaris acus	*Phoxinus phoxinus*	Dobsina Dam, Czechoslovakia	Žitňan (1973)
		Alburnus alburnus	Lake Leman (Geneva), Switzerland	Zschokke (1884)
	Spiroxys contortus	*Misgurnus fossilis*	River Danube, Bulgaria	Kakacheva-Avramova (1977)
	Undetermined nematode cysts	*Enneachanthus gloriosus* *Lepomis gibbosus*	artificial lake, Lakeview settling pond, near Durham, North Carolina, U.S.A.	Holl (1932)
3aii. HUMID COOL SUMMERS			temperate grassland, mixed forest	
	Anisakis sp.	*Abramis brama*	River Volga, U.S.S.R.	Bogdanova (1958)
	Contracaecum microcephalum	*Abramis brama* *Tinca tinca*	River Volga, U.S.S.R. River Donets, U.S.S.R.	Bogdanova (1958) Komarova (1957)
	Contracaecum rudolphii	*Perca flavescens*	Bay of Quinte, Lake Ontario, Canada	Tedla and Fernando (1969)
	Desmidocercella sp.	*Lucioperca lucioperca* *Pelecus cultratus* *Perca fluviatilis*	Rybinsk Reservoir, U.S.S.R.	Izyumova (1958)
		Gymnocephalus cernua	Rybinsk Reservoir, U.S.S.R.	Izyumova (1959)
		Blicca bjoerkna	Rybinsk Reservoir, U.S.S.R.	Izyumova (1960)
		Perca fluviatilis	Lake Dusia, Lithuania, U.S.S.R.	Rautskis (1970a)
	Eustrongylides excisus	*Esox lucius*	River Volga, U.S.S.R.	Bogdanova (1958)
	Eustrongylides sp.	*Lepomis gibbosus*	Ryan Lake, Ontario, Canada	Cone and Anderson (1977)

	Raphidascaris acus	*Abramis brama* *Lucioperca lucioperca*	Rybinsk Reservoir, U.S.S.R.	Izyumova (1958)
		Gymnocephalus cernua	Rybinsk Reservoir, U.S.S.R.	Izyumova (1959)
		Abramis ballerus *Blicca bjoerkna*	Rybinsk Reservoir, U.S.S.R.	Izyumova (1960)
		Abramis brama	Kuybyshev Reservoir, U.S.S.R.	Lyubarskaya (1970)
		Perca fluviatilis	Lake Dusia, Lithuania, U.S.S.R.	Rautskis (1970a)
	Spiroxys sp.	*Lepomis gibbosus*	Ryan Lake, Ontario, Canada temperate grassland, deciduous forest	Cone and Anderson (1977)
3aiii. EAST COAST				
	Spiroxys japonica	*Misgurnus anguillicaudatus*	Hachiro-Gata, Akita Prefecture, Japan temperate grassland, deciduous forest	Hasegawa and Otsuru (1978)
3b. MARINE WEST COAST				
	Contracaecum aduncum	*Gasterosteus aculeatus*	Hadleigh Marsh, England	Dartnall (1972)
	Desmidocercella numidica	*Perca fluviatilis*	Lake Dargin, Poland	Wierzbicki (1970)
	Eustrongylides ? excisus	*Perca fluviatilis*	Serpentine, London, England	Lee (1977)
	Eustrongylides sp.	*Salmo trutta*	Loch Leven, Scotland	Campbell (1974)
		Gasterosteus aculeatus	Shoulder of Mutton Pond, England	Dartnall (1972)
		Salmo trutta	Fernworthy Reservoir, Devon, England	Kennedy and Lie (1976)
		Salmo trutta	Dunalastair Reservoir, Scotland	Robertson (1953)
	Raphidascaris acus	*Leuciscus cephalus* *Leuciscus leuciscus* *Rutilus rutilus*	River Lugg, Hereford, England	Davies (1967)

TABLE 2 (*continued*)

Climate zones	Nematode species	Host species	Locality	References
3c. SEMI-DESERT			prairie and steppe	
	Agamospirura sp.	*Lucioperca lucioperca* *Silurus glanis*	Volga Delta, U.S.S.R.	Dubinina (1949)
		Esox lucius *Pelecus cultratus*	Dnepr Delta, U.S.S.R.	Komarova (1964)
	Anisakis sp.	*Silurus glanis*	Volga Delta, U.S.S.R.	Dubinina (1949)
	Contracaecum microcephalum	*Cyprinus carpio*	Volga Delta, U.S.S.R.	Dubinina (1949)
		Abramis brama	Mingechaur Reservoir, Azerbaidan, U.S.S.R.	Mikailov (1963)
	Contracaecum rudolphii	*Siluris glanis*	Volga Delta, U.S.S.R.	Dubinina (1949)
	Desmidocercella sp.	*Cyprinus carpio*	Volga Delta, U.S.S.R.	Dubinina (1949)
	Eustrongylides excisus	*Lucioperca lucioperca* *Silurus glanis*	Volga Delta, U.S.S.R.	Dubinina (1949)
	Eustrongylides sp.	*Lucioperca lucioperca* *Silurus glanis*	Volga Delta, U.S.S.R.	Dubinina (1949)
		Esox lucius *Lucioperca lucioperca* *Pelecus cultratus* *Rutilus rutilus heckeli*	Dnepr Delta, U.S.S.R.	Komarova (1964)
	Porrocaecum sp.	*Lucioperca lucioperca*	Volga Delta, U.S.S.R.	Dubinina (1949)
	Raphidascaris acus	*Abramis brama* *Cyprinus carpio* *Lucioperca lucioperca* *Silurus glanis*	Volga Delta, U.S.S.R.	Dubinina (1949)
		Rutilus rutilus	Iriklin Reservoir, U.S.S.R.	Kashkovski (1967)
		Abramis brama	Dnepr Delta, U.S.S.R.	Komarova (1964)

3d. DESERT			cool desert	
	Raphidascaris acus	*Atherina mochon pontica*	Aral Sea	Dergaleva and Markevich (1976)
3e. SUB-POLAR			coniferous forest	
	Raphidascaris acus	*Perca fluviatilis* *Rutilus rutilus*	Lake Konche, Karelia, U.S.S.R.	Malakhova (1961)
		Rutilus rutilus	Lake Kuĭto, Karelia, U.S.S.R.	Shul'man *et al.* (1974)
		Perca fluviatilis	forest-steppe lakes, Siberia, U.S.S.R.	Sous (1968)
		Abramis brama	Lake Ubinsk, Siberia, U.S.S.R.	Titova (1957)
4. Polar				
4a. POLAR	no seasonal studies		tundra	
4b. ICE-CAPS	no suitable habitats for freshwater nematodes		icefields and glaciers	
5. Mountain	no seasonal studies		heath, rocks and scree	

2. *Order Spirurida*

(a) *Family Gnathostomatidae*

Spiroxys contortus (Rudolphi, 1819)

Kakacheva-Avramova (1977) found two specimens in *Misgurnus fossilis* in the autumn in the River Danube, Bulgaria. The life cycle was investigated by Hedrick (1935) in the Ann Arbor Region, Michigan, U.S.A. At 23–25°C the eggs hatched in about 1 week. A temperature of 8–12°C retarded hatching, as did 37°C. The sheathed larvae vibrated in the water, attached to some object as a pivot. The first intermediate hosts were *Cyclops* species, and development occurred in their body cavities to third-stage invasive larvae. The natural second intermediate hosts were *Ictalurus nebulosus*, *Umbra limi* and other fishes. Several species of turtles served as definitive hosts: in these adult worms were seldom seen before 1 June, and old worms in process of passage out from the host were found in late summer and autumn.

Spiroxys japonica Morishita, 1926

The adult worms occur in Amphibia. The life cycle of *Spiroxys japonica* was studied by Hasegawa and Otsuru (1978) at Hachiro-Gata, Akita Prefecture, Japan: adult worms were found in *Rana nigromaculata* and eggs were discharged into water with the faeces to be ingested by *Mesocyclops leuckarti*. The invasive larvae travelled from the copepods to the frogs by one of several alternative pathways: direct to the frog, or through a fish host, or through a tadpole, in which, after metamorphosis to a frog, the worm larvae become adults, or, the most usual route according to Hasegawa and Otsuru, through a tadpole that was eaten by a frog so allowing the development of the adult worms. An annual rhythm was postulated. Larvae ingested late summer or autumn may remain overwinter as juveniles to mature the following summer. The cyclopoid phase occurred in summer. However, Hasegawa and Otsuru provided no details about the annual presence of *S. japonica* in fishes.

Spiroxys species undetermined

Cone and Anderson (1977) investigated the occurrence of *Spiroxys* sp. in *Lepomis gibbosus* in Ryan Lake, Ontario, Canada. Incidence increased with host age, and was similar before and after winter, but increased during summer. The intensities in fishes of age group I and older were similar. Cone and Anderson showed that the invasion of fishes occurred during the summer. Incidence in age-group III *L. gibbosus* was significantly higher in autumn than in the same fishes the previous spring. There was no evidence of invasion during the winter, when the food consumption of the fishes was minimal.

At several habitats in the San Marcos region of Texas, U.S.A., Davis and Huffman (1978) did not find any significant seasonal distribution of the larvae of *Spiroxys* sp. in *Gambusia affinis*.

(b) *Family Acuariidae*

Streptocara species undetermined

The adult worms of *Streptocara* species occur in a wide range of aquatic birds. Fishes can serve as paratenic hosts. Kažič *et al.* (1977) frequently found

Streptocara sp. in *Gobio gobio lepidolaemus* at Lake Skadar, Yugoslavia, during the summer months.

(c) *Family Desmidocercidae*

Desmidocercella numidica (Seurat, 1920)

The adult worms are found in aquatic birds. The larvae parasitize the vitreous body of the eyes of fishes. Dubinin (1949) experimentally demonstrated that larvae from fishes fed to *Ardea cinerea* resulted in adult worms. Wierzbicki (1970) at Lake Dargin, Poland, did not find any seasonal fluctuations in the occurrence of larvae in the eyes of *Perca fluviatilis*. The incidence varied from year to year; it was more prominent in 1960.

Desmidocercella species undetermined

The larvae of *Desmidocercella* sp. may be found sporadically through the seasons in some fishes: *Blicca bjoerkna* (Izyumova, 1960), *Cyprinus carpio* (Dubinina, 1949) and *Gymnocephalus cernua* (Izyumova, 1959) (see Table 2 for localities). In other species of fishes, larvae occurred all or most of the year: *Lucioperca lucioperca* (Izyumova, 1958), *Pelecus cultratus* (Izyumova, 1958) and *Perca fluviatilis* (Izyumova, 1958; Rautskis, 1970a) (see Table 2 for localities). Izyumova (1964) suggested that maximum incidence at the Rybinsk Reservoir, U.S.S.R., occurred in autumn and winter, with a considerable decrease during spring and summer. However, at Lake Dusia, Lithuania, U.S.S.R., Rautskis (1970a) found the lowest incidence in *Perca fluviatilis* in January–March (6·6%) increasing to a maximum in October–November (40%).

(d) *Family unknown*

Agamospirura species undetermined

This genus contains undetermined larval spirurid nematodes whose definitive hosts have not been discovered or demonstrated. Seasonal information for *Agamospirura* have been provided by Dubinina (1949) and Komarova (1964) (see Table 2 for hosts and localities). Mikailov (1963) also made brief mention of these larvae in a seasonal context.

F. NEMATODE LARVA OF UNCERTAIN AFFINITY

Holl (1932) reported unidentified nematode cysts from *Enneachanthus gloriosus* and *Lepomis gibbosus* in North Carolina, U.S.A. In *E. gloriosus* at an artificial lake, Lakeview, North Carolina, the worms were absent during the summer months, but they showed a gradual increase during the autumn to a reach maximum in December, with a decrease in spring. Owing to the small size and large numbers of the larvae, Holl only made an estimate of their intensity, the estimated average for December being 300. Also, he found cysts containing many worms in the intestine wall and mesenteries of *L. gibbosus* during December to February at a settling pond, near Durham, North Carolina (Holl, 1932).

IV. Seasonal Studies in World Climatic Zones

The justifications for the division of the information about seasonal studies of helminth parasites into world climatic zones have already been indicated and a map provided to show the zones, in Chubb (1977). A further comment was made in Section IV of Chubb (1979). At least, such discussion highlights those regions of the world where no seasonal studies have been carried out.

Table 1 (Cestoda) and Table 2 (Nematoda) list the studies on seasonal occurrence of larvae in the world climate zones.

A. Tropical (climate zone 1)

There is an unfortunate scarcity of information about seasonal variations of helminths of freshwater fishes in the tropical regions of the world.

1. *Rainy* (*Climate zone* 1a)

As far as the author is aware no seasonal studies on larvae have been carried out in this climate zone.

2. *Savanna* (*Climate zone* 1b)

The work of Pal (1963) on the migratory fish *Hilsa ilisha* from the River Hooghly, India provided some information for the larval stages of two species of cestodes. *Ilisha parthenogenetica* occurred almost throughout the year, with 100% incidences during July and August (monsoon months) of both years of study, and in September 1962. Lower incidences were found in early winter, about 33% in November and December, but heavier infections again in December 1961 and January to March 1962. No explanation for these variations was offered. With *Pterobothrium filicolle*, Pal (1963) found heavy infections of these capsules, most commonly in the gradient zone, during August and the late winter months February and March. All *H. ilisha* were infected during September 1962. As these cestode plerocercoids are presumably long-lived in the fish host, the variations in incidence must reflect the different populations of the migratory host species moving up or down the river system, rather than actual changes in overall occurrence of the cestodes.

3. *Highland* (*Climate zone* 1c)

At Lake Naivasha, Kenya, Malevestuto and Ogambo-Ongoma (1978) found larvae of the nematode *Contracaecum* sp., probably *C. rudolphii* (= *C. spiculigerum*), in *Tilapia leucosticta* during September 1972 to February 1974. They suggested that the invasion of the fishes was direct, without zooplanktonic intermediate host. In these tropical conditions the third-stage larvae were unencapsulated in the pericardial cavity of the host, whereas in temperate climates they were encapsulated in the body cavity.

4. *Semi-desert* (*Climate zone* 1d)

Larval nematodes, *Contracaecum* sp., were found in large numbers in *Mugil abu* and *Silurus triostegus* during winter in Iraq by Shamsuddin *et al.* (1971), but not in six other species during this season, whereas during late spring and summer almost all fish species examined contained infections. Possibly these differences represent variation in host populations sampled, owing to spawning or other migrations of the fishes.

5. *Desert* (*Climate zone* 1e)

No seasonal studies of larval cestodes or nematodes are known to the author from this climate zone.

B. SUBTROPICAL (CLIMATE ZONE 2)

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

Three species of cestode larvae have been studied. At Lake Paleostomi, Georgia, U.S.S.R., Cernova (1975) found *Ligula intestinalis* in *Rutilus rutilus* in autumn and winter (6·7% in each season), but not during spring and summer. Kažič *et al.* (1977) found a single incidence of *L. intestinalis* in *Gobio gobio lepidolaemus* in Lake Skadar, Yugoslavia, in January. Presumably, owing to the longevity of these plerocercoids in temperate conditions, it may be assumed that the low incidences caused the apparent seasonal distribution.

The embryonic development of the eggs of *Triaenophorus meridionalis* was reported to occur at water temperatures of 15–22°C in May at Lake Paleostomi, Georgia, U.S.S.R. (Kuperman, 1967a). At Lake Piediluco, Italy, Aisa (1975) reported a 100% incidence of *Triaenophorus nodulosus* plerocercoids in the ovaries of *Tinca tinca* from September 1973 to August 1975. This report is interesting, as *T. nodulosus* appears to be much further south in this location than would otherwise be expected.

Larval nematodes studied in this zone include *Eustrongylides excisus* (Cernova, 1975), *Eustrongylides* sp. and *Streptocara* sp. (Kažič *et al.*, 1977), the adult worms of which occur in birds. However, rather low incidences of these larvae render it difficult to generalize about their occurrence. Kažič *et al.* (1977) found larvae of *Raphidascaris* sp. in *Gobio gobio lepidolaemus* in Lake Skadar, Yugoslavia. The adults of this species occurs in predatory fishes. Overall for the year, 20% of *G. gobio lepidolaemus* were infected, but about 56% were infected from April to September. In June, as many as 60 larvae per host were found. These data suggest that there may be a main summer season of invasion of the fishes similar to that found in mid-latitude climate zones.

2. *Humid* (*Climate zone* 2b)

Two studies on nematode larvae *Contracaecum* sp. have been carried out in this climate zone. Davis and Huffman (1978) found *Contracaecum* sp. in *Gambusia affinis* at several habitats near San Marcos, Texas, U.S.A.

However, no clear pattern of occurrence was seen. Eure and Esch (1974) at Par Pond, Savannah River Plant, Aiken, South Carolina, U.S.A., noted *Contracaecum* sp. in *Micropterus salmoides*. Occurrence reached a peak during the summer and early autumn. Davis and Huffman (1978) also found larvae of *Spiroxys* sp. in *G. affinis*. In this species no significant seasonal distribution was seen, the overall level of incidence was 37%.

C. MID-LATITUDE (CLIMATE ZONE 3)

1. *Humid warm summers* (*Climate zone* 3ai)

Information is available for nine species of larval cestodes and eleven species of larval nematodes. Bogitsch (1958) studied the occurrence of *Bothriocephalus* sp. larvae in the intestine of *Lepomis macrochirus macrochirus* in ponds in Albemarle County, Virginia, U.S.A. Although unable to mature in this host, the worms showed an annual pattern of occurrence and growth. Maximum incidence (37%) and growth was found mid-winter, but all the worms were lost by mid-June without maturation. The definitive host was not discovered.

In five species of cestodes, *Dilepis unilateralis* (Sapozhnikov, 1975), *Gryporhynchus* sp. (Ivasik, 1953), *Ligula intestinalis* (Kakacheva-Avramova and Naidenov, 1974), *Triaenophorus crassus* (Scheuring, 1929), *T. nodulosus* (Ergens, 1966; Scheuring, 1923; Vojtkova, 1959) the larvae were found throughout the year. Larvae of *Diphyllobothrium latum* were also presumably present all year, as Borroni and Grimaldi (1974) found an increasing incidence of plerocercoids in the musculature of *Perca fluviatilis* with length, from 1·4% for fishes less than 4·4 cm. in length, to 50% for those greater than 29·5 cm. in length. A similar progressive accumulation of plerocercoids of *L. intestinalis* but in relation to age was noted in *Rutilus rutilus* by Žitňan (1964) at Košice, Czechoslovakia.

The periods of invasion are not well-established for this climate zone. Sapozhnikov (1975) found that age-group 0 *Cyprinus carpio* were invaded by *Dilepis unilateralis* mainly in April and May when *Cyclops* and *Diaptomus* were present in large numbers. Janicki and Rosen (1917) found young *Diphyllobothrium latum* larvae in *Perca fluviatilis* from the end of April to the end of May at Neuchâtel, Switzerland.

Secondary transfer of cestode larvae from a planktivorous to a piscivorous fish was postulated by Borroni and Grimaldi (1974) and Grimaldi (1974) for *Diphyllobothrium latum* in *Perca fluviatilis* at Lake Maggiore, Italy. In the above example, the transfer was intraspecific, but at Lake Erie, U.S.A., Stromberg and Crites (1974) suggested that *Morone chrysops*, which were chiefly piscivores, might have acquired *Triaenophorus nodulosus* plerocercoids by interspecific transfer of larvae. Such a transfer could partially explain the increased incidence of *T. nodulosus* in older *M. chrysops* from Lake Erie.

Buck *et al.* (1976) noted adult *Diphyllobothrium dendriticum* in migrant gulls in the Bass Island area of Western Lake Erie, U.S.A., during late October–November. In many areas in this climate zone the potential definitive

hosts may remain at the habitat throughout the year. Thus, at the Iskar Dam Lake, Bulgaria, Kakacheva-Avramova and Naidenov (1974) found that *Corvus cornix*, *Larus ridibundus* and *Podiceps cristatus* the definitive hosts for *Ligula intestinalis* at that reservoir were present all year.

Kakacheva-Avramova (1977) noted larvae of the nematode *Contracaecum aduncum* in *Alosa pontica pontica* during spring and summer in the River Danube, Bulgaria. These fishes enter freshwaters in spring to spawn and occur in the Black Sea during winter. Invasion by *C. aduncum* presumably occurs during the marine phase of life.

Larval nematodes of the following species were reported at all times of the year in climate zone 3ai: with avian definitive hosts, *Contracaecum rudolphii* (= *C. spiculigerum*), Cloutman (1975), Spall and Summerfelt (1969); *Eustrongylides tubifex*, Cooper *et al.* (1978): with fish definitive hosts, *Raphidascaris acus*, Žitňan (1973). Holl (1932) found an unidentified larval nematode in *Enneachanthus gloriosus* in North Carolina, U.S.A., during October to May, but they were absent from June to September. The other species reported for this zone were of low incidence and sporadic occurrence, so that no conclusions are possible.

Invasion of *Ictalurus punctatus* and *Pomoxis annularis* by *Contracaecum rudolphii* at Lake Carl Blackwell, Oklahoma, U.S.A., was established from about the fourth or fifth year of life when they began eating small fishes (Spall and Summerfelt, 1969). However, with *Micropterus salmoides* and *Pylodictis olivaris*, which became piscivores at a very young age, the incidence was high in all age classes. The capsules varied in size and appearance, the dark, nodular, fibrous capsules containing dead worms, indicating progressive senility of worms, especially in older fishes. Spall and Summerfelt (1969) considered that the larvae of *C. rudolphii* were long-lived in the fish intermediate host. Cooper *et al.* (1978) found that young of the year and I+ *Perca flavescens* at Lake Erie, U.S.A., exhibited increasing monthly larval burdens of *Eustrongylides tubifex*, but there was no significant increase in II+ *P. flavescens*. The increasing infection was seen in July to October. As with *C. rudolphii*, extreme larval longevity was suggested by the data, although dead worms were found in *Aplodinotus grunniens*. Experimental transfer of *E. tubifex* from fish to fish were invariably successful, so that Cooper *et al.* (1978) suggested that *P. flavescens* and *A. grunniens* acquired the infections primarily by eating the intermediate host oligochaetes, whereas *Ictalurus punctatus* and *Micropterus dolomieui* obtained their infections by predation on other fishes.

The incidence of *Raphidascaris acus* in *Phoxinus phoxinus* in Czechoslovakia increased through the life of the hosts. The data for all fishes did not indicate the season of invasion (Žitňan, 1973).

Hedrick (1935) studied the life cycle of *Spiroxys contortus* in this climate zone. Adult worms in turtles were seldom found, but old worms in the process of passage out were seen in late summer and autumn. This suggests that the hatching of the eggs, the invasion of the copepod hosts, and thereafter, the invasion of the fish second intermediate hosts might occur on a seasonal basis.

Larvae of *Eustrongylides tubifex* were reported as a seasonal economic factor in Lake Erie, U.S.A., by Bangham (1972). However, this remarks refers to the capture of heavily infected fishes by fishermen at a particular time of the year, rather than to any seasonality in the worm larval populations.

2. *Humid cool summers* (*Climate zone* 3aii)

In this climate zone the following larval cestodes have been shown to occur in fishes at all times of the year: *Digramma interrupta* (Dubinina, 1953; Kosheva, 1956; Lyubina, 1970); Dilepididae, species undetermined (Cone and Anderson, 1977); *Diphyllobothrium latum* (Bogdanova, 1958; Izyumova, 1958, 1960; Kuperman, 1977; Tell, 1971; Wikgren, 1963); *Ligula intestinalis* (Kosheva, 1956); *Paradilepis scolecina* (Komarova, 1957); *Triaenophorus amurensis* (Kuperman, 1968, 1973b); *T. crassus* (Kuperman, 1967a, 1973b); *T. nodulosus* (Bogdanova, 1958; Izyumova, 1958, 1959; Kuperman, 1973b; Rautskis, 1970a, b; Tell, 1971); and *T. orientalis* (Kuperman, 1973b). The larvae of *Diphyllobothrium dendriticum*, *Schistocephalus solidus* and *S. pungitii* were of sporadic occurrence in the investigation of Banina and Isakov (1972), but are known from studies elsewhere to be present in the fish hosts all year. The larvae of *Bothriocephalus* sp. (Cannon, 1973) and *Eubothrium* sp. (Izyumova, 1959) showed no seasonal pattern, perhaps on account of low incidence. Both are transmitted to fishes from copepods and are resident in the intestines of their hosts so may survive there for relatively short periods. In remaining in the intestine lumen of the host, these cestode larvae are subjected to a different set of conditions from those applying to larvae found in the somatic tissues (see Section V A).

The eggs of the species of tapeworms having fish definitive hosts are released from the matured worms at particular times of the year. In this climate zone, the period from May to early June has been shown to be the time when the eggs of *Triaenophorus amurensis*, *T. crassus*, *T. nodulosus* and *T. orientalis* embryonate and mass-emergences of coracidia occurred as the water temperatures rose to appropriate levels. This limited period of egg release is followed in sequence, by hatching of the eggs, ingestion of the coracidia by copepod hosts, growth of the procercoid to an invasive stage, and finally to invasion of the fish intermediate hosts by their feeding on infected copepods. Temperature influences all these processes, so that they occur, in turn, for only limited times during each year. The time of invasion of *Perca fluviatilis* at Rybinsk Reservoir, U.S.S.R., by *T. nodulosus* was determined as from about 21–24 May. The growth of the plerocercoids to the stage of hook formation on the scolex was completed in *T. nodulosus* from mid-July to mid-August, so that invasion by procercoids must have continued until about 5–10 July (Kuperman, 1973b). In *T. crassus* all scolex hook formation was completed by 30 August at the Rybinsk Reservoir (Kuperman, 1973b).

In species with warm-blooded avian or mammalian definitive hosts, ingestion of plerocercoids can, in theory, occur at any time of the year, provided that the plerocercoids retain their invasive condition. However, in practice limitations will apply, including migrations of the potential host species away from the area during the winter months when ice covers

the water. Thus seasonal limitations will apply. In climate zone 3aii adult *Digramma interrupta, Ligula intestinalis* and *Diphyllobothrium latum*, for instance, could occur at any season, but with the avian hosts of *D. interrupta* and *L. intestinalis* they migrate to more favoured climates during winter (Dubinina, 1964a). In man, *D. latum* will continue to produce eggs overwinter, so that it is the fate of these eggs which is of interest. In all the species, *D. interrupta, L. intestinalis* and *D. latum* the presence of low water temperatures slows or prevents embryonic development and hatching (Dubinina, 1953; Guttowa, 1963). Of particular importance, Guttowa (1963) showed at Lake Karperö, Finland, a sequence of events that is likely to apply to many species of cestodes whose eggs are liberated at cold times of the year. At Lake Karperö, Guttowa (1963) demonstrated that the eggs of *D. latum*, which had accumulated overwinter in the waters of the lake, underwent a mass-hatching in the third part of May as water temperatures rose to an appropriate level. This was followed by a peak incidence of procercoids in *Cyclops strenuus* and *Thermocyclops oithonoides* about 1 month later, mid-June. By the end of June the procercoid incidence fell, owing to the now diminished concentration of coracidia, to achieve in all probability a level of stability from mid-July until autumn when water temperatures fell below 8°C and no further hatching of *D. latum* eggs could occur. It is noteworthy that at the River Svir, U.S.S.R., also in climate zone 3aii, Razumova and Gutkovskaya (1959) also found that *D. latum* eggs were most numerous in spring and the beginning of summer. These data clearly show that in the majority of instances invasive cestode larvae will again be most abundant in the early summer, although it is possible that some larvae could overwinter in copepods. As many fishes reduce or cease their feeding activities during cold winter conditions this would reduce the likelihood of their significant invasion by cestode larvae during this season.

Thomas (1949) was able to summarize the seasonal interrelationships of *Diphyllobothrium oblongatum* in the Beaver Island area of Lake Michigan, U.S.A. Adult worms were probably shed into the lake waters in large numbers around the nesting sites of the gull and tern hosts during late July and August. The eggs would hatch according to temperature, but Thomas postulated that a continuous source of invasion might be available for the first intermediate host *Diaptomus oregonensis* because the eggs could remain viable for 5 years at 2°C. In turn, the fish second intermediate host *Coregonus artedii* would be exposed to invasion, as *D. oregonensis* was one of the chief food items of this fish species. *C. artedii* appeared over deep water, but near the surface in June and July when the gulls and terns were nesting, and would therefore be available as food for these birds at that time.

In the habitat of *Diphyllobothrium oblongatum* described above, the gulls dispersed in numbers to inland lakes during August and September.

Dubinina (1953, 1959a, 1964a) has shown that *Digramma interrupta* and *Ligula intestinalis* larvae in fishes require some 12–14 months to become invasive. Therefore fishes that acquire the infection during one summer will not contain invasive plerocercoids until the following summer (Bauer, 1959), or perhaps later.

Secondary transfer of plerocercoids of *Diphyllobothrium* from fish to fish via feeding activities of predatory *Salvelinus namaycush* was postulated by Freeman and Thompson (1969) at lakes in the Algonquin Park, Ontario, Canada. Presumably such transfers are likely to be most common during the warmer times of the year when the feeding activities of the *S. namaycush* are greatest, although Halvorsen and Wissler (1973) found experimentally that low temperatures did not prevent such transfers, they merely took longer. Wikgren (1963), in Finland, found more plerocercoids of *Diphyllobothrium latum* in the viscera of *Esox lucius* and *Lota lota* than in the musculature during the spring, whereas in autumn most larvae were located in the musculature. Wikgren (1963) speculated that the plerocercoids had accumulated in the body cavity organs during the low winter temperatures, but suggested that the problem of accumulation of plerocercoids in large predatory fishes required further investigation.

In some instances, once the larvae are established in the fish host they can survive for the remainder of the life of that host. Such may occur in the Ligulidae, although Kosheva (1956), at the Kutuluks Reservoir, U.S.S.R., suggested that *Digramma interrupta* and *Ligula intestinalis* died after 2 years. Deksbakh and Shchupakov (1954) stated that these plerocercoids lived 2 to 3 years in the Central Ural and Transural Regions of the U.S.S.R. At McKaskill Lake, Algonquin Park, Ontario, Canada, MacLulich (1943) found dead and degenerating plerocercoids of *Diphyllobothrium* sp. in *Salvelinus namaycush* caught under winter ice and living plerocercoids were not found until July. Cone and Anderson (1977) observed that with Dilepididae larvae in *Lepomis gibbosus* at Ryan Lake, Ontario, Canada, invasion was mostly acquired by the end of the second summer of life of the fishes, and thereafter there was a gradual increase with age in the percentage of degenerate larvae.

In this climate zone epizootics of *Ligula intestinalis* may be unusual. In the Central Ural and Transural region of the U.S.S.R., Deksbakh and Shchupakov (1954) found that epizootics only occurred during hot summers when water temperatures enabled the incubation of the eggs and the hatching of the coracidia to occur within the shortest possible time.

Fishes infected by *Ligula intestinalis* at Wolf Lake, New York, U.S.A., were shown to have different behaviour patterns from uninfected individuals (Dence, 1958). Thus heavily infected *Notropis cornutus frontalis* migrated shoreward throughout summer, especially in calm weather, where they were readily available to the potential avian definitive hosts. Dence (1958) also noted that the young fishes occurred in the shallows during summer and were therefore exposed to invasion by *L. intestinalis*.

Three lakes with different thermal regimes, one shallow and two deep, were investigated in Lithuania, U.S.S.R., for their effect on plerocercoids of *Triaenophorus nodulosus* and other parasites in *Perca fluviatilis* (Rautskis, 1977). Overall the incidences and intensities of the parasites were higher in the shallow, warmer lake than in the deeper, cooler ones. However, this overall effect did not seem to apply to *T. nodulosus*.

One long-term study, at the Rybinsk Reservoir, U.S.S.R., has been carried

out in this climate zone by Kuperman (1977). The incidence of plerocercoids of *Diphyllobothrium latum* in its host fishes remained stable from 1966 to 1976. However, Lawler (1969a), at Lake Mälaren, Sweden, produced evidence that the year class strength of *Triaenophorus nodulosus* varied from year to year. Thus annual differences existed in the incidence of infection in the second intermediate hosts. Lawler (1969a) stated that in 1965 the water level was high, ensuring more favourable suitable spawning habitats for *Esox lucius*, therefore production of this fish was high, and there was evidence that the incidence of *T. nodulosus* in *Perca fluviatilis* that year was also high. Ecological conditions in 1965 were generally favourable for the early stages of the life cycle of *T. nodulosus* in Lake Mälaren (Lawler, 1969a).

There is considerably less detailed information about the occurrence of larval nematodes in the fishes of climate zone 3aii. Of the species found as adults in avian hosts, larvae were found in fishes as follows: *Contracaecum microcephalum*, River Donets, U.S.S.R., present all year, no pattern of incidence (Komarova, 1957), River Volga, U.S.S.R., present February/March, May, not July/August, but of low incidence (Bogdanova, 1958); *C. rudolphii* (= *C. spiculigerum*), Bay of Quinte, Lake Ontario, Canada, present all year, no pattern of incidence (Tedla and Fernando, 1969); *Desmidocercella* sp., Rybinsk Reservoir, present all year in some species of fishes, no pattern of incidence, irregular or sporadic in other fishes (Izyumova, 1958, 1959, 1960), but Izyumova (1964) stated that maximum infection occurred autumn and winter, with a considerable decrease in spring and summer, Lake Dusia, Lithuania, U.S.S.R., present all year, no pattern of incidence (Rautskis, 1970a); *Eustrongylides excisus*, River Volga, U.S.S.R., present February/March and May, not August, but of low incidence (Bogdanova, 1958); and *Eustrongylides* sp., Ryan Lake, Ontario, Canada, present all year, incidence increased in summer (Cone and Anderson, 1977). Bogdanova (1958) at the River Volga, U.S.S.R., found *Anisakis* sp. during February and March, but not during May or July and August, hence not enough information is available for a conclusion. In summary, the data above suggest that these nematodes occur as larvae in fishes at all times of the year, but more information is required for most of the species.

Raphidascaris acus adults occur in predatory fishes. Over the range of species of hosts she examined at the Rybinsk Reservoir, U.S.S.R., Izyumova (1958, 1959, 1960) found the larvae in all seasons, although incidences within the species of host fishes were irregular and not at all times of the year. The data of Lyubarskaya (1970) from the Kuybyshev Reservoir, U.S.S.R., and of Rautskis (1970a) from Lake Dusia, Lithuania, U.S.S.R., also show occurrences, but not at all times of the year. The adults of *Spiroxys* sp. occur in turtles. At Ryan Lake, Ontario, Canada, Cone and Anderson (1977) found *Spiroxys* sp. in *Lepomis gibbosus* throughout the year. Incidence was similar before and after winter, but increased during summer.

Rather few details are available about other aspects of the biology of these nematode larvae in climate zone 3aii. Tedla and Fernando (1969), in *Perca flavescens*, found that the capsules of *Contracaecum rudolphii* (= *C. spiculigerum*) were of two types, thin-walled, containing active worms, and

hard-walled, containing coagulated material surrounding non-motile or degenerated larvae. Scars in the liver tissue may have represented totally degenerated nematode capsules. The finding of active and degenerate larval nematodes simultaneously in most *P. flavescens* seemed to Tedla and Fernando (1969) to indicate successive invasions throughout the year, or two seasons of invasion, or infestations from different years. Cone and Anderson (1977), in *Lepomis gibbosus*, noted that *Eustrongylides* sp. was predominant in larger fish individuals. Its rarity in 0 to II age-group fishes suggested that oligochaetes, the likely intermediate hosts, were not a significant item of diet, and the incidence in III and IV age groups indicated that these older fishes acquired the infection by eating paratenic hosts containing larvae. The invasion occurred during summer, since incidence in age-group III *L. gibbosus* was significantly higher in the autumn than in those same fishes the previous spring. During winter the fish food consumption was minimal, therefore the possibility of transmission was greatly reduced. With *Spiroxys* sp. at the same habitat, Cone and Anderson (1977) found that incidence increased with the age of *L. gibbosus*, but in this nematode the intensities of occurrence were similar in fishes age groups I and older. Invasions were acquired during summer, as shown by a significantly higher incidence in age-group III fishes in autumn compared with the previous spring. As with *Eustrongylides* sp., there was no evidence of invasion during the winter.

3. *East coast* (*Climate zone* 3aiii)

In this climate zone Noble (1970) found larval *Bothriocephalus* sp. in *Perca flavescens* at Lake Oneida, New York, U.S.A., most commonly during April to June. These larvae occurred in the host intestines. Noble (1970) also frequently noted *Ligula intestinalis* plerocercoids in the body cavities of young *P. flavescens* in late summer of 1968, although they did not occur in a 1966 survey. Awakura *et al.* (1976) observed that *Carassius carassius* at Lake Ōnuma, Hokkaido, Japan, had a low infection rate by *Digramma interrupta* in spring and a high one by autumn. Young worms, less than 1 g in weight, were seen in mid-July and late November, so that Awakura *et al.* (1976) suggested that invasion of the fishes occurred in early summer to late autumn.

Extensive studies of *Diphyllobothrium sebago* have been carried out in climate zone 3aiii. The species is closely related to *D. dendriticum* (Meyer and Robinson, 1963). The life cycle was completed experimentally by Meyer and Vik (1963), and of relevance here, this study showed that the only significant growth of the plerocercoids occurred when the water temperature was about 9°C, i.e. during the summer months. Meyer (1967) hatched eggs of *D. sebago* at various depths in Branch Lake, Maine. Within the temperatures 9 to 20°C, to a depth of 27 m, the percentage of eggs hatching increased with time (see Fig. 1, p. 37). Meyer concluded that for the conditions he investigated, regardless of where the eggs were deposited, there was juxtaposition in habitat between the coracidia and the copepod intermediate

hosts. Johnson (1975), at a Maine fish hatchery, examined *Cyclops scutifer* for *D. sebago* procercoids. A 1·7% incidence was found in July only. *Salmo salar* fry first became invaded during July. Meyer and Vik (1968) showed that landlocked *S. salar* in the Rangeley Lakes, Maine, U.S.A., had an increasing incidence and intensity of infection by plerocercoids of *D. sebago* with host age, so that successive invasions occurred. The definitive hosts for *D. sebago* at the Rangeley Lakes were *Larus argentatus* (Vik and Meyer, 1962; Meyer and Vik, 1972). The birds were present from spring to autumn, and had the highest infections of adult *D. sebago* in June and the summer months. The seasonal circulation of *D. sebago* in the Rangeley Lakes was summarized by Meyer (1972). In essence, a die-off of *Osmerus mordax* containing plerocercoids occurred in May, at about the time of return of the *L. argentatus* which ate the dead fishes, to give maximal occurrence of the worm eggs in June. These hatched to give coracidia, invasion of *Cyclops* species and procercoids in July. The *Cyclops* were a major food of *O. mordax* which completed the circulation of the parasite in this habitat.

Some data are available for one nematode in climate zone 3aiii, *Spiroxys japonica*, from Hachiro-Gata, Akita Prefecture, Japan (Hasegawa and Otsuru, 1978). The *S. japonica* were transmitted to the definitive hosts, amphibians, by several pathways, one of which used *Misgurnus fossilis*. An annual rhythm of development in the life cycle was postulated, with a cyclopoid phase in summer, but no details were provided about the annual presence of the larvae in the fishes.

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

In parts of this zone in the more coastal regions the potential avian definitive hosts for both cestodes and nematodes may remain on freshwater through most normal winters.

Information is available for nine species of larval cestodes. All have been shown to be present in the fishes throughout the year: *Diphyllobothrium dendriticum* (Duguid and Sheppard, 1944; Fraser, 1951, 1960a; Hickey and Harris, 1947; Kuhlow, 1953c; Vik, 1957), *D. ditremum* (Aderounmu, personal communication; Chubb, 1961; Hickey and Harris, 1947; Powell, 1966), *D. ursi* (Rausch, 1954), *D. vogeli* (Kuhlow, 1953b), *Diphyllobothrium* sp. (Andrews, 1977b; Becker, 1967; Becker and Brunson, 1967; Campbell, 1974; Mamer, 1978), *Ligula intestinalis* (Arme and Owen, 1968; Orr, 1968; Owen and Arme, 1965; Sweeting, 1976, 1977), *Schistocephalus solidus* (Arme and Owen, 1964, 1967; Chappell, 1969; Clarke, 1954; Dartnall, 1972; Hopkins, 1950; Hopkins and Smyth, 1951; Pennycuick, 1971a, b, c, d), *Triaenophorus nodulosus* (Andrews, 1977b; Borgström, 1970; Chubb, 1964; Lee, 1977; Lien, 1970; Mishra, 1966; Rizvi, 1964; Rumpus, 1975; Wierzbicki, 1970, 1971), *Valipora campylancristrota* (Jara and Olech, 1964).

Temperature has been shown to affect the developmental rates of the embryonating eggs in *Diphyllobothrium dendriticum* (Kuhlow, 1953c) and *Schistocephalus solidus* (Clarke, 1954; Hopkins and Smyth, 1951) in this

zone, and has been established elsewhere by experimental studies for the majority of the species. The growth of the procercoids in the zooplanktonic hosts are influenced in the same way by temperature.

The times of invasion of the fishes in this climate zone have been demonstrated for six species. Fraser (1951, 1960a) considered the main period of invasion of *Salmo trutta* in Yeo Reservoir, Blagdon, Somerset, England, by *Diphyllobothrium dendriticum* to be March to May. With *Diphyllobothrium* sp. in Goodwin Lake, Washington State, U.S.A., Becker (1967) found that heavy invasions appeared in young salmonids soon after their release into the lake in spring. Invasions of autumn planted fishes were lighter than in the spring-stocked individuals. Becker and Brunson (1967), working in the same lake, and other lakes, stated that the plerocercoids were acquired during summer when subsurface water temperatures (0–6 m) were maximal. Small plerocercoids (2–5 mm) in spring developed rapidly in the summer soon to reach about 20 mm. In autumn-stocked *Salmo gairdneri* the plerocercoid length of 20 mm was usually reached only by the following April–May, when growth was accelerated as the water warmed up in spring. Mamer (1978) working at Silver Lake, Washington State, U.S.A., also stated that the *Diphyllobothrium* sp. were acquired by *Salmo clarki* and *S. gairdneri* during the summer. Details, to be published elsewhere, were not given.

Arme and Owen (1968) showed that invasion of *Rutilus rutilus* and other species of fishes at Thryberg Reservoir, Yorkshire, by *Ligula intestinalis* occurred in many individuals before the age of 3 months, i.e. during the first summer, so that by September more than 50% were infected. Sweeting (1976) showed that by the April following their date of hatching, the previous summer, 92% of *Rutilus rutilus* were infected by *L. intestinalis* in a gravel pit at Yateley, Hampshire, England. The 1972 year class of fishes were invaded during their first 9 months of life, no invasions occurred during the following 10 months, probably because of a change of diet by the fishes. These data suggest that invasion of the *R. rutilus* may have continued during the winter. Clarke (1954) at a pond in Stourton, Leeds, Yorkshire, England, suggested that for *Schistocephalus solidus* the prolonged existence of the procercoid in *Cyclops* spp. did not seem sufficiently extensive for this stage to act as a reservoir of infection throughout the winter. Hopkins and Smyth (1951), at a small lake in Hunslet, Yorkshire, England, found very small *S. solidus* larvae (less than 10 mm in length), rarely, except during the summer months. An analysis of plerocercoid lengths clearly suggested that the peak period of invasion occurred during the summer months. Meakins and Walkey (1973), however, using growth rates of surgically implanted plerocercoids compared with growth rates *in vivo*, deduced that the majority of invasions occurred in the spring. Pennycuick (1971b) at Priddy Pool, Somerset, England, found new invasions in December 1966 and January to July 1967, with a rapid and large rise in infection from the end of July to September. In summary, therefore, it appears that the main invasions of *Gasterosteus aculeatus* occurred in summer, but could also occur, at a lower rate, during the remainder of the year if procercoids were available.

The above picture is quite different from that reported for *Triaenophorus nodulosus*, which has a fish definitive host. Halvorsen (1968) attempted to find natural infections in copepods from Bogstad Lake, Norway, but was unsuccessful. However, a number of authors have shown the period of invasion by reference to the presence of developing plerocercoids in the fish intermediate host. Chubb (1964) found developing plerocercoids in *Perca fluviatilis* at Llyn Tegid, Wales, from March to June. Andrews (1977b) at the same habitat and with the same host also found developing plerocercoids from March to June. In Bogstad Lake, Norway, Lien (1970) found developing plerocercoids in *P. fluviatilis* in July to September. The colder conditions of the Norwegian winter probably explain the delayed time of invasion. It must be noted that the actual period of invasion will have started a little earlier, and ended rather sooner, than the times shown by the presence of the developing plerocercoids. Nonetheless, it is clear that invasion of the fishes was limited to something about a 3 month period each year in climate zone 3b.

Jara and Olech (1964), at fish ponds in the Chojnów region of Poland, showed that the invasion of *Cyprinus carpio* by *Valipora campylancristrota* started about the beginning of July, when small plerocercus larvae appeared in the gall-bladders of these fishes.

The longevity of the larvae was in all the species greater than 1 year. Powell and Chubb (1966) found degenerating plerocercoids of *Diphyllobothrium dendriticum* and *D. ditremum* in *Salmo trutta*, but not *Salvelinus alpinus* at Llyn Padarn, Wales. At Kodiak Island, Alaska, U.S.A., *Oncorhynchus nerka* was the main host for *D. ursi*. Rausch (1954) suggested that invasion of these fishes probably occurred during their 3 years of life in Karluk Lake, and that the plerocercoids survived for the 2 to 3 years at sea in the life cycle of *O. nerka*.

The plerocercoids of *Ligula intestinalis* probably have a longevity equal to that of the remaining duration of life of the host (Arme and Owen, 1968; Orr, 1967a, 1968; Owen and Arme, 1965; Sweeting, 1976). However, significant mortality of infected fishes can be shown (Harris and Wheeler, 1974; Sweeting, 1976). The plerocercoids of *Schistocephalus solidus* also survive for the remaining life of the host (Pennycuick, 1971a).

Degenerating plerocercoids of *Triaenophorus nodulosus* have been found by Andrews (1977a,b), Chubb (1964) and Lien (1970). All these authors agreed that the plerocercoids can remain invasive for 2 to 3 years. The data of Jara and Olech (1964) for *Valipora campylancristrota* suggest that the plerocercus larvae lived for at least 9 months.

The avian or mammalian definitive hosts have been shown to carry adult worms as follows: *Diphyllobothrium dendriticum*, in *Lutra lutra*, April and May, at Yeo Reservoir, Blagdon, Somerset (Fraser, 1951); in *Larus argentatus*, *L. fuscus*, *L. marinus*, dates not given, Poulaphouca Reservoir, Eire (Hickey and Harris, 1947); and in *Larus ridibundus*, negative November–January, positive February–April, Hamburg area, Germany (Kuhlow, 1953c); *D. ditremum*, in *Phalacrocorax carbo*, *P. graculus*, *Ardea cinera*, dates not given, Poulaphouca Reservoir, Eire (Hickey and Harris, 1947); *D. ursi*, in *Ursus*

arctos middendorfii, July to September, Kodiak Island, Alaska (Rausch, 1954); and *Diphyllobothrium* sp., in *Mergus merganser*, December, Silver Lake, Washington State, U.S.A. (Mamer, 1978). No seasonal information about the occurrence of *Ligula intestinalis*, *Schistocephalus solidus* or *Valipora campylancristrota* in their definitive hosts is available for climate zone 3b. However, as noted at the start of this section, in areas such as the British Isles, the potential hosts are present on freshwaters at all seasons.

The fish definitive host of *Triaenophorus nodulosus* can be invaded at all seasons. Details will be provided later in Part IV of this review.

The behaviour of the fishes infected by cestode plerocercoids can change, and thereby facilitate availability of the plerocercoids to the potential definitive host. Examples from this climate zone are: *Ligula intestinalis* (Harris and Wheeler, 1974; Orr, 1966) and *Schistocephalus solidus* (Arme and Owen, 1967; Clarke, 1954; Lester, 1971; Pennycuick, 1971b). These behavioural differences were most pronounced during warm or hot conditions, at a time of maximum reproductive potential for the eggs which would be produced by the adult parasites.

Mortality of fishes, caused by the activities of the plerocercoids, can also aid availability of the infected fishes to the potential avian or mammalian definitive hosts. Some examples in climate zone 3b are: *Diphyllobothrium dendriticum*, mortality of *Salmo trutta* in warm conditions (Duguid and Sheppard, 1944; Fraser, 1951, 1960a; Hickey and Harris, 1947) and *Diphyllobothrium* sp., mortality in winter of *Salmo gairdneri*, and in warm conditions in small fishes soon after stocking (Becker, 1967; Becker and Brunson, 1967). A mortality of *Gasterosteus aculeatus* containing plerocercoids of *Schistocephalus solidus* was reported by Vik (1954) in August at a water temperature of 25°C.

A rather small amount of information about nematode larvae in fishes is available for climate zone 3b. Wierzbicki (1970) found *Desmidocercella numidica* at all seasons in *Perca fluviatilis* at Lake Dargin, Poland. In the British Isles, Lee (1977) found *Eustrongylides*? *excisus* in *P. fluviatilis* in the Serpentine, London, from October to March only. Dartnall (1972), however, found *Eustrongylides* sp. in *Gasterosteus aculeatus* at Shoulder of Mutton Pond, England, at all seasons, until, after eight samples, no more of these fishes were caught. In *Salmo trutta*, *Eustrongylides* sp. occurred in Dunalastair Reservoir, Scotland (Robertson, 1953), Loch Leven, Scotland (Campbell, 1974) and in Fernworthy Reservoir, Devon, England (Kennedy and Lie, 1976) and showed no marked seasonal changes in incidence through the periods of the investigations. At Fernworthy Reservoir, Kennedy and Lie (1976) noted a greater frequency of smaller larvae during the winter months. The nematodes discussed above all have avian definitive hosts.

Davies (1967), at the River Lugg, Herefordshire, England, found larval *Raphidascaris acus* in *Leuciscus cephalus*, *L. leuciscus* and *Rutilus rutilus* during all months of the year. A slight decrease in incidence was noted in July and August. Some larvae were dead. The adult worms were in *Esox lucius*.

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

As noted in Chubb (1979) the terms prairie or steppe convey a better impression of climate conditions in this zone.

Three species of cestodes with avian definitive hosts have been investigated. The plerocercoids of *Digramma interrupta* were shown to occur all year (Kamenskii, 1962, 1964) and were found in fishes up to 8 or 9 years old (Reshetnikova, 1965). An epizootic focus of *Schistocephalus pungitii* was described by Kafanov and Yazykova (1971). *Ligula intestinalis* plerocercoids were found at all seasons by Kamenskii (1962, 1964) and Mikhailov (1963). It is of interest to note that Mikailov (1963) at the Mingechaur Reservoir, Azerbaidan, U.S.S.R., reported that the avian hosts of *D. interrupta* and *L. intestinalis* were present in larger numbers in winter than in summer. The copepod intermediate hosts also reproduced all year owing to the absence of large changes in water temperature in the reservoir. At fish farms in the Akhtarskii Estuary, Azov Sea, U.S.S.R., Denisov and Vorob'ev (1974) found that young of the year *Ctenopharyngodon idella*, *Cyprinus carpio* and *Hypophthalmichthys molitrix* were invaded with plerocercoids of Ligulidae. Most larvae in *C. idella* and *C. carpio* were destroyed during the winter, but in *H. molitrix* they remained viable.

Kuperman (1967a, 1973b) noted that the development of the embryos of *Triaenophorus meridionalis* occurred at 11–18°C in the delta of the River Dnepr and at 16°C in the River Kuban. Optimum temperature requirements were 22–24°C. The worms containing the eggs were passed from the definitive host *Esox lucius* in the Volga Delta in May.

Fragments of seasonal information for *Agamospirura* sp. (Dubinina, 1949; Komarova, 1964; Mikailov, 1963), *Anisakis* sp. (Dubinina, 1949), *Contracaecum microcephalum* (Dubinina, 1949; Mikailov, 1963), *C. rudolphii* (Dubinina, 1949), *Desmidocercella* sp. (Dubinina, 1949), *Eustrongylides excisus* (Dubinina, 1949), *Eustrongylides* sp. (Dubinina, 1949; Komarova, 1964) and *Porrocaecum* sp. (Dubinina, 1949) have been collected in climate zone 3c, but in most instances low incidences of the larvae were found. Presumably, these larvae occur all year, as has been demonstrated for many of them in some other climate zones. The larvae of *Raphidascaris acus* were found during all seasons in the Volga Delta, U.S.S.R., by Dubinina (1949).

6. *Desert* (*Climate zone* 3d)

Dergaleva and Markevich (1976) reported *Raphidascaris acus* larvae in *Atherina mochon pontica* from the Aral Sea, U.S.S.R., in 0+ and 1+ fishes during June, August, September and October, 1966 to 1970. In data collected in 1971, the infection of older 1+ and 2+ fishes increased from spring to summer, but decreased slightly in autumn. No other seasonal studies from this climate zone are known to the author.

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

Seasonal data are available for the larvae of eight species of cestodes in climate zone 3e. The plerocercoids of the following have been shown to be

present all year: *Diphyllobothrium dendriticum* (Henricson, 1978), *D. ditremum* (Henricson, 1978), *D. latum* (Malakhova, 1961; Nicholson, 1932), *Diphyllobothrium* sp. (Leong, 1975), *Ligula intestinalis* (Titova, 1957), *Triaenophorus crassus* (Miller, 1943a, 1945b, 1952), *T. nodulosus* (Malakhova, 1961; Miller, 1943b, 1945a) and *T. stizostedionis* (Miller, 1945c). The first five species listed above have avian or mammalian definitive hosts, the latter three fish definitive hosts.

Henricson (1978) at Lake Bjellojaure in Swedish Lapland showed that there were no changes apparent in the seasonal incidences of *Diphyllobothrium dendriticum* or *D. ditremum*, but the dynamics were revealed by the seasonal changes in size ranges of the plerocercoids. New invasions of *Salvelinus alpinus* occurred in October and June, corresponding to procercoids in copepods in August to October and to a lesser extent in spring. At Lake Bjellojaure the potential definitive hosts, *Larus canus* for *D. dendriticum* and *Gavia arctica* for *D. ditremum*, arrived at ice-break in June and left the area in September. Adult worms in these hosts, which were not examined, could only deposit eggs in the lake during June to September.

Degeneration of *Diphyllobothrium latum* plerocercoids was reported to occur in winter in *Esox lucius* in Lake Winnipeg, Canada (Nicholson, 1932). Invasive potentials of summer and winter plerocercoids were tested using dogs as experimental definitive hosts. Nicholson (1932) obtained a 27% growth to adult worms from winter plerocercoids but a 60% growth from summer plerocercoids. Nicholson (1932) suggested that invasion of *E. lucius* took place in summer and that the worms died after 5 or 6 months, during the winter.

The majority of the eggs of *Triaenophorus crassus* were passed from the definitive host *Esox lucius* in Lesser Slave Lake, Alberta, Canada, during 1–15 May (Miller, 1943a). Miller (1952) collected together the data from other Canadian habitats, and in most *T. crassus* spawned in the first half of May. By contrast, embryonic development of the eggs of *T. crassus* did not occur until late June or mid-July in the River Yenisei, Siberia, U.S.S.R. (Kuperman, 1967a; 1973b). *T. nodulosus* in Lesser Slave Lake deposited its eggs mostly in early June (Miller, 1943b) and *T. stizostedionis* during the first 2 weeks of June, 1 or 2 weeks later than *T. nodulosus* (Miller, 1945c). As a consequence of the limited season of egg release in these *Triaenophorus* species the invasion of the copepods, growth of the procercoids to an invasive condition, invasion of the fish intermediate hosts and the growth of the plerocercoids all occur, in the sequence as above, within a relatively short span of time. Arnason (1948) found procercoids of *T. crassus* in *Cyclops bicuspidatus* from Heming Lake, Manitoba, Canada, in early June to late June only. Watson (1963a) at the same habitat found an incidence of procercoids of about 1% and observed that the small numbers of *Cyclops* eaten by *Coregonus clupeaformis* seemed sufficient to establish the infection of *T. crassus*. Miller (1945a) estimated that the procercoids of *T. nodulosus* and *T. stizostedionis* in Lesser Slave Lake would be present about mid-June to mid-July. Watson and Lawler (1965) found that the procercoids of *T. crassus*, *T. nodulosus* and *T. stizostedionis* were apparently confined to one brood of

Cyclops bicuspidatus thomasi in Heming Lake, that hatched in early May and matured in mid-July. A 1–2% incidence overall was found, most procercoids were seen in the second half of June, and the incidence fell rapidly in mid-July.

The newly established generations of plerocercoids of *Triaenophorus* species were seen as follows: *T. crassus*, in Lesser Slave Lake, Alberta, Canada, July (Miller, 1945b, 1952), Baptiste Lake, Alberta, July to October (Miller and Watkins, 1946), Heming Lake, Manitoba, Canada, July and August (Lawler, 1950), Nesslin Lake, Saskatchewan, Canada, early August (Rawson and Wheaton, 1950). Miller (1952) suggested that these times were a function of climate and limnological conditions in each habitat. *T. nodulosus*, in Lesser Slave Lake, Alberta, early July (Miller, 1945a), Heming Lake, Manitoba, July (Lawler, 1969b), River Bol'shaya Rechka, U.S.S.R., July (Pronin *et al.*, 1976); and *T. stizostedionis*, in Lesser Slave Lake, Alberta, July (Miller, 1945c). The development of these plerocercoids was completed by September in both *T. crassus* (Miller, 1945b, 1952) and *T. nodulosus* (Miller, 1945a) and thereafter progressive degeneration commenced, as shown by cauda formation (Miller, 1945a, b, 1952). Overall, the duration of life of the plerocercoids was deduced to be at least 3 years in *T. crassus* (Miller, 1945b, 1952) and probably only 2 to 3 years in *T. nodulosus*.

It is interesting to see that in Heming Lake, Manitoba, Canada, *Perca flavescens* was the second intermediate host for *Triaenophorus nodulosus* (Lawler, 1969b). However, in Lesser Slave Lake, Alberta, Canada, *Lota lota maculosa* in their first summer of life served as the fish intermediate host (Miller, 1945a). In the latter habitat, the *P. flavescens* were not utilized, owing to their late date of spawning, June, so that the fry in July were not invaded because the incidence of procercoids of *Triaenophorus* species reached a peak in the latter part of June (Miller, 1945a; Watson and Lawler, 1965).

Very little information is available for nematode larvae in fishes in this climate zone. *Raphidascaris acus* larvae were present all year (Malakhova, 1961; Shul'man *et al.*, 1974; Titova, 1957). At Lake Konche, Karelia, U.S.S.R., Malakhova (1961) found a uniform incidence throughout the year. but Shul'man *et al.* (1974) at Lake Kuĭto, Karelia, found marked variations in incidence.

D. POLAR (CLIMATE ZONE 4)

1. *Polar* (*Climate zone* 4a)

Kuperman (1967a, 1973b) reported that the eggs of the cestode *Triaenophorus crassus* embryonated at water temperatures of 8–12°C in late June or even as late as the middle of July in the River Anadyr, Chukotka, U.S.S.R.

2. *Ice-caps* (*Climate zone* 4b)

Such conditions are unsuitable habitats for cestodes and nematodes in freshwaters. However, it is possible that the eggs of some species carried by avian definitive hosts and dropped on ice would survive for some time.

E. MOUNTAIN (CLIMATE ZONE 5)

No seasonal studies of larval cestodes or nematodes from mountain areas are known to the author.

F. SPECIES STUDIED IN MORE THAN ONE CLIMATE ZONE

Table 3 provides a list of larval cestodes and nematodes that have been studied in more than one climate zone.

The overall analysis of the information for those species for which it is available shows that the cestode and nematode larvae are present in the intermediate fish hosts at all times of the year in all climate zones in which they have been found. These larvae are invasive, usually for a year or more, although with increasing age their vitality decreases in many species. This situation occurs regardless of the climatic conditions, and is similar to that seen for the metacercariae of the trematodes (Chubb, 1979).

It is the processes of invasion of the intermediate fish hosts, growth of the larvae to an invasive condition within these hosts and subsequent invasion of the definitive hosts which change with the climate zones. Some examples of each of these processes will be briefly summarized. All the examples are taken from the cestodes, as the data for the nematodes are fragmentary. Further, most examples are from the mid-latitude climate zones (zone 3), owing to the small amount of information that is available for the tropical (zone 1) and sub-tropical regions (zone 2) of the world.

It is convenient to start with the egg stage. With species in warm-blooded hosts, eggs could be released into the habitat at all times of the year. This probably occurs with *Diphyllobothrium latum* in man. Development of these eggs would commence once water temperatures rose above 8°C (Guttowa, 1961), in milder climate zones, for example parts of zone 3b, from April to November (J. Chubb, personal observations), whereas in the colder climate zones, zone 3e for instance, water temperatures may not reach 8°C until late May, June or even later. At Lake Karperö, Finland, a mass-hatching of the eggs of *D. latum* occurred in the third part of May (Guttowa, 1963). Eggs deposited in the colder periods of the year, i.e. below 8°C for *D. latum*, probably remain viable until temperature conditions suitable for hatching return (see for *D. latum* von Bonsdorff, 1977). Thus the invasion of the fish intermediate hosts commences once copepods are invaded and procercoids grow to maturity, in Lake Karperö, Finland, *D. latum* procercoid occurrence peaked in mid-June, and Guttowa (1963) postulated that a low incidence of procercoids continued thereafter until the autumn when the water temperatures fell to 8°C once more, and egg hatching ceased.

In cold-blooded hosts the eggs of cestodes and nematodes may be produced only at one time of the year. This is shown especially clearly for the species of the genus *Triaenophorus*. Details need not be repeated here, as they have already been given, for each of the species in Section III A and for the relevant climate zones earlier in Section IV. In other species in cold-blooded hosts egg production may occur over a much longer period, sometimes throughout the year. With the one time species, e.g. *Triaenophorus*, the

TABLE 3

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

Larval species	Climate zone											
	1		2		3							4
	c	d	a	b	ai	aii	aiii	b	c	d	e	a
CESTODA												
Bothriocephalus sp.					S	S	S					
Digramma interrupta						S	S		S			
Diphyllobothrium dendriticum					S			S			S	
Diphyllobothrium ditremum								S			S	
Diphyllobothrium latum					S	S					S	
Diphyllobothrium sp.						S		S			S	
Ligula intestinalis			S		S	S	S	S	S		S	
Schistocephalus pungitii						S			S			
Schistocephalus solidus					S	S		S				
Triaenophorus crassus					S	S					S	S
Triaenophorus meridionalis			S						S			
Triaenophorus nodulosus			S		S	S		S			S	
NEMATODA												
Anisakis sp.						S			S			
Contracaecum aduncum					S			S				
Contracaecum microcephalum						S			S			
Contracaecum rudolphii	S				S	S			S			
Contracaecum sp.		S		S	S							
Desmidocercella sp.						S			S			
Eustrongylides excisus			S		S	S		S	S			
Eustrongylides sp.			S		S	S		S	S			
Raphidascaris acus					S	S		S	S	S	S	
Spiroxys sp.				S		S						

coracidia and procercoids will only occur for a short time too, see for instance Watson and Lawler (1965), whereas in the species producing eggs over long periods, e.g. *Bothriocephalus* (see Part IV of review), the coracidia and procercoids will also occur for longer periods. It is the availability of the procercoids that will determine the times of invasion of the fish second intermediate hosts.

The invasion times of the fishes show some correlation with conditions prevailing in the various climate zones. In the genus *Diphyllobothrium* the following times have been demonstrated: 3ai, *D. latum* end April to end of May (Janicki and Rosen, 1917); 3b, *D. dendriticum* March to May (Fraser, 1951, 1960a), *Diphyllobothrium* sp. spring to autumn (Becker, 1967), summer (Mamer, 1978); 3e, *D. dendriticum* October and June (Henricson, 1978). From evidence of procercoid occurrence, it may be inferred that invasion times are: 3aii, *D. latum* mid-June to autumn (Guttowa, 1963); and 3aiii, *D. sebago* July (Johnson, 1975). With *Triaenophorus nodulosus*, invasion times have been shown: 3aii, about 21–24 May to 5–10 July (Kuperman, 1973b); 3b, March to June (Chubb, 1964; Andrews, 1977b), July to September (Lien, 1970); and 3e, early July (Miller, 1945a), July (Lawler, 1969b; Pronin *et al.*, 1976). In general it can be seen that in the climate zones with milder winter conditions (3b) invasion of fishes can occur earlier in the year than in those zones with harsher winter conditions (3aii, 3aiii) or very prolonged cold conditions (3e). That such should be so is no surprise, as it of course reflects the temperature minima and optima required by each of the species for development of a particular stage in the life cycle. Such temperature requirements have been experimentally demonstrated, for instance, for *D. dendriticum* (Halvorsen, 1966, 1970; Kuhlow, 1953c), *D. ditremum* (Wikgren, 1966a, b), *D. latum* (Guttowa, 1961; Morozova, 1955; Neu'min, 1952), *D. sebago* (Meyer, 1967; Meyer and Vik, 1963), *T. amurensis* (Kuperman, 1973b), *T. crassus* (Kuperman, 1967a, 1973b; Watson, 1963b; Watson and Lawler, 1963), *T. meridionalis* (Kuperman, 1973b), *T. nodulosus* (Guttowa, 1955, 1958; Kuperman, 1973b; Lawler and Watson, 1963; Michajłow, 1951; Vogt, 1938; Watson, 1963b; Watson and Lawler, 1963) and *T. orientalis* (Kuperman, 1973b).

Although the invasion of fishes by the procercoid larvae may be seasonal, secondary transfer of plerocercoids from smaller fishes to larger predatory fishes could occur at all seasons. Halvorsen and Wissler (1973) have shown that transfer and re-establishment can experimentally occur at 4°C in *Diphyllobothrium latum*. However, as feeding activity of many fish species falls or ceases in cold conditions, secondary transfer may occur mainly at warmer seasons. In the nematodes, secondary transfer of *Eustrongylides* sp. to *Lepomis gibbosus* was thought to occur mostly in summer as feeding activity of *L. gibbosus* was minimal in winter (Cone and Anderson, 1977). None the less, in the milder mid-latitude climate zones such transfers could occur where fishes continue active feeding.

The attainment of invasive potential in the majority of species of cestodes and nematodes discussed in this review is achieved within the season of invasion, although, details of rates of growth are sparse, except for some

cestodes. In general, the speed of growth increases with temperature (see for example, *Diphyllobothrium ditremum*; Wikgren, 1966a, b). In some Ligulidae, however, the attainment of the invasive condition is not achieved until a period of 12 to 14 months has passed, for example in *Digramma interrupta* and *Ligula intestinalis* (see Dubinina, 1964a).

In migratory fish hosts, although larvae may be potentially invasive at all seasons, they may only attain contact with the definitive host for a limited time. This probably applies as much to *Ilisha parthenogenetica* in *Hilsa ilisha* in the tropics (climate zone 1b) (Pal, 1963) as to *Diphyllobothrium ursi* on Kodiak Island, Alaska (climate zone 3b) (Rausch, 1954). For *D. ursi* plerocercoids, the definitive host can take the fishes, *Oncorhynchus nerka*, only during their spawning migration.

The potential avian definitive hosts for many cestode and nematode larvae are frequently involved in annual migrations, to and from winter feeding quarters and summer breeding grounds. Thus actual or potential definitive hosts have been noted to occur as follows: climate zone 3ai, *Ligula intestinalis* at Iskar Dam Lake, Bulgaria, present all year (Kakacheva-Avramova and Naidenov, 1974); 3aii, *Diphyllobothrium oblongatum* at Lake Michigan, U.S.A., present spring to late-September (Thomas, 1949); 3aiii, *Diphyllobothrium sebago* at Rangeley Lakes, Maine, U.S.A., present spring to autumn (Meyer, 1972); 3b, *Diphyllobothrium dendriticum*, *D. ditremum* at Llyn Padarn, Wales, present all year (J. Chubb, personal observations); 3c, *Digramma interrupta*, *Ligula intestinalis* at Mingechaur Reservoir, Azerbaidan, U.S.S.R. present all year, commonest in winter (Mikailov, 1963); and 3e, *Diphyllobothrium dendriticum*, *D. ditremum* at Lake Bjellojaure, Swedish Lapland, present from ice-break in June, to September (Henricson, 1978). It is evident that ice formation prevents feeding by aquatic birds, therefore most individuals will arrive as ice break-up is completed in the spring and leave before the onset of freezing conditions in the autumn. The period of open water will be shortest in northern climatic regions (3e) and ice formation may not occur in normal winters in milder conditions near the sea in the oceanic areas (3b) or in the more southern latitudes (3c) of the mid-latitude climate zones. In the southern hemisphere colder conditions naturally occur to the south. In sub-tropical and tropical conditions migrations of potential definitive hosts may occur in response to wet or dry conditions, but the author is unaware of any examples where these have been shown to influence the occurrence of cestode or nematode larvae in fishes.

V. General Conclusions

A. Incidence and Intensity of Occurrence

The information for incidence and intensity of occurrence is given in Section III, but it is necessary to appreciate the limitations of these data and to comprehend the seasonal changes in the larval populations in fish hosts.

Two genera of cestode larvae, *Bothriocephalus* sp. and *Eubothrium* sp.

occurred in the intestines of their hosts. *Bothriocephalus* sp. showed an annual pattern of growth during the winter and elimination without reaching maturity in *Lepomis macrochirus macrochirus* (Bogitsch, 1958). Incidence increased from 0% in June to about 37% in January–February and was 0% again by June. The annual biology of the species found by Bogitsch (1958) closely resembles that of many adult cestodes (see Part IV of review), except for the absence of maturation. Other *Bothriocephalus* sp. (Cannon, 1973; Noble, 1970) and *Eubothrium* sp. (Izyumova, 1959) records show varied patterns of no seasonality, perhaps owing to low incidence (Cannon, 1973), winter–spring incidence (Izyumova, 1959) or maximum occurrence from April to June (Noble, 1970). No general conclusion is possible, owing to the small amount of information.

The remaining 26 species of cestode and 21 species of nematode larvae occurred in the somatic tissues of their hosts. All the cestode species and 17 of the nematode species have been shown or can be inferred to have larvae present in the fish host throughout the year. One nematode larva, an unidentified species, was found only in winter (Holl, 1932), and the three other nematode species probably occurred all year but the information is too fragmentary to be certain. Most of the larvae live at least 12 months (see Section V F) in the fish, often much longer, so that infections can be cumulative through the life of the fish from the time of initial invasion, and thereafter as long as invasion continues and the larvae retain their viability. Unfortunately, except for recent invasions in most cestodes, it is impossible to establish the age, or different year classes of larvae if invasion occurs on a seasonal basis. Degenerate and dead larvae are seen for some cestode genera, for instance, *Diphyllobothrium* and *Triaenophorus*, and a few nematodes, *Contracaecum rudolphii* (= *C. spiculigerum*) (Spall and Summerfelt, 1969) and *Eustrongylides tubifex* (Cooper *et al.*, 1978), but it is rarely possible to determine precisely how long these have been in the naturally infected fish host.

In view of the accumulation of larvae through the life of a host individual it is important to study the seasonal biology of the larval parasite in relation to the age of the host rather than the length. Such use of age as the parameter of comparison with larval biology has been made in studies on the following species: Dilepididae, species undetermined (Cone and Anderson, 1977), *Diphyllobothrium dendriticum* and *D. ditremum* (Henricson, 1977, 1978), *Gryporhynchus* sp. (Ivasik, 1953), *Ligula intestinalis* (Arme and Owen, 1968; Sweeting, 1976; Titova, 1957), *Schistocephalus solidus* (Pennycuick, 1971d), *Triaenophorus crassus* (Miller, 1952), *T. nodulosus* (Miller, 1945a), *Contracaecum rudolphii* (= *C. spiculigerum*) (Spall and Summerfelt, 1969), *Eustrongylides tubifex* (Cooper *et al.*, 1978), *Eustrongylides* sp. (Cone and Anderson, 1977), *Raphidascaris acus* (Titova, 1957; Žitňan, 1973) and *Spiroxys* sp. (Cone and Anderson, 1977). The information for these species has been presented in outline in Section III. An overall summary of the patterns of occurrence for some species are presented in Table 4.

It can be seen from Table 4 that there is a variety of possible patterns of incidence, determined in each instance by the specific biology of the

parasite and the particular relationship of the host species to the manifestations of the life pattern of the parasite. It is evident that the incidence and intensity of occurrence of a larval cestode or nematode can vary quite dramatically during the life of each potential fish host at an intraspecific level, and if more than one fish species can serve as host, the pattern of occurrence is also likely to be different at the interspecific level. It is the interaction of behaviour and ecology of both host and parasite that determines the biology of a parasite in a given host species, and as these relationships almost always change through the life of the host individual, it means that a greater or lesser part of the host population may be affected at any one time.

The acceptance of the reality of considerable variations of incidence and intensity of larval parasites through the host populations, in the manner described above and as shown in Table 4, means that the data collected by many authors for total host populations, or more often, for an unspecified part of a population, have only limited meaning. They show presence or absence of the larval form through the year, they may provide some indication of period of invasion of the hosts, but probably little more. Such studies have already been summarized in Section III. It is important therefore, for a fuller understanding of the biological interactions of the parasite and host species to identify the stage of development of the parasite, where possible the length of time it has been present in the host, and the age of the host fish.

It is of course possible to study without difficulty the process of acquisition of larval parasites during the first season of invasion of the fish host. However, once exposed to more than one season of invasion, it may be impossible to follow critically the sequence of further invasions by larvae, unless the previous history of infections are known for the particular year class of fishes, or unless it is possible to age each larval parasite accurately. The latter is rarely possible.

It should be noted that there are many studies that consider age of fishes in relation to incidence and intensity of occurrence of larval parasites. Such studies have not been reviewed in the present account unless they also included a seasonal component.

B. PRINCIPAL AND AUXILIARY HOSTS

The phenomenon of principal and auxiliary hosts (Dogiel, 1964) may be important in the seasonal biology of larval cestode and nematode parasites in fishes. It is certainly relevant at the procercoid phase of development in the copepod hosts (see for example, Michajłow, 1951). Much of the information presented in Section III will refer to principal fish hosts, as these are likely to be the species with high incidences and intensities of infection. It would be interesting to have experimental information about larval establishment and growth rates in a variety of potential hosts. Bråten (1966) used surgical-transfer techniques to demonstrate that *Schistocephalus solidus* was specific to *Gasterosteus aculeatus*, whereas Orr *et al.* (1969) showed that the procercoids would establish in *G. aculeatus*, both marine and freshwater forms, as well as *Pungitius pungitius*, but not in *Barbus* 'Schuberti'.

TABLE 4

Patterns of invasion, incidence, intensity and longevity in some species of larval cestodes and nematodes of fishes. Seasons and months refer to the northern hemisphere. The species are in alphabetical order.

Parasite species	Host species	Invasion of fishes	Incidence and intensity of parasite occurrence	Longevity of larvae	References
CESTODA					
Dilepidae, species undetermined	*Lepomis gibbosus*	Mostly during second summer of life.	Young of year not infected. Incidence and intensity highest age group I, lower to age group IV.	Active age group I, 86% degenerate age groups II, III, all dead by IV.	Cone and Anderson (1977)
Diphyllobothrium dendriticum *Diphyllobothrium ditremum*	*Salvelinus alpinus*	End August–October and May	No seasonal cycle of incidence; intensity and plerocercoid length distributions varied with season in relation to invasion times.	Long-lived	Henricson (1977, 1978)
Gryporhynchus sp.	*Cyprinus carpio*	O+ and 1+ fishes	0+ and 1+ infected, 2+, 4 and 5+ not infected	1 year?	Ivasik (1953)
Ligula intestinalis	*Rutilus rutilus*	During first year of life, a few thereafter	50% fry current season infected by September. Declining incidence with age of fishes owing to mortality and predation.	Probably remainder of life of fish	Arme and Owen (1968)
Schistocephalus solidus	*Gasterosteus aculeatus*	Much of year, but maximal summer, July to September	Cyclical fluctuations, maximal incidence in small fishes, declining with age of fishes owing to mortality and predation	Probably remainder of life of fish	Pennycuick (1971a, b, c, d)
Triaenophorus crassus	Coregonidae	July–August	Increasing incidence first 5 summers of life no increase 6th, decreased thereafter	About 3 years, up to 4–5 years	Miller (1945b, 1952)
Triaenophorus nodulosus	*Lota lota maculosa*	First summer of life, late June, July	Incidence maximal first summer of life, decreasing to probably disappear by third summer	2–3 years	Miller (1945a)

NEMATODA					
Contracaecum rudolphii (= *C. spiculigerum*)	*Micropterus salmoides* *Pylodictis olivaris*	All year, maximum summer? From young age	Incidence high all age classes as fish hosts were piscivores from a very young age	Long-lived	Spall and Summerfelt (1969)
	Ictalurus punctatus *Pomoxis annularis*	Invasion from 4th and 5th year of life	Fish hosts piscivores from 4th and 5th year of life, incidence increased thereafter		
Eustrongylides tubifex	*Perca flavescens*	July–October	Young-of-year and I+ fishes showed increasing monthly larval burdens. Invasion acquired by eating intermediate host (?)	Extreme larval longevity	Cooper *et al.* (1978)
	Micropterus dolomieui	All year, maximum summer?	Infection acquired by predation on other fishes		
Eustrongylides sp.	*Lepomis gibbosus*	Summer	Fishes age groups III–IV, once they were predatory on other fishes	Long-lived	Cone and Anderson (1977)
Raphidascaris acus	*Abramis brama*	—	3+ to 8+ fishes, maximal incidence in autumn in 5-year-old fishes	—	Titova (1957)
Raphidascaris acus	*Phoxinus phoxinus*	—	I–III-year-old fishes, incidence increasing with age (70% maximum, III year fishes in June)	—	Žitňan (1973)
Spiroxys sp.	*Lepomis gibbosus*	Summer	Incidence increased with fish age. Intensity age group I and older similar	Short-lived?	Cone and Anderson (1977)

However, in *P. pungitius* growth of the larvae was much slower at first than in *G. aculeatus*, but ceased after 6 to 8 days, and the larvae were dead by day 11 at 19.5°C and by day 14 at 10°C. A challenge infection of *S. solidus* procercoids to *P. pungitius* after two previous infections given 14 and 28 days before produced a rejection in 3 to 5 days, suggesting that an immune response was operating.

C. INVASION OF FISHES BY LARVAE

Some examples of invasion by larvae have been discussed in Section III, and selected examples are given in Table 4. Overall, two main pathways of invasion predominate, infection from the intermediate invertebrate host and infection from a fish host. In both instances invasion is via the feeding activities of the fishes. Invasion from an invertebrate intermediate host has been shown for the following: cestodes, *Digramma interrupta* (Dubinina, 1953), *Dilepis unilateralis* (Sapozhnikov, 1975), *Diphyllobothrium dendriticum* (Kuhlow, 1953c), *D. ditremum* (Bylund, 1973), *D. latum* (Janicki and Rosen, 1917), *D. oblongatum* (Thomas, 1947), *D. sebago* (Meyer and Vik, 1963), *D. vogeli* (Bylund, 1975b), *Ligula intestinalis* (Rosen, 1919b), *Paradilepis scolecina* (Jarecka, 1970b), *Schistocephalus solidus* (Clarke, 1954), *Triaenophorus amurensis* (Kuperman and Monakov, 1972c), *T. crassus* (Michajłow, 1932a), *T. meridionalis* (Kuperman, 1973b), *T. nodulosus* (Rosen, 1919a), *T. orientalis* (Kuperman and Monakov, 1972c), *T. stizostedionis* (Miller, 1945c) and *Valipora campylancristrota* (Jarecka, 1970a); nematodes, *Contracaecum microcephalum* (Mozgovoi *et al.*, 1965), *C. rudolphii* (Huizinga, 1966; Mozgovoi *et al.*, 1968), *Eustrongylides excisus* (Karmanova, 1965), *Hystrichis tricolor* (Karmanova, 1968), *Raphidascaris acus* (Moravec, 1970), *Spiroxys contortus* (Hedrick, 1935) and *S. japonica* (Hasegawa and Otsuru, 1978). Invasion can occur from a fish host in the following species: cestodes, *Diphyllobothrium dendriticum* (Halvorsen and Wissler, 1973) and *D. latum* (Halvorsen and Wissler, 1973); nematodes, *Contracaecum rudolphii* (= *C. spiculigerum*) (Spall and Summerfelt, 1969), *Eustrongylides ignotus* (von Brand, 1944) and *Eustrongylides* sp. (Cone and Anderson, 1977), Stromberg and Crites (1974) have postulated that the increased incidence of *Triaenophorus nodulosus* in older *Morone chrysops* in Lake Erie, U.S.A., might be explained by this transmission pathway.

The season of invasion in all species appears to be during the summer, although in the milder climate zones (see Section IV C 4), at least in the more coastal areas, invasion can also continue overwinter (see *Schistocephalus solidus*, Pennycuick, 1971b), but even so peak invasions occurred during the warmer summer months. For many species the season of invasion of the fishes is fairly extended, although in the genus *Triaenophorus* it may be rather brief. In Canada, one generation of copepods only each year has been shown to serve as hosts for the procercoids, that brood of *Cyclops bicuspidatus thomasi* hatched in early May and maturing mid-July (Watson and Lawler, 1965).

The invasions of fishes could potentially occur overwinter in a number of

instances, as in Lake Bjellojaure, Sweden, with *Diphyllobothrium dendriticum* and *D. ditremum* (Henricson, 1978), but do not owing to hibernation of the copepods in the cold water conditions of winter. However, a shorter, smaller period of invasion occurred in the spring resulting from overwintered procercoids. Transfers of infections from fish to fish, where appropriate, could also potentially occur in winter, but no evidence of this was found by Cone and Anderson (1977) for *Eustrongylides* sp. This was attributed to a minimal feeding level by the fishes during the winter months. However, with *Diphyllobothrium latum* there is some evidence that fish to fish transfers do occur overwinter in natural conditions (see Wikgren, 1963) and have been shown to occur in experimental conditions at 4°C (Halvorsen and Wissler, 1973).

Vogt (1938) showed that procercoids of *Triaenophorus nodulosus* must pass rapidly through the stomach of *Salmo gairdneri* and enter the intestine quickly in order to survive. Such a condition depended on the rapid passage of food through the fish stomach. At 2–4°C, the passage of food was slow so that almost all of the procercoids perished. Thus in winter conditions, even if larvae are taken with food by the potential species of fish host, establishment may not occur owing to the procercoids perishing in the stomach of the fish. Dubinina (1966) and Kuperman (1973b) considered that the conclusions of Vogt (1938) probably applied to all pseudophyllidean cestodes (including diphyllideans in classification used in this review).

It is evident that there are a variety of possibilities for invasion, although in mid-latitude climates the majority of such transfers probably occur during the warmest months, reflecting the optimum temperature conditions for both hosts and parasites. It is unfortunate that there are so few data from tropical regions (see Section IV A), and none pertaining to the actual seasons of invasion, as information from the warmer parts of the world might assist in an understanding of the factors operating in the mid-latitude climates.

D. GROWTH OF LARVAE IN FISHES

Temperature has been shown to influence the rate of growth of the plerocercoid larvae of *Diphyllobothrium ditremum* in an artificial medium (Wikgren, 1966a, b). The mitotic rate was found to be very temperature-sensitive, and generation times, which equalled the time needed by the cell population to double in number and hence was also approximately equal to the time needed by the larvae to double in mass, were calculated at a range of temperatures. At 10°C the generation time was 960 h, at 20°C 252 h, at 30°C 91 h and at 38–39°C only 54 h (Wikgren, 1966b). These growth rates suggested that the development from procercoid to full-grown plerocercoid lasted about 6–7 months at 10°C but only 20 days at 20°C. Sinha and Hopkins (1967) examined the growth rate of *Schistocephalus solidus* also in an artificial medium at temperatures between 4 and 40°C. Between 7 and 23°C the rate of growth increased nearly exponentially (Q_{10} 3·6) and between 23 and 27°C the growth rate of the plerocercoid was maximal.

Although determinations of growth rates of the larvae in fishes at a range of temperatures are rather unusual, evidence from growth studies of the

procercoids in the copepod hosts (for instances see, *Digramma interrupta*, Dubinina, 1953; *Diphyllobothrium dendriticum*, Halvorsen, 1966; Kuhlow, 1953c; *D. latum*, Guttowa, 1961; *D. oblongatum*, Thomas, 1947; *Ligula intestinalis*, Dubinina, 1964a; *Schistocephalus solidus*, Dubinina, 1957; *Triaenophorus crassus*, Kuperman, 1973b; and *T. nodulosus*, Michajłow, 1951; Vogt, 1938) show that low temperatures inhibit growth and that there is an optimum temperature for each species. In the light of the work of Wikgren (1966a, b) and Sinha and Hopkins (1967) it is reasonable to conclude that low temperatures will produce minimal growth and that optimum temperatures for larval growth in fishes will be reached in natural conditions in mid-latitude climates during the summer months. In fact, field observations and experimental studies on cestode larvae at one temperature confirm this conclusion.

Some examples of growth times from procercoid to invasive plerocercoid are: *Digramma interrupta*, 12–14 months (Dubinina, 1953); *Diphyllobothrium dendriticum*, about 38 days (Kuhlow, 1953b); *D. ditremum*, about 153 days (Bylund, 1973); *D. latum*, appeared completely developed at 2–7 months, but not invasive up to 7 months (Kuhlow, 1953b), but in *Gymnocephalus cernua* an invasive plerocercoid was obtained in $3\frac{1}{2}$ months (Kuhlow, 1955); *D. oblongatum*, 135 days (Thomas, 1947); *D. sebago*, about 173 days (Meyer and Vik, 1963); *Ligula colymbi*, more than 365 days (Dubinina, 1964b); *Ligula intestinalis*, 12–14 months (Dubinina, 1953), but Orr and Hopkins (1969b) obtained plerocercoids with genital primordia after 60 days; *Schistocephalus pungitii*, 2 months (Dubinina, 1964b); *S. solidus*, after 177 days (Dubinina, 1957), 3 months (Hopkins and McCaig, 1963), or as little as 2 months, with full invasiveness attained in 3 months (Orr and Hopkins, 1969a); *Triaenophorus amurensis*, 42 days (Kuperman, 1973b); *T. crassus*, about 1–2 months (Miller, 1952); *T. nodulosus*, about 41–44 days (Kuperman, 1973b); and *T. orientalis*, about 39 days (Kuperman, 1973b). For the conditions under which each of these species was studied, reference should be made to the original sources. It can be seen that as temperature influences development, to be exactly comparable all the above growth times should relate to one temperature. However, this is not so, therefore these times are at best only an indication of the duration of growth. In natural conditions some species have plerocercoids which all attain invasiveness during the summer of establishment in the fish host (e.g. *Triaenophorus* spp.), some may attain this condition during the same summer, or the second summer, depending on time of establishment in the fish host and temperature (e.g. *Diphyllobothrium* spp., *Schistocephalus* spp.), whereas others do not reach an invasive condition until the second summer (e.g. *Digramma* spp., *Ligula* spp.).

The details of growth of the individual species of plerocercoids, where known, have already been summarized in Section III.

The fact that growth rate of the cestode larvae is determined by temperature, allows and explains the variations reported within some species in different climatic conditions in which the species can occur. In colder conditions all phases of the life cycle will be prolonged, and the life span of each increased. Strazhnik and Davydov (1975), with *Ligula*

intestinalis plerocercoids, have shown that the life duration decreases with increasing temperature and is in reverse relation to their metabolism.

To this point the discussion in this section has centered on cestode larvae. This is because few details of development of nematode larvae in the fish host are available. Moravec (1970) experimentally infected *Noemacheilus barbatulus* with eggs containing second-stage larvae of *Raphidascaris acus*, and described their development. Third-stage larvae had a length of 3·02–5·20 mm at 153 days post-infection, and were invasive to *Salmo gairdneri*. The rate of development of the eggs and other stages was influenced by temperature, low temperatures greatly prolonging the duration of each stage in the life cycle (Moravec, 1970). It is likely, and this is indicated by studies on nematodes in invertebrates, that temperature influences the growth rates and longevity of each stage in the nematode life cycle in freshwater fishes.

E. MORPHOLOGICAL DIFFERENCES

Morphological changes in larvae occur after invasion of the fish host as a result of the growth of the larvae and may also be found as a function of progressive degeneration leading to death. The process of invasion of hosts has been discussed in Section V C. The importance of these changes in the present context is that they represent one way of determining whether or not recurring annual, seasonal invasions are taking place in a natural population of fishes. Differences in larval morphology have been used in this manner for the following species: cestodes, Dilepididae, degeneration (Cone and Anderson, 1977); *Diphyllobothrium dendriticum*, size of larvae (Fraser, 1960a; Halvorsen, 1970; Henricson, 1978), degeneration (Powell and Chubb, 1966); *D. ditremum*, size of larvae (Fraser, 1960a; Henricson, 1978), degeneration (Powell and Chubb, 1966); *D. latum*, activity and degeneration (Nicholson, 1932), presence in viscera compared with muscle (Wikgren, 1963); *Diphyllobothrium* sp., degeneration (MacLulich, 1943); *Ligula intestinalis*, weight changes (Arme and Owen, 1968; Žitňan, 1964), length of parasite (Lawler, 1964); *Schistocephalus solidus*, parasite index (Arme and Owen, 1967), weight changes (Clarke, 1954; Hopkins, 1950; Hopkins and Smyth, 1951; Pennycuick, 1971b); *Triaenophorus crassus*, presence of cauda and degeneration (Miller, 1943b, 1952; Scheuring, 1929); *T. nodulosus*, presence of cauda and degeneration (Andrews, 1977a, b; Chubb, 1964; Lawler, 1969b; Lien, 1970; Miller, 1945a; Mishra, 1966; Scheuring, 1923); nematodes, *Contracaecum rudolphii* (= *C. spiculigerum*), viability and degeneration (Spall and Summerfelt, 1969; Tedla and Fernando, 1969); and *Eustrongylides tubifex*, degeneration (Cooper *et al.*, 1978).

F. LONGEVITY

It was stated in Section V A that some 26 species of cestodes and 17 species of nematodes had larvae present in the somatic tissues of the fish hosts throughout the year. The total longevity has been determined or estimated for the following species: cestodes, *Digramma interrupta*, 3 years or more (Bauer

et al., 1969), 2 years (Kosheva, 1956); *Dilepis unilateralis*, about 1 year (Sapozhnikov, 1975); Dilepididae species undetermined, 1–2 years (Cone and Anderson, 1977); *Diphyllobothrium dendriticum*, at least 2–3 years (Halvorsen, 1970); *D. latum*, 5 to 6 months (Nicholson, 1932); *D. ursi*, at least 2–3 years (Rausch, 1954); *Gryporhynchus* sp., about 1–2 years (Ivasik, 1953); *Ligula colymbi*, at least 2 years (Bauer, 1959); *L. intestinalis*, remainder of life of host (Arme and Owen, 1968; Orr, 1967a; Owen and Arme, 1965; Sweeting, 1976), 2 years (Kosheva, 1956), 2–3 years (Deksbakh and Shchupakov, 1954) or longer at lower temperatures (Strazhnik and Davydov, 1975); *Schistocephalus solidus*, life of host (Pennycuick, 1971a); *Triaenophorus crassus*, at least 3 years (Miller, 1945b), 4–5 years (Miller, 1952); *T. nodulosus*, about 3 years (Andrews, 1977b; Chubb, 1964), 2–3 years (Lien, 1970), capsules gone by third summer (Miller, 1945a); and *Valipora campylancristrota*, about 9 months (Jara and Olech, 1964); nematodes, *Contracaecum rudolphii* (= *C. spiculigerum*), more than 1 year (Spall and Summerfelt, 1969; Tedla and Fernando, 1969); *Eustrongylides ignotus*, 4 years (von Brand, 1944), more than 1 year (Cooper *et al.*, 1978); and *Spiroxys* sp., at least 1 year (Cone and Anderson, 1977).

It will be noted that where more than one investigation of a species has been undertaken, some variation of estimates of longevity may occur. Differences such as these cannot be explained owing to the relatively small amount of information available. It is probable that longer survival times may occur in colder regions of the world, owing to the slower development of all stages in cooler conditions. The longevity of the plerocercoids of *Diphyllobothrium latum* estimated as 5 to 6 months by Nicholson (1932) is too short, as Kuhlow (1953b) found that at 6 months of age the larvae were scarcely 1 cm long, although some were invasive at 3½ months (Kuhlow, 1955).

G. MORTALITY OF HEAVILY INFECTED FISHES

Mortality, or disappearance of infected fishes from the population, has been reported by several authors who have studied the details of the larval–host interaction. In the genus *Diphyllobothrium*, mortalities of host fishes occur in some species, for instance, *D. dendriticum*, but not in others, *D. latum*. The mortalities associated with *D. dendriticum* have been described by Duguid and Sheppard (1944), Hickey and Harris (1947), Fraser (1951, 1960a) and Henricson (1978). Duguid and Sheppard (1944) observed an epizootic in South Wales in July, which decimated the population of *Salmo trutta*. Hickey and Harris (1947) found dead *S. trutta* at Poulaphouca Reservoir, Eire from May 1943, with epizootic conditions in the period May–September 1944 and again in the summer of 1945. Hickey and Harris (1947) demonstrated by experiment in saline that higher temperatures (15°C) caused the plerocercoids to leave their capsules, hence explaining why the mortalities were confined to the summer months. Fraser (1951, 1960a) also reported dead *S. trutta* at Yeo Reservoir, Blagdon, Somerset, England, in the spring and summer of 1947; maximum mortality occurred in July–August. The number of plerocercoids present in the *S. trutta* as well as the higher summer water

temperatures were considered important, together with the seasonal invasion of the fishes in spring and early summer, the additional infection load proving fatal to older more heavily infected *S. trutta* (Fraser, 1960a). Henricson (1977, 1978) at Lake Bjellojaure, Sweden described the dynamics of infection of *Salvelinus alpinus* by *D. dendriticum*. Chronic losses of *S. alpinus* from the population were suggested by Henricson (1977) because intensities of infection were not cumulative over a certain age of fish. Henricson (1978) found further support for the deleterious effect of *D. dendriticum* on its fish intermediate host from the seasonal variation in the structure of the plerocercoid population. During the winter, October to April, there was no recruitment to the plerocercoid population, so that the mean intensity of infection, the overdispersion and the frequency distribution of the plerocercoids should have remained constant in the same age group of fishes. However, a lowering in intensity and overdispersion and the changed frequency distributions during winter showed that there was a loss of parasites, caused by the death of heavily infected hosts. The loss of parasites probably continued from May to October, but was complicated owing to the periods of recruitment of procercoids, so that the mortality was not easily shown. Henricson (1978) also demonstrated that the mortality was correlated not only with the number of plerocercoids, but also with their location in the fishes, as shown from the seasonal variation in the proportion of plerocercoids in different organs. Where such lethal levels are reached, feedback controls operate, and such a system has also been described by Pennycuick (1971b, c) for *Schistocephalus solidus* in *Gasterosteus aculeatus*. With the epizootics of the *S. trutta* during the summer months the plerocercoids are probably made more readily available to scavenging gulls, potential definitive hosts, but the mortality of *S. alpinus* in Lake Bjellojaure during the winter months cannot help in this way, as the *Larus canus* were present June–September only (Henricson, 1978).

In many localities plerocercoids of both *Diphyllobothrium dendriticum* and *D. ditremum* occur in the same host individual. However, it is generally accepted that it is the plerocercoids of *D. dendriticum* which cause the mortalities (Duguid and Sheppard, 1944; Hickey and Harris, 1947; Henricson, 1978).

Transfer of *Diphyllobothrium sebago* plerocercoids from the fish *Osmerus mordax* to the definitive host *Larus argentatus* in Maine, U.S.A., was also assisted by mortality of *O. mordax*, however, in this area, the fish mortalities were due to the spawning activities of these fishes (Meyer, 1972).

Epizootics of *Diphyllobothrium* sp. reported by Becker (1967) and Becker and Brunson (1967) in *Salmo gairdneri* at Goodwin Lake, Washington State, U.S.A., occurred in the autumn and winter of 1963–64. Chronic mortality also occurred in association with warm temperatures during the spring, summer and autumn, after the fishes were stocked, when the *S. gairdneri* were relatively small and plerocercoid growth rapid.

It is clear that ligulids reduce the longevity of the host fishes. This is shown by the disappearance of infected fishes from a population, for instances, *Abramis brama* with *Digramma interrupta* (Reshetnikova, 1965), *Alburnus alburnus* (Harris and Wheeler, 1974) and *Rutilus rutilus* (Sweeting, 1976) with

Ligula intestinalis, and *Gasterosteus aculeatus* with *Schistocephalus solidus* (Pennycuick, 1971b). These progressive losses occur at all seasons. Epizootics caused by *D. interrupta*, *L. intestinalis* and *S. solidus* appear to occur in summer conditions (Bauer *et al.*, 1969; Deksbakh and Shchupakov, 1954; Vik, 1954). The altered behaviour patterns of fishes infected by *L. intestinalis* (Dence, 1958; Harris and Wheeler, 1974; Sweeting, 1977) and *S. solidus* (Lester, 1971; Pennycuick, 1971b) greatly increase the likelihood of predation by potential avian definitive hosts, as well as by predatory fishes such as *Esox lucius*.

Epizootics of fishes which have been attributed to plerocercoids of *Triaenophorus nodulosus* have been summarized by Bauer (1959). It seems likely that these epizootics in natural waters represent an unusual combination of ecological factors leading to a mass occurrence of the parasite. In pond fish culture conditions epizootics can usually be avoided by changed procedures. No doubt some lower level of natural mortality occurs in most populations of fishes, caused by plerocercoids of *T. nodulosus* and other cestodes, but these are more difficult to demonstrate except where regular and rigorous fish and parasite sampling techniques are adopted.

Mortalities caused by larval nematodes in freshwater fishes appear insignificant in a seasonal context, perhaps from a lack of detailed studies.

H. SPORADIC POPULATION CHANGES

Changes in status of both host and parasite populations are to be expected, but owing to the rarity of long-term investigations are reported relatively infrequently. Some changes have been noted for larval cestode parasites in freshwater fishes.

A stability of plerocercoid populations of *Diphyllobothrium latum*, as expressed by incidence in five species of host fishes, was seen by Kuperman (1977) in the Rybinsk Reservoir, U.S.S.R., from 1966 to 1976. Campbell (1974) found no marked changes of incidence of *Diphyllobothrium* spp. (*D. dendriticum* and *D. ditremum* combined) in Loch Leven, Scotland, from 1967 to 1972. Thus incidence in *Salmo trutta* of length 34·6 to 39·5 mm was: 1967–68, 22%; 1968–69, 23·8%; 1969–70, 30·2%; 1970–71, 29·2%; and 1971–72, 24·5%. However, Powell and Chubb (1966) at Llyn Padarn, Wales, found evidence of significant changes. *D. dendriticum* occurred as follows [% (mean number plerocercoids per fish)]: in *Salvelinus alpinus*, 1963, 100% (4·6); 1964, 96% (7·8); and 1965, 100% (12·0); in *Salmo trutta*, 1963, 46% (14·7); 1964, 65% (11·6); and 1965, 31% (0·6). *D. ditremum* also showed changes: *S. alpinus*, 1963, 100% (66·2); 1964, 100% (24·3); and 1965, 93% (36·5); *S. trutta*, 1963, 77% (84·6); 1964, 77% (38·3); and 1965, 65% (3·1). Powell and Chubb (1966) considered that their observations had been made during a period of decline in a cycle of changes between high and low levels of infections. Henricson (1978) at Lake Bjellojaure, Sweden also found long-term fluctuations in the infections of *D. dendriticum* and *D. ditremum* in *S. alpinus*. Reference has already been made to feedback controls operating if lethal levels of *D. dendriticum* occur (see Section V G). In summary, Henricson

(1978) suggested that the plerocercoid populations of *D. dendriticum* and *D. ditremum* at Lake Bjellojaure were limited through success of transmission, but that changes in the ecosystem of biotic or climatic origin may favour transmission and make parasite numbers build up in the hosts until lethal levels were reached. In the study at Lake Bjellojaure there was a rise in the infection level at the start of the period of investigation and a decrease at the end (Henricson, 1978).

Lawler (1969a) at Lake Mälaren, Sweden, found evidence for variations in year class strength of *Triaenophorus nodulosus* plerocercoids in *Perca fluviatilis*. He concluded that annual differences in success of transmission of *T. nodulosus* and of availability of hosts owing to favourable ecological conditions caused such variations. Lawler (1969b) has discussed the dynamics of incidence of *T. nodulosus* plerocercoids in *Perca flavescens* compared with catch per unit effort of the definitive host, *Esox lucius*, at Heming Lake, Manitoba, Canada, and has demonstrated a relationship. The incidence in *P. flavescens* in 1 year was reflected in the incidence of adult worms in *E. lucius* the following year.

	P. flavescens infection (%)		*E. lucius* infection (%)
1964	61	1965	95
1965	37	1966	30
1966	18	1967	39
1967	61	1968	98

However, as Lawler (1969b) stated, the interrelations between the species associated in the life cycle are complex. Nonetheless, it is evident that changes can be related by careful study of all aspects of the life cycle of a parasite.

Andrews (1977b) also found differences in the status of the population of *Triaenophorus nodulosus* in *Perca fluviatilis* at Llyn Tegid, Wales, between 1957–58 (Chubb, 1964) and 1975–76 (Andrews, 1977b). In 1975–76 there was a higher incidence, mean intensity and occurrence of degenerating plerocercoids, but both populations were overdispersed, especially so in 1975–76 (Andrews, 1977b).

The populations of both *Ligula intestinalis* and *Schistocephalus solidus* plerocercoids affect the reproductive capability of their host fishes. At Yeadon Tarn, near Leeds, Yorkshire, England, Sweeting (1977) saw no fry of *Rutilus rutilus* during 2 years, which was attributed to the high incidence of *L. intestinalis* seriously impairing the reproductive capacity of the infected fishes. The population of *R. rutilus* fell dramatically in spring 1969, hence further studies were suspended (Sweeting, 1977). At Hoggenfield Loch, Scotland, Orr (1967a) noted that only older *R. rutilus* were infected by *L. intestinalis* and this indicated that the life cycle of the parasite was no longer being completed, but at Milngavie Reservoir fry were infected, so that active transmission was occurring. At Oneida Lake, New York, U.S.A., Noble (1970) noted that *L. intestinalis* was not recorded in a 1966 survey, but in the

late summer of 1968 plerocercoids were frequent in the body cavities of young *Perca flavescens*. Lawler (1964) found marked annual fluctuations and also marked regional differences in occurrence of *L. intestinalis* in Heming Lake, Manitoba, Canada. The more heavily infected fishes came from an area of the lake that was highly productive, both in yield of plankton and fishes.

Schistocephalus solidus delayed or prevented gametogenesis in both sexes of *Gasterosteus aculeatus* (Arme and Owen, 1964; Pennycuick, 1971a). Evidence of cyclical fluctuations of *Gasterosteus aculeatus* and *Schistocephalus solidus* populations were seen by Arme and Owen (1964, 1967) and Pennycuick (1971a). Pennycuick (1971a) suggested that these changes were brought about by the *S. solidus* infections in the following way. The infection tended to build up until many fishes died or were eaten, or were prevented from breeding. This caused a gradual decline in the numbers of fishes and hence in the size of the parasite population. As the number of fishes decreased and the infection became less, breeding was more successful and the numbers of fishes increased, so that this in turn enabled the *S. solidus* infection to build up again. These fluctuations would be modified by other external factors such as food supply, winter temperatures, abundance of other host species, etc. (Pennycuick, 1971a). A span of 4 to 5 years per cycle was suggested (Pennycuick, 1971b).

In all of the host–parasite relationships described in this review and elsewhere, whether occurrence appears to be static or to show short- or long-term fluctuations, it must be remembered that the processes of invasion, growth of larvae, transmission, or degeneration and death are in fact dynamic. It is the sequences of these processes, modified by abiotic and biotic factors, which determine the status of the populations at any given time.

I. IMMUNITY

Halvorsen (1966) found a seasonal variation in incidence and intensity of experimental infection of *Eudiaptomus gracilis* by *Diphyllobothrium dendriticum*. Kisielewska (1957) earlier had reported that the development of *Drepanidotaenia lanceolata* in the copepod host was related to season. It is not known whether these differences relate to resistance to infection by the copepod host at some times of the year, or to an adaptation by the copepod to different habitats through the seasons, or to some variation in invasive potential by the cestode oncospheres at different times of the year. The latter is unlikely, however, as Halvorsen (1966) did not find the variation with season in all species of copepod tested. Variation in the carrying capacity of copepods with season could influence seasonal dynamics.

Parasite-specific antibodies to *Digramma interrupta* and *Ligula intestinalis* plerocercoids were demonstrated in the blood of *Abramis brama* by Molnár and Berczi (1965). Orr *et al.* (1969) have suggested that a cytotoxic antibody may be involved in the rejection of *Schistocephalus solidus* plerocercoids by *Pungitius pungitius*. However, in the principal fish host, *Gasterosteus aculeatus*, there was no evidence of resistance to superinvasion. Scheuring (1919) claimed to have found superinvasive immunity in *Salmo trutta* to infection

by procercoids of *Triaenophorus nodulosus* and Vogt (1938) attempted to demonstrate this with a series of experimental infections of *Salmo gairdneri* with *T. nodulosus* procercoids, and concluded that immunity was not developed. However, Bauer (1959) considered that Vogt's (1938) conclusion was unfounded, owing to the experiment having been incorrectly planned. Kuperman (1973b), as a result of his experimental infections, invoked a host defence mechanism on the part of the fishes to explain the low infections by plerocercoids of *T. nodulosus*, whereas Chubb (1964) explained low intensity of infections in *Perca fluviatilis* at Llyn Tegid, Wales, by the apparent absence of copepods from the food during the months in which infection could occur, and not with an immunity effect. The matter remains to be resolved by a series of experiments with proper controls. In the meantime, it seems unlikely that immune reactions on the part of the fish host play any significant role in the seasonal dynamics of larval parasites in fishes.

Kuhlow (1953c) was unable to reinfect *Larus ridibundus* with further plerocercoids of *Diphyllobothrium dendriticum* for at least 6 months after an initial experience of adult *D. dendriticum*. Should this phenomenon apply widely, it could play a significant part in seasonal dynamics.

J. SEASONAL STUDIES IN WORLD CLIMATE ZONES

Seasonal studies of larval cestodes and nematodes in relation to world climate zones have been discussed in detail in Section IV. It is important here to emphasize one point only, the scarcity of information from tropical and subtropical regions of the world. It is to be hoped that fish helminthologists in these regions will rectify this situation as soon as possible.

K. AN HYPOTHESIS FOR SEASONAL OCCURRENCE

The information presented in the previous sections of this review is frequently fragmentary for individual species, nevertheless, seen overall it is possible and helpful to produce an hypothesis to explain the seasonal dynamics of cestode and nematode larvae in fishes.

The following points can be taken as reasonably well-established. In the fishes the larvae are present at all seasons (see Section V A). They are probably potentially invasive to the definitive host through the year, although the level of establishment achieved can vary from season to season in experimental conditions [see Section III A 2(a), *Diphyllobothrium latum*]. The larvae are frequently long-lived in the fish host (see Section V F), although in the end degeneration and death of the worms can occur (see Section V E). In some species the larvae survive the remainder of the life of the fish host, although changes in host behaviour owing to the presence of the worms increases the chance of predation of the infected fishes thereby enhancing the possibility of transmission (see Section V G). Mortality of heavily infected fishes can also increase the likelihood of transmission, and can occur at either a particular season, for instance summer, or throughout the year (also see Section V G).

The seasonal dynamics of the larvae in fishes are influenced decisively by events before entry of the invasive larvae into the fish host, and after the transmission of the worms to the definitive host.

In warm-blooded avian or mammalian definitive hosts the adult cestodes or nematodes can be present at all times of the year, and can also liberate eggs into the habitat at all times of the year [see Section III A 2(a), *Diphyllobothrium latum* in man]. However, in many mid-latitude climate zones seasonal migrations of potential definitive hosts occur [see Section III A 2(a) *D. oblongatum*, *D. sebago*], which limit the actual presence of adult worms in these hosts to a particular season, thereby limiting the time of egg release. In the cold-blooded fish and amphibian definitive hosts, a limited season of maturation and egg release is normal [see Section III A 1(b) *Triaenophorus* species, Section III E 1(a), *Raphidascaris acus*, Section III E 2(a) *Spiroxys* species].

The eggs once liberated into an aquatic habitat are controlled in their development by the prevailing water temperatures. Eggs liberated at all times of the year [see Section III A 2(a) *Diphyllobothrium latum*] retain their viability and accumulate through the cold seasons, until such time arrives when water temperatures allow hatching. A rapid rise in water temperature in spring can result in a short period of mass hatching, followed by a longer period through the summer months during which egg hatching occurs at a lower more stable level. Declining water temperatures in autumn will eventually terminate hatching for that year, and overwinter egg accumulation will again occur. Eggs liberated at a particular season [see Section III A 1(b) *Triaenophorus* species] are usually released into the habitat in spring as water temperatures rise, therefore mass hatching occurs in spring or early summer. Thus the eggs are present for a limited season only. *Spiroxys* species release their eggs in summer [see Section III E 2(a)], and also hatch at summer water temperatures within a short time to give a limited season of occurrence. The times when the eggs hatch for all species are determined by the temperature minimum, optimum and maximum for each species. In mid-latitude climates, times will be earlier in the more southern latitudes, later in the more northern, but of course modified in each habitat by other factors such as altitude and water depth (see Section IV C). Overall, in many mid-latitude climates, the presence of the cold winter will mean that peak hatching activity will occur in spring and early summer, and all within the period spring to autumn. Such a constraint should not apply in sub-tropical and tropical zones.

Transmission of the hatched larvae (or eggs where relevant) to the invertebrate intermediate host (where applicable) will tend to be concentrated in spring and early summer in mid-latitude climates. Development in the invertebrate host will be facilitated at this time owing to water temperatures approaching optimum levels. The ingestion of the invertebrate host together with the invasive cestode or nematode larva will also be facilitated by warm water conditions which stimulate the feeding of most species of potential fish hosts. In addition, by summer newly hatched fry will be available for invasion. The establishment and growth of the larvae in the fish tissues also

occurs most rapidly in warm conditions enhancing the speed at which transmission back to a definitive host can occur, although in some species [see Section III A 2(b) *Digramma interrupta*, *Ligula* species] the larvae may not attain their invasive potential until the second summer.

It is suggested, therefore, that the seasonal calendar of events for larval cestodes and nematodes in freshwaters of the world is determined by the interactions of the annual life cycle pattern of the parasite with those of all its hosts, principal and auxiliary, intermediate or definitive. Water temperature, as determined by climate, the sum of weather conditions over a long period of time (Levine, 1978), or weather, environmental conditions at a particular time and place (Levine, 1978), will affect the rate and time of these interactions of parasite with potential hosts by operating on both parasite and hosts, and will control the rate of development of the parasite within each of these hosts. The exception to the control by environmental temperature will be for the adult worms of those parasites with warm-blooded definitive hosts, where the rate of development to maturity is determined by the body temperature of the host. It is possible that the hormonal condition or state of sexual development of the host will modify seasonal patterns of development of larval parasites in fishes, but at the moment there is no evidence of this.

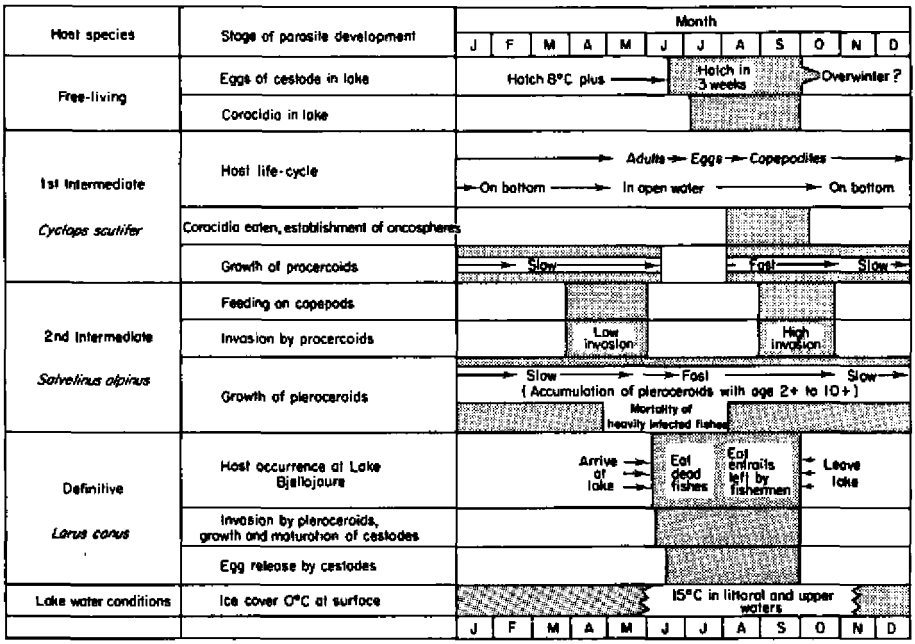

FIG. 3. Seasonal calendar for the life cycle of *Diphyllobothrium dendriticum* in Lake Bjellojaure, Sweden, based on data from Henricson (1977, 1978). Stipple indicates presence of stage. The occurrence of the adult worms in *Larus canus* was not established by sampling.

Figs 3 and 4 attempt to illustrate the points made in the preceeding discussion by reference to two species, one with an avian definitive host and one with a fish definitive host. The examples are both of cestodes, because more information is available than for nematodes. Fig. 3 shows the seasonal calendar for *Diphyllobothrium dendriticum* in Lake Bjellojaure, Sweden, using the information from Henricson (1977, 1978). In this cestode the plerocercoid larvae are cumulative through the life of the host, and fishes of ages 2+ to 10+ were infected in Lake Bjellojaure. The definitive host was thought to be *Larus canus*, which were present June to late September.

Fig. 4 gives the seasonal calendar for *Triaenophorus nodulosus* in Lesser Slave Lake, Alberta, Canada, based on the data from Miller (1943a, b, 1945a). In this habitat the intermediate host fish *Lota lota maculosa* is exposed to invasion by plerocercoids only during its first summer of life, through which time it feeds on plankton. Later in life these fishes are benthophagous and are no longer available for invasion by *T. nodulosus*. Miller (1943a, b, 1945a) did not give data for water conditions at Lesser Slave Lake, so these are taken

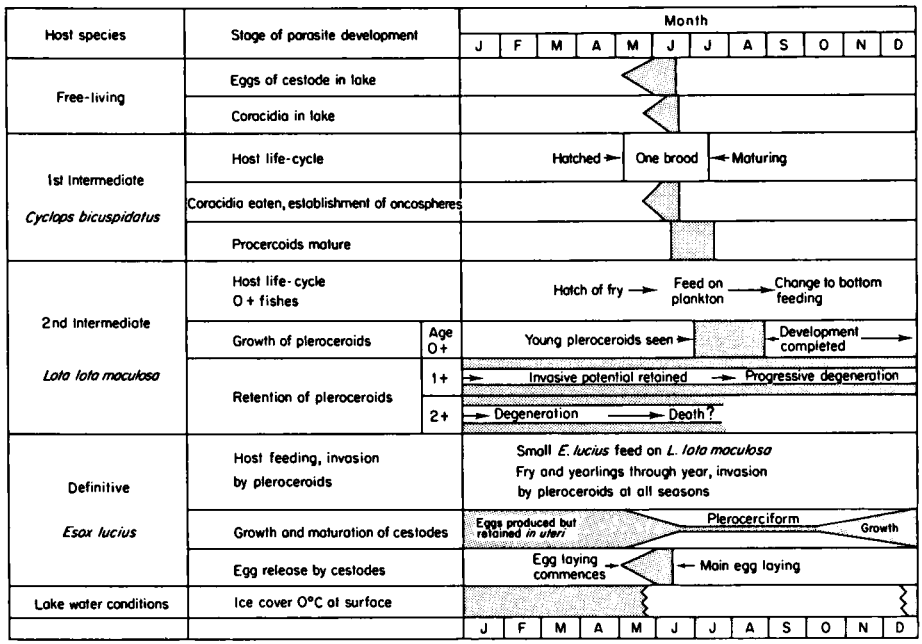

FIG. 4. Seasonal calendar for the life cycle of *Trianeophorus nodulosus* in Lesser Slave Lake, Alberta, Canada, based on data from Miller (1943a, b, 1945a). Stipple indicates presence of stage. Information for the first intermediate host is from Heming Lake, Manitoba, Canada (Watson and Lawler, 1965). Dates of ice break and formation are from Cold Lake, Alberta, Canada (Holmes *et al.*, 1977). The presence of eggs and coracidia in the lake were not established by sampling, but were deduced from experimental studies. Full information about the maturation of the adult worms in *Esox lucius* can be found in Part IV of this review.

from Holmes *et al.* (1977) for Cold Lake, Alberta, Canada, in the same climate zone. Furthermore, although Miller (1943b) established that *Cyclops bicuspidatus* was the first intermediate host for *T. nodulosus* in Lesser Slave Lake, the data for the brood of *C. bicuspidatus thomasi* invaded by procercoids of *Triaenophorus* species are taken from Watson and Lawler (1965) for Heming Lake, Manitoba, Canada, also in the same climate zone.

L. EXPERIMENTAL STUDIES

In Section III a wide variety of experimental studies concerned with the life cycles and seasonal occurrence of cestodes and nematodes have been described. It will have been noted that many more of these studies have been carried out on cestode rather than on nematode larvae in fishes. Further, it will also have been seen that the most comprehensive experimental studies have been concerned with the hatching of the eggs of the cestodes and the invasion and development of the procercoid larvae in the invertebrate intermediate host. For these aspects, for some species of cestodes, for instance, in *Diphyllobothrium latum* and *Triaenophorus nodulosus*, fairly complete data are available about development and growth rates over the range of temperatures from minimum through optimum to the maximum above which thermal death of the larvae occurs. In addition, some indications of the longevity of the eggs at below the minimum temperature for development have been determined experimentally. These data are of much importance in explaining parts of the annual rhythm of the life cycles of these parasites. Clearly, for those species for which this information is not available, experimental studies are required to provide it.

The work indicated above relates to the stages before entry of the parasite into the fish host, but is important as pertaining directly to the seasonal timing and process of invasion of the fish host by the appropriate larval stage.

The information about the seasonal biology of the larvae within the actual fish host is much more restricted, and suffers from a lack of structured experimental data. The processes of invasion, growth and maturation of the larvae in the fishes have not been examined by experiment over a range of temperatures, so that for almost all species details of minimum, optimum and maximum temperatures for invasion and growth have not been ascertained. It is much more difficult to study the fish host than the egg or invertebrate intermediate host stages in the life cycle, as it is necessary to maintain a large population of larvae in order to infect the experimental fishes, and it is more expensive and an operation needing a much longer time to study the growth of the larval stages in the fishes. Nonetheless, it would greatly facilitate the explanation of seasonal dynamics of larval development in the fishes if data about growth through the range of temperatures were available. Information about the effect of temperature on the longevity of the larvae may also be important.

Other abiotic factors, including the amount of light entering the water, depth, hydrogen ion concentration (pH), oxygen concentration, salinity and the factor of standing or running water may determine presence or absence

of the species of parasite in a given area, but have not at present been implicated as having seasonal significance.

The experimental studies carried out so far have shown the importance of temperature on the growth rates of the larvae of cestode and nematode parasites in fishes, and it seems likely that temperature is the most important single factor in controlling the seasonal dynamics of occurrence of these larvae.

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References

Aisa, E. (1975). Cisti da larve plerocercoidi di *Triaenophorus lucii* (Müller, 1776) nelle ovaie di *Tinca tinca* L. e di *Esox lucius* L. *Atti della Societa Italiana della Scienze Veterinarie*, **29**, 613–620.

Andersen, K. (1970). Morphological comparison between *Diphyllobothrium dendriticum* Nitzsch 1824 and *D. norvegicum* Vik 1957. *Nytt Magasin for Zoologi*, **18**, 102.

Andersen, K. (1971). Studies of the helminth fauna of Norway XVIII: morphological comparison of *Diphyllobothrium dendriticum* Nitzsch 1824, *D. norvegicum* Vik 1957 and *D. latum* (Linné 1758) (Cestoda: Pseudophyllidea). *Norwegian Journal of Zoology*, **19**, 21–36.

Andersen, K. (1975). Comparison of surface topography of three species of *Diphyllobothrium* (Cestoda, Pseudophyllidea) by scanning electron microscopy. *International Journal for Parasitology*, **5**, 293–300.

Anderson, R. C., Chabaud, A. G. and Willmott, S. (1974). "General Introduction. Glossary of terms. Keys to subclasses, orders and superfamilies." Commonwealth Institute of Helminthology Keys to the nematode parasites of vertebrates, Commonwealth Agricultural Bureaux, Farnham Royal, England, No. 1, 17 pp.

Andrews, C. R. (1977a). Long-term observations on the *Triaenophorus nodulosus* (Pallas, 1781) infection in perch (*Perca fluviatilis* L.) from Llyn Tegid, North Wales. *Parasitology*, **75**, ix.

Andrews, C. R. (1977b). "The biology of the parasite fauna of perch (*Perca fluviatilis* L.) from Llyn Tegid, North Wales." Ph.D. thesis, University of Liverpool.

Arme, C. and Owen, R. W. (1964). Massive infections of the three-spined stickleback with *Schistocephalus solidus* (Cestoda: Pseudophyllidea) and *Glugea anomala* (Sporozoa: Microsporidia). *Parasitology*, **54**, 11P.

Arme, C. and Owen, R. W. (1967). Infections of the three-spined stickleback, *Gasterosteus aculeatus* L., with the plerocercoid larvae of *Schistocephalus solidus* (Müller, 1776), with special reference to pathological effects. *Parasitology*, **57**, 301–314.

Arme, C. and Owen, R. W. (1968). Occurrence and pathology of *Ligula intestinalis* infections in British fishes. *Journal of Parasitology*, **54**, 272–280.

Arnason, I. G. (1948). Infestation of *Cyclops bicuspidatus* with *Triaenophorus* at Heming Lake, Manitoba, during summer of 1948. Fisheries Research Board of Canada Annual Report of the Central Fisheries Research Station for 1948. Appendix 16, pp. 26–27.

Awakura, T., Tonosaki, H. and Ito, T. (1976). (Ligulosis of cyprinids in lakes of Hokkaido, Japan.) Scientific Reports of the Hokkaido Fish Hatchery No. 31, 67–81. (In Japanese.)

Baer, J. G. and Bona, F. (1960). Révision des cestodes Dilepididae Fuhrm., 1907 des ardéiformes. Note préliminaire. *Bollettino dell' Istituto e Museo di Zoologia dell' Universita di Torino*, **6**, 1–53.

Bangham, R. V. (1972). A resurvey of the fish parasites of western Lake Erie. *Bulletin of the Ohio Biological Survey* new series Vol. 4 (No. 2), 1–20.

Banina, N. N. and Isakov, L. S. (1972). (Experiment in ecological analysis of parasitofauna in sticklebacks.) *Izvestiya Gosudarstvennogo Nauchno-Issledovatel' skogo Instituta Ozernogo i Rechnogo Rȳbnogo Khozyaistva*, **80**, 89–112. (In Russian: translation British Library RTS 8523.)

Baruš, V., Sergeeva, T. P., Sonin, M. D. and Ryzihkov, K. M. (1978). "Helminths of fish-eating birds of the Palaearctic Region 1. Nematoda." pp. 1–318. Dr. W. Junk, The Hague, Academia, Prague.

Bauer, O. N. (1959). (Ecology of the parasites of freshwater fish. Interrelationships between the parasite and its habitat.) *Izvestiya Gosudarstvennogo Nauchno-Issledovatel'skogo Instituta Ozernogo i Rechnogo Rȳbnogo Khozyaistva*, **49**, 5–206. (In Russian: translation Israel Program for Scientific Translations Cat. No. 622.)

Bauer, O. N. and Solomatova, Y. P. (1978). Influence of thermal pollution on fish parasites. Fourth International Congress of Parasitology 19–26 August 1978 Warsaw Short Communications Section H p. 22.

Bauer, O. N., Musselius, V. A. and Strelkov, Yu. A. (1969). ("Diseases of pond fish.") pp. 1–335. Izdatel'stvo "Kolos", Moscow. (In Russian: translation Israel Program for Scientific Translations Cat. No. 60118 9).

Becker, C. D. (1967). The parasite fauna of teleosts in six Washington lakes. *Northwest Science*, **41**, 160–168.

Becker, C. D. and Brunson, W. D. (1967). *Diphyllobothrium* (Cestoda) infections in salmonids from three Washington lakes. *Journal of Wildlife Management*, **31**, 813–824.

Bibby, M. C. (1972). Population biology of the helminth parasites of *Phoxinus phoxinus* (L.), the minnow, in a Cardiganshire lake. *Journal of Fish Biology*, **4**, 289–300.

Bogdanova, E. A. (1958). (Seasonal changes in the parasite fauna of pike and bream in the Volga river.) *In* "Papers on Helminthology presented to Academician K. I. Skryabin on his 80th Birthday", pp. 72–78. Izdatel'stvo Akademii Nauk SSSR, Moscow. (In Russian.)

Bogitsch, B. J. (1958). Observations on the seasonal occurrence of a pseudophyllidean tapeworm infecting the alimentary tract of *Lepomis macrochirus macrochirus* Raf. in Albemarle County, Virginia. *American Midland Naturalist*, **60**, 97–99.

Borgström, R. (1970). Studies of the helminth fauna of Norway XVI. *Triaenophorus nodulosus* (Pallas, 1760) (Cestoda) in Bogstad Lake. III. Occurrence in pike, *Esox lucius* L. *Nytt Magasin for Zoologi*, **18**, 209–216.

Borroni, I. and Grimaldi, E. (1974). Ecologia dell'infestione da larve plerocercoidi di *Diphyllobothrium latum* (Cestoda: Pseudophyllidea) a carico delle specie ittiche recettive del Lago Maggiore. *Rivista di Parassitologia*, **35**, 261–276.

Bråten, T. (1966). Host specificity in *Schistocephalus solidus*. *Parasitology*, **56**, 657–664.

Buck, O. D., Cooper, C. L. and Crites, J. L. (1976). Helminth parasites of the herring gull, *Larus argentatus*, from the Bass Island region of western Lake Erie. *Proceedings of the Helminthological Society of Washington*, **43**, 233–234.

Bykhovskaya-Pavlovskaya, I. E., Gussev, A. V., Dubinina, M. N., Izyumova, N. A., Smirnova, T. S., Sokolovskaya, I. L., Shtein, G. A., Shul'man, S. S. and Epshtein, V. M. (1962). ("Key to the parasites of freshwater fishes of the U.S.S.R.") Izdatel'stvo Akademii Nauk SSSR, Moscow and Leningrad. (In Russian: translation Israel Program for Scientific Translations Cat. No. 1136.)

Bylund, G. (1969). Experimentell undersökning av *Diphyllobothrium dendriticum* (= *D. norvegicum*) från norra Finland. *Tiedoksianto-Information*, **10**, 3–17.

Bylund, G. (1971). Experimentell undersökning av *Diphyllobothrium ditremum* (= *D. osmeri*). *Tiedoksianto-Information*, **12**, 10–18.

Bylund, G. (1973). Observations on the taxonomic status and the biology of *Diphyllobothrium ditremum* (Creplin, 1825), [= *D. osmeri* (von Linstow, 1878)]. *Acta Academiae Aboensis B*, **33**, 18 pp.

Bylund, G. (1975a). "Delimitation and characterization of European *Diphyllobothrium* species." Dissertation, Department of Biology, Abo Akademi, Abo, Finland. 23 pp.

Bylund, G. (1975b). Studies on the taxonomic status and biology of *Diphyllobothrium vogeli* Kuhlow, 1953. *Commentationes Biologicae Societas Scientiarum Fennica*, **79**, 22 pp.

Bylund, G. (1975c). The taxonomic significance of embryonic hooks in four European *Diphyllobothrium* species (Cestoda, Diphyllobothriidae). *Acta Zoologica Fennica*, **142**, 1–22.

Bylund, G. (1978). Protein taxonomical methods applied in the genus *Diphyllobothrium*. Fourth International Congress of Parasitology 19–26 August 1978 Warsaw Short Communications Section B p. 31.

Bylund, G. and Djupsund, B. M. (1977). Protein profiles as an aid to taxonomy in the genus *Diphyllobothrium*. *Zeitschrift für Parasitenkunde*, **51**, 241–247.

Campbell, A. D. (1974). The parasites of fish in Loch Leven. *Proceedings of the Royal Society of Edinburgh B*, **74**, 347–364.

Cannon, L. R. G. (1973). Diet and intestinal helminths in a population of perch, *Perca flavescens*. *Journal of Fish Biology*, **5**, 447–457.

Cernova, T. N. (1975). (Seasonal dynamics of the parasite fauna of pike and roach in lakes Dzhapana and Paleostomi.) *Trudȳ Vsesoyuznogo Nauchno-Issledovatel'skogo Instituta Morskogo Rybnogo Khozaistva i Okeanografii* (*Biologicheskie osnovy produktivnosti vodoemov Gruzinskoi SSR*), **105**, 108–120. (In Russian.)

Chappell, L. H. (1969). The parasites of the three-spined stickleback *Gasterosteus aculeatus* L. from a Yorkshire pond. 1. Seasonal variation of parasite fauna. *Journal of Fish Biology*, **1**, 137–152.

Chizhova, T. P. (1958). (On the specific identity of *Diphyllobothrium strictum* Talysin, 1932 and *D. dendriticum* Nitzsch, 1824) *In* "Papers on Helminthology presented to Academician K. I. Skyrabin on his 80th Birthday", Izdatel'stvo Akademii Nauk SSSR, Moscow pp. 384–386. (In Russian.)

Chizhova T. P. (1965). (Plerocercoids from fish in Kirgizia and Siberia.) *Trudy Moskovskoi Ordena Lenina Meditsinskogo Instituta*, **41**, 63–66. (In Russian.)

Chizhova, T. P. and Gofman-Kadoshnikov, P. B. (1959). (Anatomical and histological structure of plerocercoids of diphyllobothriids from Lake Baikal.) *Meditsinskaya Parazitologiya i Parazitarnie Bolezni*, **28**, 728–733. (In Russian.)

Chizhova, T. P. and Gofman-Kadoshnikov, P. B. (1960). (The pattern of the natural focus of diphyllobothriasis in Lake Baikal.) *Meditsinskaya Parazitologiya i Parazitarnie Bolezni*, **29**, 168–176. (In Russian.)

Chizhova, T. P., Gofman-Kadoshnikov, P. B. and Kravtsov, E. G. (1962). (Plerocercoids in fish in Karelia and their epidemiological significance.) *Meditsinskaya Parazitologiya i Parazitarnie Bolezni*, **31**, 213–223. (In Russian: translation British Library RTS 5269.)

Chubb, J. C. (1961). "A preliminary investigation of the parasite fauna of the fish of Llyn Tegid (Bala Lake), Merionethshire." Ph.D. thesis, University of Liverpool.

Chubb, J. C. (1963). Seasonal occurrence and maturation of *Triaenophorus nodulosus* (Pallas, 1781) (Cestoda: Pseudophyllidea) in the pike, *Esox lucius* L. of Llyn Tegid. *Parasitology*, **53**, 419–433.

Chubb, J. C. (1964). Observations on the occurrence of the plerocercoids of *Triaenophorus nodulosus* (Pallas, 1781) (Cestoda: Pseudophyllidea) in the perch *Perca fluviatilis* L. of Llyn Tegid (Bala Lake), Merionethshire. *Parasitology*, **54**, 481–491.

Chubb, J. C. (1968). Tapeworms of the genus *Diphyllobothrium* in the British Isles. *Parasitology*, **58**, 22 P.

Chubb, J. C. (1970). The parasite fauna of British freshwater fish. *In* "Aspects of fish parasitology" (A. E. R. Taylor and R. Muller, eds.). Symposium of the British Society for Parasitology 8, pp. 119–144. Blackwell Scientific Publications, Oxford.

Chubb, J. C. (1977). Seasonal occurrence of helminths in freshwater fishes. Part I. Monogenea. *In* "Advances in Parasitology" (B. Dawes, ed.), Vol. 15, pp. 133–199. Academic Press, London and New York.

Chubb, J. C. (1979). Seasonal occurrence of helminths in freshwater fishes. Part II. Trematoda. *In* "Advances in Parasitology" (W. H. R. Lumsden, R. Muller and J. R. Baker, eds.), Vol. 17, pp. 141–313. Academic Press, London and New York.

Clarke, A. S. (1954). Studies on the life cycle of the pseudophyllidean cestode *Schistocephalus solidus*. *Proceedings of the Zoological Society of London*, **124**, 257–302.

Cloutman, D. G. (1975). Parasite community structure of largemouth bass, warmouth, and bluegill in Lake Fort Smith, Arkansas. *Transactions of the American Fisheries Society*, **104**, 277–283.

Cone, D. K. and Anderson, R. C. (1977). Parasites of the pumpkinseed (*Lepomis gibbosus* L.) from Ryan Lake, Algonquin Park, Ontario. *Canadian Journal of Zoology*, **55**, 1410–1423.

Cooper, C. L., Crites, J. L. and Sprinkle-Fastkie, D. J. (1978). Population biology and behaviour of larval *Eustrongylides tubifex* (Nematoda: Dicotophymatida) in poikilothermous hosts. *Journal of Parasitology*, **64**, 102–107.

Dartnall, H. J. G. (1972). "Variations in the parasite fauna of the three-spined stickleback related to salinity and other parameters." Ph.D. thesis, University of London.

Davies, E. H. (1967). "Parasite fauna of the fish of the River Lugg, a tributary of the River Wye, Herefordshire." Ph.D. thesis, University of Liverpool.

Davis, J. R. and Huffman, D. G. (1978). Some factors associated with the distribution of helminths among individual mosquitofish, *Gambusia affinis*. *Texas Journal of Science*, **30**, 43–53.

Deksbakh, N. K. and Shchupakov, I. G. (1954). (*Ligula* in fish in the waters of the Central Ural and Transural regions.) *Zoologicheskii Zhurnal*, **33**, 544–548. (In Russian.)

Dence, W. A. (1958). Studies on *Ligula*-infected common shiners (*Notropis cornutus frontalis* Agassiz) in the Adirondacks. *Journal of Parasitology*, **44**, 334–338.

Denisov, A. I. and Vorob'ev, V. A. (1974). (Liguliasis in carp and plant-eating fish.) *Veterinariya* No. 8, 77–78. (In Russian.)

Dergaleva, Zh. T. and Markevich, N. B. (1976). The dynamics of the infestation of the silverside *Atherina mochon pontica* by the larvae of *Raphidascaris acus* (Nematoda, Ascaridata) in the Aral Sea and its influence on the physiological state of the fish. *Journal of Ichthyology*, **16**, 866–868. (English translation of *Voprosy Ikhtiologii*.)

Dogiel, V. A. (1964). "General Parasitology." (Translation Z. Kabata), pp. i–ix, 1–516. Oliver and Boyd, Edinburgh and London.

Dubinin, V. B. (1949). (Experimental studies on the life-cycles of some parasitic worms of animals in the Volga delta.) *Parazitologicheskii Sbornik*, **11**, 126–160. (In Russian.)

Dubinina, M. N. (1949). (Influence of hibernation on parasitic fauna of fishes in hibernating grounds of the Volga delta.) *Parazitologicheskii Sbornik*, **11**, 104–125. (In Russian.)

Dubinina, M. N. (1953). (Specificity in ligulids at various stages of their life cycle.) *Parazitologicheskii Sbornik*, **15**, 234–251. (In Russian.)

Dubinina, M. N. (1957). (Experimental study of the life cycle of *Schistocephalus solidus* (Cestoda: Pseudophyllidea).) *Zoologicheskii Zhurnal*, **36**, 1647–1658. (In Russian.)

Dubinina, M. N. (1959a). Fish ligulosis and its control. Proceedings of the Conference on Fish Diseases. Conference Proceedings No. 9, Akademiya Nauk SSSR, Ikhtiologicheskaya Komissiya. Translation Israel Program for Scientific Translations Cat. No. 620, pp. 144–149.

Dubinina, M. N. (1959b). (The natural classification of the genus *Schistocephalus* Creplin (Cestoda, Ligulidae).) *Zoologicheskii Zhurnal*, **38**, 1498–1517. (In Russian.)

Dubinina, M. N. (1960). (The possibility of progenesis in plerocercoids of Ligulidae (Cestoda).) *Zoologicheskii Zhurnal*, **39**, 1467–1477. (In Russian.)

Dubinina, M. N. (1964a). "Ligulidae (Cestoda: Pseudophyllidae) and their evolution." Report presented at the First International Congress of Parasitology. "Nauka", Leningrad pp. 1–11.

Dubinina, M. N. (1964b). Cestodes of the family Ligulidae and their taxonomy. *In* "Parasitic worms and aquatic conditions", (R. Ergens and B. Ryšavý, eds.), pp. 173–186. Czechoslovak Academy of Sciences, Prague.

Dubinina, M. N. (1964c). (Geographical forms of cestodes of the genus *Triaenophorus* Rudolphi (Pseudophyllidea).) *Zoologicheskii Zhurnal*, **43**, 412–417. (In Russian.)

Dubinina, M. N. (1965). ("Ligulidae (Cestoda) in the fauna of the U.S.S.R.") Avtoreferat pp. 1–31 (In Russian, original not seen, quoted from Kuperman, 1973b.)

Dubinina, M. N. (1966). ("Ligulidae (Cestoda) of the U.S.S.R.") Izdatel'stvo "Nauka", Moscow and Leningrad pp. 1–261. (In Russian.)

Duguid, J. B. and Sheppard, E. M. (1944). A *Diphyllobothrium* epidemic in trout. *Journal of Pathology and Bacteriology*, **56**, 73–80.

Edwards, R. W., Harley, J. P. and Williams, J. C. (1977). Parasites of channel catfish from the Kentucky River, with a comparative note on the Ohio River. *Transactions of the Kentucky Academy of Science*, **38**, 132–135.

Ekbaum, E. (1937). On the maturation and the hatching of the eggs of the cestode *Triaenophorus crassus* Forel from Canadian fish. *Journal of Parasitology*, **23**, 293–295.

Ergens, R. (1966). Results of parasitological investigations on the health of *Esox lucius* L. in the Lipno reservoir. *Folia Parasitologica*, **13**, 222–236.

Eure, H. E. and Esch, G. W. (1974). Effects of thermal effluent on the population dynamics of helminths in largemouth bass. *In* "Thermal Ecology" (J. W. Gibbons and R. R. Sharitz, eds.), pp. 207–215, AEC Symposium series.

Fraser, P. G. (1951). *Diphyllobothrium* in trout. Final Report to the Development Commission, pp. i–xxii, 1–141.

Fraser, P. G. (1960a). The occurrence of *Diphyllobothrium* in trout, with special reference to the outbreak in the West of England. *Journal of Helminthology*, **34**, 59–72.

Fraser, P. G. (1960b). On *Diphyllobothrium medium* (Fahmy, 1954) parasitic in trout in Great Britain. *Journal of Helminthology*, **34**, 193–204.

Freeman, R. S. (1973). Ontogeny of cestodes and its bearing on their phylogeny and systematics. *In* "Advances in Parasitology" (B. Dawes, ed.), Vol. 11, pp. 481–557. Academic Press, London and New York.

Freeman, R. S. and Thompson, B. H. (1969). Observations on transmission of *Diphyllobothrium* sp. (Cestoda) to lake trout in Algonquin Park, Canada. *Journal of the Fisheries Research Board of Canada*, **26**, 871–878.

Freze, V. I. (1977). (European *Diphyllobothrium*. (Experimental study of polymorphism.).) *Trudȳ Gel'mintologicheskoi Laboratorii*, **27**, 174–204. (In Russian.)

Freze, V. I., Sidorov, V. S. and Gur'yanova, S. D. (1974). (Amino-acids of the plerocercoids from the genus *Diphyllobothrium* (Cestoidea: Pseudophyllidae) and of their hosts.) *Helminthologia*, **15**, 487–494. (In Russian.)

Gofman-Kadoshnikov, P. B., Khodakova, B. I., Chizhova, T. P. and Kravtsov, E. (1963). (The role of *Pungitius pungitius* in the spread of *Diphyllobothrium* infection.) *Meditsinskaya Parazitologiya i Parazitarnie Bolezni*, **32**, 460–465. (In Russian.)

Grimaldi, E. (1974). Dipendenza della infestione viscerale del pesce persico (*Perca fluviatilis*) da larve di *Diphyllobothrium latum* (Cestoda: Pseudophyllidea) dal fenomeno della reinfestione. *Bollettino di Zoologia*, **41**, 497.

Gur'yanova, S. D., Sidorov, V. S. and Freze, V. I. (1976). (The amino acid composition of the plerocercoids of *Diphyllobothrium*, related to ecological factors.) *In* "Parazitologicheskie issledovaniya Karel'skoi ASSR i Murmanskoi oblasti." Karel'skoi filial Akademii Nauk SSSR, Institut Biologii. Petrozavodsk pp. 222–230. (In Russian.)

Guttowa, A. (1955). O inwazyjności onkosfer *Triaenophorus lucii* (Müll.) i jej zmienności. *Acta Parasitologica Polonica*, **3**, 447–465.

Guttowa, A. (1958). Dalsze badania nad wpływen temperatury rozwój zarodków tasiemca *Triaenophorus lucii* w jajeczkach, oraz na inwazyjność powstałych z nich onkosfer. *Acta Parasitologica Polonica*, **6**, 367–381.

Guttowa, A. (1961). Experimental investigations on the systems "procercoids of *Diphyllobothrium latum* (L.)—Copepoda". *Acta Parasitologica Polonica*, **9**, 371–408.

Guttowa, A. (1963). Natural focus of infection of plankton crustaceans with procercoids of *Diphyllobothrium latum* L. in Finland. *Acta Parasitologica Polonica*, **11**, 145–152.

Halvorsen, O. (1966). Studies of the helminth fauna of Norway. VIII. An experimental investigation of copepods as first intermediate hosts for *Diphyllobothrium norvegicum* Vik (Cestoda). *Nytt Magasin for Zoologi*, **13**, 83–117.

Halvorsen, O. (1968). Studies of the helminth fauna of Norway. X. *Triaenophorus nodulosus* (Pallas, 1760) (Cestoda) in Bogstad Lake. I. Copepods as first intermediate hosts. *Nytt Magasin for Zoologi*, **15**, 124–129.

Halvorsen, O. (1970). Studies of the helminth fauna of Norway XV: on the taxonomy and biology of plerocercoids of *Diphyllobothrium* Cobbold, 1858 (Cestoda, Pseudophyllidea) from north-western Europe. *Nytt Magasin for Zoologi*, **18**, 113–174.

Halvorsen, O. and Wissler, K. (1973). Studies of the helminth fauna of Norway XXVIII: an experimental study of the ability of *Diphyllobothrium latum* (L.), *D. dendriticum* (Nitzsch), and *D. ditremum* (Creplin) (Cestoda, Pseudophyllidea) to infect paratenic hosts. *Norwegian Journal of Zoology*, **21**, 201–210.

Harris, M. T. and Wheeler, A. (1974). *Ligula* infestation of bleak *Alburnus alburnus* (L.) in the tidal Thames. *Journal of Fish Biology*, **6**, 181–188.

Hartwich, G. (1964). Revision der vogelparasitischen Nematoden Mitteleuropas II. Die Gattung *Contracaecum* Raillet & Henry, 1912 (Ascaridoidea). *Mitteilungen aus dem Zoologischen Museum in Berlin*, **40**, 15–53.

Hasegawa, H. and Otsura, M. (1978). Notes on the life cycle of *Spiroxys japonica* Morishita, 1926 (Nematoda: Gnathostomatidae). *Japanese Journal of Parasitology*, **27**, 113–122.

Hedrick, L. R. (1935). The life history and morphology of *Spiroxys contortus* (Rudolphi); Nematoda: Spiruridae. *Transactions of the American Microscopical Society*, **54**, 307–335.

Henricson, J. (1977). The abundance and distribution of *Diphyllobothrium dendriticum* (Nitzsch) and *D. ditremum* (Creplin) in the char *Salvelinus alpinus* (L.) in Sweden. *Journal of Fish Biology*, **11**, 231–248.

Henricson, J. (1978). The dynamics of infection of *Diphyllobothrium dendriticum* (Nitzsch) and *D. ditremum* (Creplin) in the char *Salvelinus alpinus* (L.) in Sweden. *Journal of Fish Biology*, **13**, 51–71.

Hickey, M. D. and Harris, J. R. (1947). Progress of the *Diphyllobothrium* epizootic at Poulaphouca Reservoir, Co. Wicklow, Ireland. *Journal of Helminthology*, **22**, 13–28.

Hobmaier, M. (1927). Wie kommt die Infektion der Raubfische mit dem Plerocerkoid von *Dibothriocephalus latus* zustande? *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene (Abteilung 2)*, **72**, 268–273.

Holl, F. J. (1932). The ecology of certain fishes and amphibians with special reference to their helminth and linguatulid parasites. *Ecological Monographs*, **2**, 83–107

Holmes, J. C., Hobbs, R. P. and Leong, T. S. (1977). Populations in perspective: community organization and regulation of parasite populations. *In* "Regulation of Parasite Populations" (G. W. Esch, ed.), pp. 209–245. Academic Press, London and New York.

Hopkins, C. A. (1950). Studies on cestode metabolism. I. Glycogen metabolism in *Schistocephalus solidus* in vivo. *Journal of Parasitology*, **36**, 384–390.

Hopkins, C. A. and McCaig, M. L. O. (1963). Studies on *Schistocephalus solidus*. I. The correlation of development in the plerocercoid with infectivity to the definitive host. *Experimental Parasitology*, **13**, 235–243.

Hopkins, C. A. and Smyth, J. D. (1951). Notes on the morphology and life history of *Schistocephalus solidus* (Cestoda: Diphyllobothriidae). *Parasitology*, **41**, 283–291.

Huizinga, H. W. (1966). Studies on the life cycle and development of *Contracaecum spiculigerum* (Rudolphi, 1809) (Ascaroidea: Heterocheilidae) from marine piscivorous birds. *Journal of the Elisha Mitchell Scientific Society*, **82**, 181–195.

Ivasik, V. M. (1953). (Carp parasites and the diseases they cause in fish farms in the western districts of the Ukrainian S.S.R.) *Trudȳ Nauchno-Issledovatel'skogo Instituta Prudovogo i Ozerno Rechnogo Rybnogo Khozyaistva Ukrainskoi SSR*, **9**, 85–122. (In Russian.)

Izyumova, N. A. (1958). (Seasonal dynamics of the parasites of fish in the Rybinsk water reservoir.) *Trudȳ Biologicheskoi Stantsii "Borok", Moscow*, **3**, 384–398. (In Russian.)

Izyumova, N. A. (1959). (Seasonal dynamics of the parasites of the fishes of the Rybinsk Reservoir. 2. Roach, ruff.) *Trudȳ Instituta Biologii Vodokhranilishch. Akademiya Nauk SSSR. Moscow*, **1**, 332–342. (In Russian.)

Izyumova, N. A. (1960). (Seasonal dynamics in the parasite fauna of fish in the Rybinsk water reservoir. Part III. Pike, blue bream and *Blicca bjoerkna*.) *Trudȳ Instituta Biologii Vodokhranilishch. Akademiya Nauk SSSR. Moscow*, **3**, 283–300. (In Russian.)

Izyumova, N. A. (1964). The formation of the parasitofauna of fishes in the Rybinsk reservoir. *In* "Parasitic worms and aquatic conditions", (R. Ergens and B. Ryšavý, eds.), pp. 49–55. Czechoslovak Academy of Sciences, Prague.

Janicki, C. (1917). Experimentelle Untersuchungen zur Entwicklung von *Dibothriocephalus latus* L. I. Ueber negative Versuche, junge Forellen, Hechte und Barsche direkt mit Flimmerembryonen zu infizieren. *Zentralblatt für Bakteriologie. Parasitenkunde, Infektionskrankheiten und Hygiene (Abteilung 1 Originale)*, **79**, 443–461.

Janicki, C. and Rosen, F. (1917). Le cycle évolutif du *Dibothriocephalus latus* L. Recherches expérimentales et observations. *Bulletin de la Société Neuchâteloise des Sciences Naturelles*, **42**, 19–53.

Jara, Z. and Olech, W. (1964). Dynamika inwazji *Cystericercus dilepidis campylancistrotae* (Aubert) u karpi hodowanych (*Cyprinus carpio* L.) *Wiadomości Parazytologiczne*, **10**, 518–520.

Jarecka, L. (1970a). Life cycle of *Valipora campylancristrota* (Wedl, 1855) Baer and Bona 1958–60 (Cestoda—Dilepididae) and the description of cercoscolex—a new type of cestode larva. *Bulletin de l'Académie Polonaise des Sciences. Classe II. Série des Sciences Biologiques*, **18**, 99–102.

Jarecka, L. (1970b). On the life cycles of *Paradilepis scolecina* (Rud., 1819) Hsu, 1935, and *Neogryporhynchus cheilancristrotus* (Wedl, 1855) Baer and Bona, 1958–1960 (Cestoda: Dilepididae). *Bulletin de l'Académie Polonaise des Sciences. Classe II. Série des Sciences Biologiques*, **18**, 159–163.

Johnson, S. E. (1975). First intermediate host of *Diphyllobothrium sebago* (Cestoda: Pseudophyllidea) in nature. *Journal of Parasitology*, **61**, 74.

Jones, J. W. (1953). Part I. The scales of roach. *Fishery Investigations Series I*, **5** (7), 3–6.

Kafanov, A. I. and Yazykova, I. M. (1971). (Epizootiology of *Schistocephalus pungitii* (Ligulidae) in Lake Manych-Gudilo.) *Zoologicheskii Zhurnal*, **50**, 1572–1574. (In Russian.)

Kakacheva-Avramova, D. (1973). (Helminth fauna in fish in rivers of the central and eastern Balkan mountains.) *Izvestiya na Tsentralnata Khelmintologichna Laboratoriya*, **16**, 87–110. (In Bulgarian.)

Kakacheva-Avramova, D. (1977). (Helminths of fish from the Bulgarian part of the Danube.) *Khelmintologiya, Sofia* No. 3, 20–45. (In Bulgarian.)

Kakacheva-Avramova, D. and Naidenov, V. (1974). (The biological factors involved in maintaining a focus of liguliasis (*Ligula intestinalis*) in the Iskar dam lake.) *Izvestiya na Tsentralnata Khelmintologichna Laboratoriya*, **17**, 73–79. (In Bulgarian.)

Kamenskii, I. V. (1962). (Age and seasonal dynamics of liguliasis in fish of the Kakhovsk water-reservoir.) *Tezisy Dokladȳ Nauchnogo Konferentsii Vsesoyuznoi Obshchestva Gel'mintologii* Year 1962, Part I, pp. 71–72.

Kamenskii, I. V. (1964). (Study of the epizootiology of ligulid infections of fish in the Kakhovsk water reservoir.) *Trudȳ Vsesoyuznogo Instituta Gel'mintologii*, **11**, 62–70. (In Russian.)

Kamo, H. (1978). (Reconsideration on taxonomic status of *Diphyllobothrium latum* (Linnaeus, 1758) in Japan with special regard to species specific characters.) *Japanese Journal of Parasitology*, **27**, 135–142. (In Japanese.)

Karmanova, E. M. (1965). (Intermediate hosts of *Eustrongylides excisus*, parasite of aquatic birds.) *Trudȳ Gel'mintologicheskoi Laboratorii*, **15**, 86–87. (In Russian.)

Karmanova, E. M. (1968). (Principles of nematology. Vol. XX. Dioctophymata of animals and man and the diseases caused by them.) (K. I. Skryabin, ed.), Izdatel'stvo "Nauka", Moscow. pp. 1–261.

Kashkovski, V. V. (1967). (Seasonal changes in the parasite fauna of *Rutilus rutilus* in the Iriklin reservoir.) *Voprosȳ Ikhtiologii. Otdelenie Biologicheskikh Nauk. Akademiya Nauk SSSR*, **7**, 378–386. (In Russian.)

Kažić, D., Ubelaker, J. and Čanković, M. (1977). Observations and seasonal variations of the helminth fauna of *Gobio gobio lepidolaemus* (Kessler, 1872) (Cyprinidae, Pisces) from Lake Skadar, Yugoslavia. *Glasnik Republ. Zavoda Zašt Prirode-Prirodnjačkog Muzeja Titograd*, **10**, 5–29.

Keleher, J. J. (1952). Growth and *Triaenophorus* parasitism in relation to taxonomy of Lake Winnipeg ciscoes (*Leucichthys*). *Journal of the Fisheries Research Board of Canada*, **8**, 469–478.

Kennedy, C. R. and Lie, S. F. (1976). The distribution and pathogenicity of larvae of *Eustrongylides* (Nematoda) in brown trout *Salmo trutta* L. in Fernworthy Reservoir, Devon. *Journal of Fish Biology*, **8**, 293–302.

Kerr, T. (1948). The pituitary in normal and parasitized roach (*Leuciscus rutilus* Flem.). *Quarterly Journal of Microscopical Science*, **89**, 129–137.

Kiseliene, V., Volskis, G., Astrauskas, B. and Usinene, B. (1978). Influence of the changed thermic regime on the formation of helminthosis foci in water reservoir. Fourth International Congress of Parasitology 19–26 August 1978 Warsaw Short Communications Section H. p. 23.

Kisielewska, K. (1957). On intra-population relations of larvae *Drepanidotaenia lanceolata* (Bloch) in some intermediate hosts. *Acta Parasitologica Polonica*, **5**, 63–90.

Komarova, M. S. (1957). (Seasonal parasitofauna dynamics of the tench in the Donets River.) *Zoologicheskii Zhurnal*, **36**, 654–657. (In Russian.)

Komarova, T. I. (1964). (Seasonal dynamics of the helminth fauna of some species of fish in the Dnepr Delta.) *Problemȳ Parazitologii* (*Trudȳ Ukrainskogo Respublikanskogo Nauchnogo Obshchestva Parazitologov*), **3**, 90–105. (In Russian.)

Kosheva, A. F. (1956). (Influence of the parasites *Ligula intestinalis* and *Digramma interrupta* on their fish hosts.) *Zoologicheskii Zhurnal*, **35**, 1629–1632. (In Russian.)

Kozicka, J. (1958). Plerocercoid *Diphyllobothrium* species from *Coregonus albula* L. *Bulletin de l'Académie Polonaise des Sciences. Classe II. Série des Sciences Biologiques*, **6**, 209–213.

Kuhlow, F. (1953a). Bau und Differentialdiagnose heimischer *Diphyllobothrium*-Plerocercoide. *Zeitschrift für Tropenmedizin und Parasitologie*, **4**, 186–202.

Kuhlow, F. (1953b). Beiträge zur Entwicklung und Systematik heimischer *Diphyllobothrium*-Arten. *Zeitschrift für Tropenmedizin und Parasitologie*, **4**, 203–234.

Kuhlow, F. (1953c). Über die Entwicklung und Anatomie von *Diphyllobothrium dendriticum* Nitzsch, 1824. *Zeitschrift für Parasitenkunde*, **16**, 1–35.

Kuhlow, F. (1955). Untersuchungen über die Entwicklung des Breiten Bandwurmes (*Diphyllobothrium latum*). *Zeitschrift für Tropenmedizin und Parasitologie*, **6**, 213–225.

Kuperman, B. I. (1965). (Variability of species of *Triaenophorus* (Cestoda, Pseudophyllidea).) *Trudy Zoologicheskogo Instituta. Akademiya Nauk SSSR. Leningrad*, **35**, 175–186. (In Russian.)

Kuperman, B. I. (1967a). (Cestodes of the genus *Triaenophorus*.) Avtoreferat Zoologicheskogo Instituta. Akademiya Nauk SSSR. Leningrad. (In Russian: translation Fisheries Research Board of Canada Translation No. 1483).

Kuperman, B. I. (1967b). (Morphology and biology of cestodes of the genus *Triaenophorus* from the Amur basin.) *Zoologicheskii Zhurnal*, **46**, 486–494. (In Russian: translation British Library RTS 4813.)

Kuperman, B. I. (1968). (New species of the genus *Triaenophorus* Rud. (Cestoda, Pseudophyllidea).) *Parazitologiya*, **2**, 495–501. (In Russian: translation Fisheries Research Board of Canada Translation No. 1479).

Kuperman, B. I. (1971). (The influence of temperature on the embryonic development of the tapeworm *Triaenophorus* (Pseudophyllidea).) *Informatsionnyi Byulleten', Institut Biologii Vnutrennikh Vod* No. 11, 32–37. (In Russian.)

Kuperman, B. I. (1973a). Infection of young perch by the tapeworm *Triaenophorus nodulosus*. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandt Limnologie*, **18**, 1697–1704.

Kuperman, B. I. (1973b). (Cestodes of the genus *Triaenophorus*, parasites of fish. Experimental taxonomy, ecology.) Izdatel'stvo "Nauka", Leningrad pp. 1–207. (In Russian: translation British Library RTS 10341–10347).

Kuperman, B. I. (1977). (*Diphyllobothrium latum* in fish in the Rybinsk Reservoir.) *Meditsinskaya Parazitologiya i Parazitarnie Bolezni*, **46**, 736. (In Russian.)

Kuperman, B. I. and Monakov, A. V. (1972a). (Copepoda, the first intermediate hosts of *Triaenophorus* in the Rybinsk water reservoir.) *Informatsionnyi Byulleten', Institut Biologii Vnutrennikh Vod*, No. 14, 6–9. (In Russian.)

Kuperman, B. I. and Monakov, A. V. (1972b). (Experimental study of Copepoda as first intermediate hosts of members of *Triaenophorus*.) *In* "Parazity vodnykh bespozvonochnykh zhivotnykh." I Vsesoyuznyi Simpozium pp. 49–51. Izdatel'stvo L'vovskogo Universiteta, L'vov (In Russian.)

Kuperman, B. I. and Monakov, A. V. (1972c). (First intermediate hosts of cestodes of the genus *Triaenophorus* (Pseudophyllidea).) *Parazitologiya*, **6**, 274–282. (In Russian.)

Lawler, G. H. (1950). *Triaenophorus* studies at Heming Lake, 1950. Fisheries Research Board of Canada Annual Report of the Central Fisheries Research Station for 1950. Appendix 7, 24–28.

Lawler, G. H. (1964). Incidence of *Ligula intestinalis* in Heming Lake fish. *Journal of the Fisheries Research Board of Canada*, **21**, 549–554.

Lawler, G. H. (1969a). Occurrence of *Triaenophorus* spp. in Lake Mälaren fishes. *Report of the Institute of Freshwater Research Drottningholm*, No. 49, 120–128.

Lawler, G. H. (1969b). Aspects of the biology of *Triaenophorus nodulosus* in yellow perch, *Perca flavescens*, in Heming Lake, Manitoba. *Journal of the Fisheries Research Board of Canada*, **26**, 821–831.

Lawler, G. H. and Scott, W. B. (1954). Notes on the geographical distribution and the hosts of the cestode genus *Triaenophorus* in North America. *Journal of the Fisheries Research Board of Canada*, **11**, 884–893.

Lawler, G. H. and Watson, N. H. F. (1963). Measurements of immature stages of *Triaenophorus*. *Journal of the Fisheries Research Board of Canada*, **20**, 1089–1093.

Lee, R. (1977). The Serpentine fish and their parasites. *London Naturalist*, **56**, 57–70.

Leong, R. Tak Seng (1975). Metazoan parasites of fishes of Cold Lake, Alberta: a community analysis. Ph.D. thesis, University of Alberta.

Lester, R. J. G. (1971). The influence of *Schistocephalus* plerocercoids on the respiration of *Gasterosteus* and a possible resulting effect on the behaviour of the fish. *Canadian Journal of Zoology*, **49**, 361–366.

Levine, N. D. (1978). The influence of weather on the bionomics of the free-living stage of nematodes. *In* "Weather and parasitic animal disease" (T. E. Gibson, ed.), pp. 51–57, references pp. 78–80. World Meteorological Organization Technical Note No. 159, Geneva.

Libin, M. L. (1951). Laboratory experiments on the control of the tapeworm, *Triaenophorus crassus*. M.Sc. thesis, University of Alberta.

Lien, L. (1970). Studies of the helminth fauna of Norway XIV: *Triaenophorus nodulosus* (Pallas, 1760) (Cestoda) in Bogstad Lake. II. Development and life span of the plerocercoids in perch (*Perca fluviatilis* L., 1758). *Nytt Magasin for Zoologi*, **18**, 85–96.

Locke, L. N., de Witt, J. B., Menzie, C. M. and Kerwin, J. A. (1964). A merganser die-off associated with larval *Eustrongylides*. *Avian Diseases*, **8**, 420–427.

Lyubarskaya, O. D. (1970). (Seasonal dynamics of the parasite fauna of *Abramis brama* in the Kuybyshev reservoir, at the inlet of the Volga.) *In* "Voprosy evolyutsionnoi morfologii i biogeografii", pp. 40–49. Izdatel'stvo Kazanskogo Universiteta, Kazan'. (In Russian.)

Lyubina, T. V. (1970). (Parasite fauna of fish in Lake Bol'shoe in the Omsk region (U.S.S.R.).) *Sbornik Nauchnykh Rabot Sibirskogo Nauchno-issledovatel'skogo Veterinarnogo Instituta*, No. 18, 211–216. (In Russian.)

MacLulich, D. A. (1943). Parasites of trout in Algonquin Provincial Park, Ontario. *Canadian Journal of Research D*, **21**, 405–412.

Malakhova, R. P. (1961). (Seasonal changes in the parasitofauna of certain freshwater fishes from Karelian lakes (Lake Konche).) *Trudȳ Karelskogo Filiala. Akademiya Nauk SSSR*, **30**, 55–78. (In Russian.)

Malvestuto, S. P. and Ogambo-Ongoma, A. (1978). Observations on the infection of *Tilapia leucosticta* (Pisces: Cichlidae) with *Contracaecum* (Nematoda: Heterocheilidae) in Lake Naivasha, Kenya. *Journal of Parasitology*, **64**, 383–384.

Mamer, B. E. (1978). The parasites of trout in northwest Washington. *Journal of Parasitology*, **64**, 314.

Marits, N. M. and Tomnatik, E. N. (1971). (Parasite fauna of *Abramis brama* in the Dubossary water-reservoir.) *In* "Parazity zhivotnykh i rastenii", No. 6, 33–38. RIO Akademii Nauk Moldavskoi SSSR, Kishinev. (In Russian.)

Marits, N. M. and Vladimirov, M. Z. (1969). (Parasite fauna of *Vimba vimba vimba* natio *carinata* (Pallas) in Dubossary reservoir.) *In* ("Parasites of vertebrates") (A. A. Spasski, ed.), pp. 37–40. Izdatel'stvo "Kartya Moldovenyaske", Kishinev. (In Russian.)

Markevich, A. P. (1943). (Epizootic of perch triaenophorosis in Lake Kandry-Kul (Bashkir A.S.S.R.).) *Trudy Bashkirskoi Veterinarnoi Stantsii*, **4**, 111–117. (In Russian, original not seen.)

Markevich, A. P., Iskov, M. P., Koval', V. P. and Chernogorenko, M. I. (1976). (The effect of hydroconstruction on the parasite fauna of the Dnepr.) *Gidrobiologicheskii Zhurnal*, **12**, 5–12. (In Russian, translation *Hydrobiological Journal*, **12**, 1–6.)

Markowski, S. (1933). Die eingeweidewürmer der fische des polnischen Balticums. (Trematoda, Cestoda, Nematoda, Acanthocephala). *Archiwum Hydrobiologji i Rybactwa*, **7**, 1–58.

Markowski, S. (1949). On the species of *Diphyllobothrium* occurring in birds, and their relation to man and other hosts. *Journal of Helminthology*, **23**, 107–126.

Matthey, R. (1963). Rapport sur les maladies des poissons en Suisse. *Bulletin de l'Office International des Épizooties*, **59**, 121–126.

McDonald, M. E. (1969). Catalogue of helminths of waterfowl (Anatidae). *Bureau of Sport Fisheries and Wildlife, Special Scientific Report Wildlife* No. 126, *Washington D.C.* (I) i–viii, 1–692. (II) i–ii, 1–333.

Meakins, R. H. (1974a). A quantitative approach to the effects of the plerocercoid of *Schistocephalus solidus* Müller 1776 on the ovarian maturation of the three-spined stickleback *Gasterosteus aculeatus* L. *Zeitschrift für Parasitenkunde*, **44**, 73–79.

Meakins, R. H. (1974b). The bioenergetics of the *Gasterosteus/Schistocephalus* host–parasite system. *Polskie Archiwum Hydrobiologii*, **21**, 455–466.

Meakins, R. H. and Walkey, M. (1973). Aspects of *in vivo* growth of the plerocercoid stage of *Schistocephalus solidus*. *Parasitology*, **67**, 133–141.

Meakins, R. H. and Walkey, M. (1975). The effects of parasitism by the plerocercoid of *Schistocephalus solidus* Müller 1776 (Pseudophyllidea) on the respiration of three-spined stickleback *Gasterosteus aculeatus* L. *Journal of Fish Biology*, **7**, 817–824.

Meyer, M. C. (1967). Hatching of *Diphyllobothrium sebago* eggs in nature. *Transactions of the American Microscopical Society*, **86**, 239–243.

Meyer, M. C. (1972). The pattern of circulation of *Diphyllobothrium sebago* (Cestoda: Pseudophyllidea) in an enzootic area. *Journal of Wildlife Diseases*, **8**, 215–220.

Meyer, M. C. and Robinson, E. S. (1963). Description and occurrence of *Diphyllobothrium sebago* (Ward, 1910). *Journal of Parasitology*, **49**, 969–973.

Meyer, M. C. and Vik, R. (1963). The life cycle of *Diphyllobothrium sebago* (Ward, 1910). *Journal of Parasitology*, **49**, 962–968.

Meyer, M. C. and Vik, R. (1968). Observations on *Diphyllobothrium sebago* in the fish hosts. *Proceedings of the Helminthological Society of Washington*, **35**, 92–96.

Meyer, M. C. and Vik, R. (1972). Final hosts of *Diphyllobothrium sebago* (Cestoda: Pseudophyllidea) in nature. *Proceedings of the Helminthological Society of Washington*, **39**, 1–4.

Michajłow, W. (1932). Les adaptations graduelles des copépods comme premiers hôtes intermediares de *Triaenophorus nodulosus* Pall. *Annales de Parasitologie Humaine et Comparée*, **10**, 334–344.

Michajłow, W. (1933). Les stades larvaires de *Triaenophorus nodulosus* (Pall.) I. Le coracidium. *Annales de Parasitologie Humaine et Comparée*, **11**, 339–358.

Michajłow, W. (1951). Stadialnost rozwoju niectorych tasiemcow (Cestoda). *Annales Universitatis Mariae Curie-Sklodowska Sectio C Biologia*, **6**, 77–147.

Mikailov, T. K. (1963). (Seasonal variations in the parasite fauna of fish in the Mingechaur reservoir.) *Izvestiya Akademii Nauk Azerbaidzhanskoi SSR (Azärbajčan SSR Elmär Akademijasynyn Häbärläri), Biologicheskie i Meditsinskie Nauki*, No. 4, 35–40. (In Russian.)

Miller, R. B. (1943a). Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave Lake, Alberta. I. Introduction and the life of *Triaenophorus crassus* Forel and *T. nodulosus* (Pallas) in the definitive host, *Esox lucius*. *Canadian Journal of Research Section D Zoological Sciences*, **21**, 160–170.

Miller, R. B. (1943b). Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave Lake, Alberta. II. The eggs, coracidia, and life in the first intermediate host of *Triaenophorus crassus* Forel and *T. nodulosus* (Pallas). *Canadian Journal of Research Section D Zoological Sciences*, **21**, 284–291.

Miller, R. B. (1945a). Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave Lake, Alberta. III. Notes on *Triaenophorus nodulosus* (Pallas) in the second intermediate host. *Canadian Journal of Research Section D Zoological Sciences*, **23**, 1–5.

Miller, R. B. (1945b). Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave Lake, Alberta. IV. The life of *Triaenophorus crassus* Forel in the second intermediate host. *Canadian Journal of Research Section D Zoological Sciences*, **23**, 105–115.

Miller, R. B. (1945c). Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave Lake, Alberta. V. Description and life history of *Triaenophorus stizostedionis* n. sp. *Canadian Journal of Research Section D Zoological Sciences*, **23**, 117–127.

Miller, R. B. (1952). A review of the *Triaenophorus* problem in Canadian lakes. *Bulletin of the Fisheries Research Board of Canada*, No. 95, i–iv, 1–42.

Miller, R. B. and Watkins, H. B. (1946). An experiment on the control of the cestode, *Triaenophorus crassus* Forel. *Canadian Journal of Research Section D Zoological Sciences*, **24**, 175–179.

Mishra, T. N. (1966). "The parasite fauna of the fish of the Shropshire Union Canal, Cheshire." Ph.D. thesis, University of Liverpool.

Molnár, K. (1966). Untersuchungen über die Jahreszeitlichen Schwankungen in der Parasitenfauna des Kaulbarsches und des Zanders im Balaton mit besonderer Berücksichtigung der Gattung *Proteocephalus*. *Angewandte Parasitologie*, **7**, 65–77.

Molnár, K. (1968). Jahreszeitliche Schwankungen der Parasitenfauna bei der Elritze (*Phoxinus phoxinus*). *Zeitschrift für Fischerei und deren Hilfswissenschaften*, **16**, 197–206.

Molnár, K. and Berczi, I. (1965). Nachweis von parasitenspezifischen Antikörpern im Fischblut mittels der Agar-Gel-Präzipitationsprobe. *Zeitschrift für Immunitäts- und Allergieforschung*, **129**, 263–267.

Moravec, F. (1970). Studies on the development of *Raphidascaris acus* (Bloch, 1779) (Nematoda: Heterocheilidae). *Věstník Československé Společnosti Zoologické*, **34**, 33–49.

Morozova, M. E. (1955). ("The biology of the early phases of development of the broad tapeworm in conditions of the Karelo-Finnish SSR.") Avtoreferat Karelo-Finnish Filiala. Academiya Nauk SSR. (In Russian, original not seen, quoted from Kuperman, 1973b.)

Mozgovoi, A. A., Shakhmatova, V. I. and Semenova, M. K. (1965). (The life cycle of *Contracaecum spasskii* (Ascaridata: Anisakidae)—a parasite of fish-eating birds.) *In* "Raboty po parazitofauna yugo-zapada SSSR.", pp. 96–103. Instituta Zoologii Academiya Nauk Moldavskoi SSSR. (In Russian.)

Mozgovoi, A. A., Shakhmatova, V. I. and Semenova, M. K. (1968). (Life cycle of *Contracaecum spiculigerum* (Ascaridata: Anisakidae), a parasite of domestic and economically important birds.) *Trudy Gel'mintologicheskoi Laboratorii. Akademiya Nauk SSSR*, **19**, 129–136. (In Russian.)

Neu'min, I. V. (1952). (The effect of external environmental factors on the development and survival of broad tapeworm coracidia.) *Chteniya Pamyati N. A. Kholodkovskogo 1952 goda. Izydatel'stvo Akademiya Nauk SSSR*, pp. 22–31. (In Russian.)

Newton, M. V. B. (1932). The biology of *Triaenophorus tricuspidatus*, (Bloch, 1779), in western Canada. *Contributions to Canadian Biology and Fisheries new series*, **7**, 341–360.

Nicholson, D. (1932). *Diphyllobothrium* infection in *Esox lucius*. *Canadian Journal of Research*, **6**, 166–170.

Noble, R. L. (1970). Parasites of yellow perch in Oneida Lake New York. *New York Fish and Game Journal*, **17**, 95–101.

Nyberg, W. (1966). Immunological identification of *Diphyllobothrium* species. First International Congress of Parasitology 21–26 August 1964 Rome Proceedings Vol. 1, pp. 135–136.

Orr, T. S. C. (1966). Spawning behaviour of rudd, *Scardinius erythrophthalmus*, infested with plerocercoids of *Ligula intestinalis*. *Nature (London)*, **212**, 736.

Orr, T. S. C. (1967a). Parasites of freshwater fish in the Glasgow area Part I. *Glasgow Naturalist*, **18**, 503–504.

Orr, T. S. C. (1967b). Distribution of the plerocercoid of *Ligula intestinalis*. *Journal of Zoology, London*, **153**, 91–97.

Orr, T. S. C. (1968). Distribution and specificity of the plerocercoid of *Ligula intestinalis* (L) in the Northamptonshire area. *Journal of Helminthology*, **42**, 117–124.

Orr, T. S. C. and Hopkins, C. A. (1969a). Maintenance of *Schistocephalus solidus* in the laboratory with observations on rate of growth of, and proglottid formation in, the plerocercoid. *Journal of the Fisheries Research Board of Canada*, **26**, 741–752.

Orr, T. S. C. and Hopkins, C. A. (1969b). Maintenance of the life cycle of *Ligula intestinalis* in the laboratory. *Journal of the Fisheries Research Board of Canada*, **26**, 2250–2251.

Orr, T. S. C., Hopkins, C. A. and Charles, G. H. (1969). Host specificity and rejection of *Schistocephalus solidus*. *Parasitology*, **59**, 683–690.

Owen, R. W. and Arme, C. (1965). Some observations on the distribution of *Ligula* plerocercoids in British freshwater fishes. *Parasitology*, **55**, 6P.

Pal, R. N. (1963). Observations on fluctuations in parasitisation of the Indian shad, *Hilsa ilisha* (Hamilton), of the Hooghly estuary. *Indian Journal of Helminthology*, **15**, 119–126.

Paperna, I. (1974). Larval *Contracaecum* (Nematoda, Anisakidae) in the pericardium of fishes from East African lakes. *Proceedings of the Helminthological Society of Washington*, **41**, 252.

Pennycuick, L. (1971a). Quantitative effects of three species of parasites on a population of three-spined sticklebacks, *Gasterosteus aculeatus*. *Journal of Zoology, London*, **165**, 143–162.

Pennycuick, L. (1971b). Seasonal variations in the parasite infections in a population
Peof three-spined sticklebacks, *Gasterosteus aculeatus* L. *Parasitology*, **63**, 373–388.
nnycuick, L. (1971c). Frequency distributions of parasites in a population of three-spined sticklebacks, *Gasterosteus aculeatus* L., with particular reference to the negative binomial distribution. *Parasitology*, **63**, 389–406.

Pennycuick, L. (1971d). Differences in the parasite infections in three-spined sticklebacks (*Gasterosteus aculeatus* L.) of different sex, age and size. *Parasitology*, **63**, 407–418.

Petrushevskii, G. K. and Shul'man, S. S. (1958). The parasitic diseases of fishes in the natural waters of the U.S.S.R. *In* "Parasitology of Fishes" (V. A. Dogiel, G. K. Petrushevskii and Yu. I. Polyanskii, eds.), pp. 299–319. Translation Z. Kabata, 1961, Oliver and Boyd, Edinburgh and London.

Pitt, C. E. and Grundmann, A. W. (1957). A study into the effects of parasitism on the growth of the yellow perch produced by the larvae of *Ligula intestinalis* (Linnaeus, 1758) Gmelin, 1790. *Proceedings of the Helminthological Society of Washington*, **24**, 73–80.

Plasota, K. (1970). Próba zastosowania rozkładu Weibulla w badaniach populacyjnych do analizy układ'u pasożyt-żywiciel: *Triaenophorus nodulosus* (Pall.)—*Cyclops vicinus* Ulj. *Wiadomości Ekologiczne*, **16**, 145–167.

Pojmańska, T. (1976). The influence of thermic pollution on parasitocenoses structure in water reservoirs. *Wiadomości Parazytologiczne*, **22**, 445–449.

Pojmańska, T., Grabda-Kazubska, B., Kazubski, S. L., Machalska, J. and Niewiadomska, K. (1978). The influence of thermal factor on formation of fish parasitofauna. Fourth Internal Congress of Parasitology 19–26 August 1978 Warsaw Short Communications Section H pp. 24–25.

Ponyi, J., Biró, P. and Murai, É. (1972). (On the food, growth and internal parasitic worms of the ruff (*Acerina cernua* L.) in Lake Balaton.) *Parasitologia Hungarica*, **5**, 383–408. (In Hungarian.)

Powell, A. M. (1966). "A preliminary investigation of the biology and parasite fauna of the charr (*Salvelinus alpinus perisii*)." Ph.D. thesis, University of Liverpool.

Powell, A. M. and Chubb, J. C. (1966). A decline in the occurrence of *Diphyllobothrium* plerocercoids in the trout *Salmo trutta* L. of Llyn Padarn, Caernarvonshire. *Nature* (*London*), **211**, 439.

Pronin, N. M., Pronina, S. V. and Shigaev, S. Sh. (1976). (Larval *Triaenophorus* infection in young-of-the-year *Esox lucius*.) *Materialy Nauchnoi Konferentsii Vsesoyuznogo Obshchestva Gel'mintologov* (*Teoreticheskie i prikladnye problemy gel'mintologii*) No. **28**, 118–126. (In Russian.)

Rausch, R. (1954). Studies on the helminth fauna of Alaska. XXI. Taxonomy, morphological variation, and ecology of *Diphyllobothrium ursi* n. sp. provis. on Kodiak Island. *Journal of Parasitology*, **40**, 540–563.

Rausch, R. L., Scott, E. M. and Rausch, V. R. (1967). Helminths in Eskimos in western Alaska, with particular reference to *Diphyllobothrium* infection and anaemia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **61**, 351–357.

Rautskis, Ė. (1970a). (Seasonal variation in the parasite fauna of perch in Lake Dusia.) *Acta Parasitologica Lituanica*, **10**, 123–128. (In Russian.)

Rautskis, Ė. (1970b). (Seasonal changes in the parasite fauna of pike in Lake Dusia.) *Acta Parasitologica Lituanica*, **10**, 129–135. (In Russian.)

Rautskis, Ė. (1977). (Seasonal dynamics of parasitic infections in *Perca fluviatilis* in the different thermal regimes of lakes Obyaliya, Shlavantas and Galtas.) *Lietuvos TSR Mokslu Akademijos Darbai* (*Trudȳ Akademii Nauk Litovskoi SSR*), **C4**, 63–73. (In Russian.)

Rawson, D. S. and Wheaton, R. R. (1950). Studies of *Triaenophorus crassus* in Nesslin Lake, Saskatchewan, 1950. *Fisheries Research Board of Canada Annual Report of the Central Fisheries Research Station for 1950* Appendix 4, 18–21.

Razumova, E. P. and Gutkovskaya, A. I. (1959). (The fauna of copepods in a focus of *Diphyllobothrium* infection of the lake-river type.) *Meditsinskaya Parazitologiya i Parazitarnie Bolezni*, **28**, 89–94. (In Russian.)

Reshetnikova, A. V. (1965). (Effect of *Digramma interrupta* plerocercoids on the growth of bream.) *Zoologicheskii Zhurnal*, **44**, 734–739. (In Russian.)

Reshetnikova, A. V. (1969). (Influence of wind, temperature and water level in a reservoir on the incidence of infection of bream with *Ligula*.) *In* ("Problems of Parasitology") (A. P. Markevich, ed.) pp. 268–271. Trudy Nauchnoi Konferentsii Parazitologii Ukrainskoi SSR (6th) Part II.

Rizvi, S. S. H. (1964). "The parasite fauna of the fish of Rostherne Mere, Cheshire." Ph.D. thesis, University of Liverpool.

Robertson, J. (1953). The parasites of brown trout (*Salmo trutta* L.) and other freshwater fish. Unpublished report of the Brown Trout Research Laboratory, Scottish Home Department pp. 1–30. (BTSC. 81 34. FW. 53.)

Romanov, I. V. (1964). (Study of foci of diphyllobothriasis in the Gorkov region.) *Meditsinskaya Parazitologiya i Parazitarni Bolezni*, **33**, 289–294. (In Russian.)

Rosen, F. (1919a). Recherches sur le développement des cestodes. I. Le cycle évolutif des Bothriocéphales. Etude sur l'origine des cestodes et leurs états larvaires. *Bulletin de la Société Neuchâteloise des Sciences Naturelles*, **43**, 241–300.

Rosen, F. (1919b). Recherches sur le développement des cestodes. II. Le cycle évolutif de la ligule et quelques questions générales sur le développement des Bothriocéphales. *Bulletin de la Société Neuchâteloise des Sciences Naturelles*, **44**, 259–280.

Rumpus, A. E. (1975). The helminth parasites of the bullhead *Cottus gobio* (L.) and the stone loach *Noemacheilus barbatulus* (L.) from the River Avon, Hampshire. *Journal of Fish Biology*, **7**, 469–483.

Ruszkowski, J. S. (1926). Materjały do fauny helmintologicznej Polski. Część I. *Sprawozdania Komisji Fizyograficznej oraz Materialy do Fizyografii Kraju. Kraków*, **60**, 173–185.

Sapozhnikov, G. I. (1975). (*Dilepis unilateralis* in carp.) In "VII Nauchnaya Konferentsiya Parazitologov Ukrainy." (Tezisy dokladov). Sentyabr' 1975, Donetsk pp. 132–135. (In Russian.)

Scheuring, L. (1919). Eine massenhafte Infektion von *Triaenophorus nodulosus* bei Forellenbrut. *Allgemeine Fischerei-Zeitung*, **44**, 202–207.

Scheuring, L. (1923). Studien an Fischparasiten. *Zeitschrift für Fischerei und deren Hilfswissenschaften*, **22**, 93–204.

Scheuring, L. (1929). Beobachtungen zur Biologie des Genus *Triaenophorus* und Betrachtungen über des Jahreszeitliche Auftreten von Bandwürmern. *Zeitschrift für Parasitenkunde*, **2**, 157–177.

Sedinkin, A. N. (1975). (Data on the crustacean fauna of Lake Batush and its role in the transmission of infection to fish.) *Voprosy Zoologii*, No. 4, 96–99. (In Russian.)

Serdyukov, A. M. (1972). (*Diphyllobothrium dendriticum* (Nitzsch, 1824)—a parasite of man in the Tyumen district.) *Parazitologiya*, **6**, 419–425. (In Russian.)

Shamsuddin, M., Nader, I. A. and Al-Azzawi, M. J. (1971). Parasites of common fishes from Iraq with special reference to larval forms of *Contracaecum* (Nematoda: Heterocheilidae). *Bulletin of the Biological Research Centre University of Baghdad*, **5**, 66–78.

Shul'man, S. S., Malakhova, R. P. and Rybak, V. F. (1974). ("Comparative ecological analysis of fish parasites in the Karelian lakes.") pp. 1–108. Izdatel'stvo "Nauka", Leningrad. (In Russian.)

Sinha, D. P. and Hopkins, C. A. (1967). Studies on *Schistocephalus solidus*. 4. The effect of temperature on growth and maturation *in vitro*. *Parasitology*, **57**, 555–566.

Sous, S. M. (1968). (Effect of winter mass mortalities on the parasite fauna of fish in the forest-steppe lakes of western Siberia.) Fifth All-Union Meeting on the Diseases and Parasites of Fish and Aquatic Invertebrates 29 November–4 December 1968, pp. 107–108. Izdatel'stvo "Nauka", Leningrad. (In Russian.)

Southwell, T. (1930). "The fauna of British India including Ceylon and Burma. Cestoda. Vol. I." i–xxxi, 1–391. Taylor and Francis, London.

Southwell, T. and Prashad, B. (1919). IX. Notes from the Bengal Fisheries Laboratory. No. 4. Cestode parasites of hilsa, *Hilsa ilisha* (Ham. Buch.). *Records of the Indian Museum*, **15**, 77–88.

Spall, R. D. and Summerfelt, R. C. (1969). Host–parasite relations of certain endoparasitic helminths of the channel catfish and white crappie in an Oklahoma reservoir. *Bulletin of the Wildlife Disease Association*, **5**, 48–67.

Strazhnik, L. V. (1975). (Effect of high temperature and biologically active substances on glycogen dynamics and development of fish tapeworms.) Avtoreferat, Kiev. (In Russian, original not seen, quoted from Markevich *et al.* 1976.)

Strazhnik, L. V. and Davydov, O. N. (1975). (On the role of high temperature in the vital activity of some fish cestodes.) *Parazitologiya*, **9**, 37–46. (In Russian.)

Stromberg, P. C. and Crites, J. L. (1974). Triaenophoriasis in Lake Erie white bass, *Morone chrysops*. *Journal of Wildlife Diseases*, **10**, 352–358.

Sweeting, R. A. (1976). Studies on *Ligula intestinalis* (L.) effects on a roach population in a gravel pit. *Journal of Fish Biology*, **9**, 515–522.

Sweeting, R. A. (1977). Studies on *Ligula intestinalis*. Some aspects of the pathology in the second intermediate host. *Journal of Fish Biology*, **10**, 43–50.

Tallqvist, J. (1965). Occurrence of plerocercoids of the fish tapeworm (*Diphyllobothrium latum*) in perch and ruff from Lake Iisvesi and Lake Niinivesi (SE Finland). *Acta Societatis Fauna Flora Fennica* Years 1961–64, **77**, (2), 1–10.

Tedla, S. and Fernando, C. H. (1969). Observations on the seasonal changes of the parasite fauna of yellow perch (*Perca flavescens*) from the Bay of Quinte, Lake Ontario. *Journal of the Fisheries Research Board of Canada*, **26**, 833–843.

Tell, H. (1971). On the parasites of predatory fish of Lake Võrtsjärv. *In* "Estonian Contributions to the International Biological Programme. Progress Report III", pp. 165–182. Academy of Sciences of the Estonian SSR, Estonian Republican Committee for I.B.P., Tartu.

Thomas, L. J. (1946). New pseudophyllidean cestodes from the Great Lakes region I. *Diphyllobothrium oblongatum* n. sp. from gulls. *Journal of Parasitology*, **32**, 1–6.

Thomas, L. J. (1947). The life cycle of *Diphyllobothrium oblongatum* Thomas, a tapeworm of gulls. *Journal of Parasitology*, **33**, 107–117.

Thomas, L. J. (1949). Interrelations of *Diphyllobothrium* with fish-eating birds of northern Lake Michigan. *Journal of Parasitology*, **35**, (6, Section 2), p. 27.

Titova, S. D. (1957). (The parasitofauna of bream (*Abramis brama*) of Lake Ubinsk.) *Izvestiya Vsesoyuznogo Nauchno-Issledovatel'skogo Instituta Ozernogo i Rechnogo Rybnogo Khozyaistva*, **42**, 166–174. (In Russian, translation Israel Program for Scientific Translations Cat. No. 105.)

Vik, R. (1954). Investigations on the pseudophyllidean cestodes of fish, birds, and mammals in the Ånøya water system in Trøndelag. Part I. *Cyathocephalus truncatus* and *Schistocephalus solidus*. *Nytt Magasin for Zoologi*, **2**, 5–51.

Vik, R. (1957). Studies of the helminth fauna of Norway. I. Taxonomy and ecology of *Diphyllobothrium norvegicum* n. sp. and the plerocercoid of *Diphyllobothrium latum* (L.). *Nytt Magasin for Zoologi*, **5**, 26–93.

Vik, R. (1964a). The genus *Diphyllobothrium*. An example of the interdependence of systematics and experimental biology. *Experimental Parasitology*, **15**, 361–380.

Vik, R. (1964b). Studies of the helminth fauna of Norway. V. Plerocercoids of *Diphyllobothrium* spp. from the Rössåga water system, Nordland County. *Nytt Magasin for Zoologi*, **12**, 1–9.

Vik, R. and Meyer, M. C. (1962). Seasonal resistance to *Diphyllobothrium* sp. infection in herring gulls (*Larus argentatus*). *Journal of Parasitology*, **48**, (2, Section 2), p. 46.

Vik, R., Halvorsen, O. and Andersen, K. (1969). Observations on *Diphyllobothrium* plerocercoids in three-spined sticklebacks, *Gasterosteus aculeatus* L., from the River Elbe. *Nytt Magasin for Zoologi*, **17**, 75–80.

Vogt, K. (1938). Experimentelle Untersuchungen über die Gründe von Massen-infectionen mit Plerocercoiden des Fischbandwurms *Triaenophorus nodulosus* (Pall.). *Zeitschrift für Fischerei und deren Hilfswissenschaften*, **36**, 193–224.

Voigt, H-R. (1975). A checklist of the parasites on the smelt (*Osmerus eperlanus* L.). *Tiedoksianto—Information*, **14**, 28–40.

Vojtkova, L. (1959). Příspěvek k poznaní cizopasniků ryb reky Svitavy a Svratky. *Spisy Prirodovedecke Fakulty University v Brne*, No. 401, 97–123.

von Bonsdorff, B. (1977). "Diphyllobothriasis in Man", pp. i–xii, 1–189. Academic Press, London and New York.

von Brand, T. (1944). Physiological observations upon a larval *Eustrongylides*. VI. Transmission to various coldblooded intermediate hosts. *Proceedings of the Helminthological Society of Washington*, **11**, 23–27.

Voore, V. N. (1950). (The distribution of tapeworms (*Ligula*) in Estonian waters.) *Zoologicheskii Zhurnal*, **29**, 323–326. (In Russian.)

Walkey, M. and Davies, P. S. (1964). Respiratory studies on *Schistocephalus solidus*. I. Effect of temperature and worm size. *Parasitology*, **54**, 6–7P.

Wardle, R. A., McLeod, J. A. and Radinovsky, S. (1974). "Advances in the Zoology of Tapeworms, 1950–1970", pp. i–xvi., 1–274 University of Minnesota Press, Minneapolis.

Watson, N. H. F. (1963a). Summer food of lake whitefish, *Coregonus clupeaformis* Mitchill, from Heming Lake, Manitoba. *Journal of the Fisheries Research Board of Canada*, **20**, 279–286.

Watson, N. H. F. (1963b). A note on the upper lethal temperature of eggs of two species of *Triaenophorus*. *Journal of the Fisheries Research Board of Canada*, **20**, 841–843.

Watson, N. H. F. and Lawler, G. H. (1963). Temperature and rate of hatching of *Triaenophorus* eggs. *Journal of the Fisheries Research Board of Canada*, **20**, 249–251.

Watson, N. H. F. and Lawler, G. H. (1965). Natural infections of cyclopid copepods with procercoids of *Triaenophorus* spp. *Journal of the Fisheries Research Board of Canada*, **22**, 1335–1343.

Watson, N. H. F. and Price, J. L. (1960). Experimental infections of cyclopid copepods with *Triaenophorus crassus* Forel and *T. nodulosus* (Pallas). *Canadian Journal of Zoology*, **38**, 345–356.

Wierzbicki, K. (1970). The parasite fauna of the perch, *Perca fluviatilis* L., of Lake Dargin. *Acta Parasitologica Polonica*, **18**, 45–55.

Wierzbicki, K. (1971). The effect of ecological conditions on the parasite fauna of perch *Perca fluviatilis* L. in Lake Dargin. *Ekologia Polska*, **19**, 73–86.

Wikgren, B-J. P. (1963). A contribution to the occurrence of plerocercoids of *Diphyllobothrium latum* in Finland. *Commentationes Biologicae Societas Scientarum Fennica*, **26**, (6), 1–11.

Wikgren, B-J. P. (1964). Notes on the taxonomy and occurrence of plerocercoids of *Diphyllobothrium dendriticum* Nitzsch, 1824 and *D. osmeri* (v. Linstow, 1878). *Commentationes Biologicae Societas Scientarum Fennica*, **27**, (6), 1–26.

Wikgren, B-J. P. (1966a). The growth of plerocercoids of *Diphyllobothrium osmeri* in relation to temperature, with special reference to their mitotic activity. First International Congress of Parasitology 21–26 September 1964 Rome, Proceedings Vol. I, pp. 554–555.

Wikgren, B-J. P. (1966b). The effect of temperature on the cell division cycle in diphyllobothrid plerocercoids. *Acta Zoologica Fennica* No. 114, 1–27.

Wikgren, B-J. and Muroma, E. (1956). Studies on the genus *Diphyllobothrium* a revision of the Finnish finds of diphyllobothrid plerocercoids. *Acta Zoologica Fennica*, **93**, 1–22.

Winterfield, R. W. and Kazacos, K. R. (1977). Morbidity and mortality of great blue herons in Indiana caused by *Eustrongylides ignotus*. *Avian Diseases*, **21**, 448–451.

Zhatkanbaeva, D. (1969). (The role of gulls in the dissemination of *Ligula* and *Digramma* infections at the Kurgal'dzhin lakes.) *Izvestiya Akaden.ii Nauk Kazakhskoi SSR (Kazak SSR Gylym Akademiyasynyn Habarlary) (Biologicheskaya*, No. 6, 38–41. (In Russian.)

Žitňan, R. (1973). Helminty rýb Dobsinskej (Hnileckej) priehrady a ich epizootologický význam. *Biologické Práce*, **19**, (6), 1–87.

Žitňan, R. (1964). Veková dynamika plerocerkoidov *Ligula intestinalis* (L.) u plotice obyčajnej (*Rutilus rutilus*) a niektoré jej zvláštnosti. *Biológia Bratislava*, **19**, 107–111.

Zschokke, F. (1884). Recherches sur l'organisation et la distribution zoologique des vers parasites des poissons d'eau douce. *Archives de Biologie*, **5**, 153–241.

Rumen Ciliate Protozoa

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I. Introduction

Rumen protozoa were first observed by Gruby and Delafond in 1843 and were assumed, from their dramatic appearance, to be important in rumen metabolism. These ciliate protozoa are present in all wild and domesticated

ruminants (Giesecke, 1970) and are of two types, the entodiniomorphid protozoa characterized by the presence of a firm pellicle and the possession of cilia only on the peristome and specialized areas elsewhere and the holotrich protozoa which are completely covered with cilia. This review includes, firstly, consideration of the apparent role of the protozoa in ruminant growth, metabolism and disease, followed by brief accounts of cultivation and structure and detailed descriptions of physiology and biochemistry of individual species. Lastly, the detailed and general findings are correlated to show the role played by protozoa in normal ruminants.

II. Role of Ciliate Protozoa *In Vivo*

A. Effect on the ruminant of the presence or absence of protozoa

1. *Effect on growth*

Although ciliate protozoa are present in all ruminants, they are not essential for the life of adult animals and in 1929, Becker *et al.* were the first to show that ruminants could be rendered protozoa-free without change in their weight or metabolism. Rumen ciliate protozoa are acquired by young ruminants directly from adult animals or by eating food contaminated with saliva from such animals (Becker and Hsiung, 1929; Eadie, 1962a). Lambs or calves reared in the absence of adult animals have no ciliate protozoa although they acquire a normal bacterial flora. Subsequently Becker and Everett (1930) showed that protozoa-free and protozoa-containing lambs grew at the same rate. Since then there have been eight other studies on the effect of protozoa on the growth of young animals of which three (Abou Akkada and El-Shazly, 1964; Christiansen *et al.*, 1965; Borhami *et al.*, 1967) showed that the average daily weight gain could be up to 30% higher in lambs containing single or mixed protozoal species whereas five studies (Pounden and Hibbs, 1950; Eadie, 1962a; Chalmers *et al.*, 1968; Eadie and Gill, 1971; Williams and Dinusson, 1973) found no effect on growth. However, Eadie and Gill (1971) and Pounden and Hibbs (1950) noted that protozoa-free animals were pot-bellied compared with protozoa-containing animals and the latter authors also noted the presence of a rougher coat. Borhami *et al.* (1967) showed that protozoa were more beneficial to the host when a poor ration was given, whereas Eadie and Gill (1971) showed that under some conditions the converse was marginally true. Recently, Bird *et al.* (1978) have shown that on a low-protein ration faunated animals grew more slowly than defaunated animals unless comparatively large amounts of fishmeal (a by-pass protein) were added to the ration.

2. *Effect on rumen and blood metabolites and bacteria*

In experiments comparing the concentration of metabolites in the rumen of faunated and protozoa-free animals, there is general but not universal

agreement that levels of ammonia and volatile fatty acids were higher in the faunated animal (e.g. Abou Akkada and El-Shazly, 1964; Borhami *et al.*, 1967; Eadie and Gill, 1971). However, there is disagreement on the effect of faunation on the relative proportions of volatile fatty acids in the rumen with Abou Akkada and El-Shazly (1964), Christiansen *et al.* (1965), Williams and Dinusson (1973) and Kurihara *et al.* (1978) believing that the acetate/propionate ratio decreased on faunation, whereas Leng (1976), Youseff and Allen (1968) and Males and Purser (1970) showed the converse. These last authors and Klopfenstein *et al.* (1966) also found a marked increase in the level of butyrate on faunation. These discrepancies suggest that other factors such as ration and animal age may be important in determining the exact effect of faunation.

As might be expected from the different results obtained in experiments on live weight gain, Chalmers *et al.* (1968) found no increased nitrogen digestibility or retention in faunated lambs, whereas Abou Akkada and El-Shazly (1965) found higher blood haemoglobin and protein levels associated with better nitrogen retention in lambs containing protozoa. Klopfenstein *et al.* (1966) found lower plasma amino acid concentrations in faunated animals and obtained evidence that lysine was the limiting amino acid for growth in defaunated animals, whereas no single amino acid was consistently limiting in faunated animals. These authors also found an apparent increase in digestibility of dry matter in faunated animals on some rations, and Kurihara *et al.* (1978) found increased digestibility of cellulose in a faunated animal fed on purified rations. In contrast, Eadie and Gill (1971) found lower digestibility of crude fibre in their faunated animals.

There is, however, agreement that faunation of a rumen decreased the bacterial population (Eadie and Gill, 1971; Eadie and Hobson, 1962; Kurihara *et al.*, 1968, 1978), probably due to engulfment of bacteria by the protozoa (described in detail in Section V).

B. EFFECT OF FEED COMPOSITION ON PROTOZOAL POPULATION

It is difficult to interpret the results of any experiment on variation in numbers of protozoa in the rumen because (a) the rumen contents are not homogeneous, having solid and liquid phases, and (b) there is up to three-fold variation in the protozoal population density in one animal kept under constant conditions on a constant ration (Boyne *et al.*, 1957). However, Purser and Moir (1959) found little difference in protozoal population density in samples taken from different parts of the rumen. Regardless of these problems, there are certain very marked effects of feed composition on protozoal numbers.

Almost all entodiniomorphid protozoa can grow on starch grains and up to a certain level any increase in the amount of starch in the feed increased the number of protozoa (Abe *et al.*, 1973). If animals were fed to repletion on a pelleted barley ration, the rumen acidity increased and all the ciliate protozoa disappeared (Eadie *et al.*, 1967). However, if a restricted (80% of appetite) high-grain ration was given, very high protozoal population

densities ($> 2 \times 10^6$ ml^{-1}) developed (Eadie *et al.*, 1970) and over 95% of these could be *Entodinium* spp. (Van der Wath and Myburgh, 1941). The number of holotrich protozoa did not parallel that of the entodiniomorphid protozoa and often increased when the total ciliate population was decreasing (Eadie *et al.*, 1970). This may reflect the ability of the holotrich protozoa to use soluble sugars. However, any feeding of a high-grain ration must be begun gradually because of the risk of the protozoa being unable to grow quickly enough to engulf all the starch. If free starch remained, it was fermented to lactic acid and the resultant increase in acidity killed the protozoa (Eadie and Mann, 1970; Schwartz and Gilchrist, 1975). On a more usual ration, decrease in water availability and feed intake also both tended to increase the numbers of holotrichs (Dehority and Purser, 1970). Certain chemical additions increased the rumen ciliate population; e.g., aureomycin and tylosin (Klopfenstein *et al.*, 1964; Purser *et al.*, 1965), diethylstilboestrol (Ibrahim *et al.*, 1970), β-sitosterol (Hino and Kametaka, 1974) and the hydroxy analogue of methionine (De Vuyst *et al.*, 1975).

C. DIURNAL VARIATION IN PROTOZOAL POPULATION DENSITY

In animals fed once a day the population density of entodiniomorphid protozoa declined by over 50% in the 6 h after feeding and then at some time during the remainder of the day increased slowly or rapidly (preceded or followed by a stable period) until the original pre-feeding population density was reached (Warner, 1962, 1966a; Michalowski and Muszynski, 1978; Michalowski, 1977). There is disagreement on the diurnal changes that occur in the numbers of the holotrich protozoon *Dasytricha ruminantium*. Purser (1961), Warner (1962) and Michalowski and Muszynski (1978) showed that the population density declined steadily for 16 h after feeding and then increased rapidly with up to a five-fold increase over 4 h. In contrast, Clarke (1965) found in animals fed on fresh red clover that there was a rapid increase (3–4 times) in the number of *D. ruminantium* in the 2 h after feeding followed by a decline to the original value in the next 3–6 h. The numbers then remained constant till the next feed.

In animals fed a proportion of their feed every 3 or 12 h, the total number of protozoa tended to be higher than in those fed once a day but the pattern of variation was similar, except that it was correspondingly shorter (Warner, 1966b; Moir and Somers, 1956; Michalowski and Muszynski, 1978; Sénaud *et al.*, 1973). The pattern of variation in animals on pasture tended to be that of animals fed once a day as, when left to itself, the animal grazed only at one time of day (Warner, 1966c).

D. INTERRELATIONSHIPS BETWEEN PROTOZOA

There are two different protozoal populations in the rumen, one (designated A) characterized by the presence of *Polyplastron multivesiculatum* and *Diploplastron affine* and another, designated B, characterized by *Epidinium* spp., *Eudiplodinium maggii* and *Eremoplastron bovis*. Certain protozoa such as

Entodinium spp. and the holotrich protozoa are common to both (Eadie, 1962b). Both populations are stable and flocks of sheep, at least, frequently contain some animals with A- and some animals with B-type protozoal populations. However, if a small quantity of rumen contents containing A-type protozoa was inoculated into the rumen of an animal with a B-type population, the characteristic B-type protozoa disappeared and were replaced by those of type A (Eadie, 1967). The change is brought about by *P. multivesiculatum* engulfing and killing the B-type protozoa (see below).

Other antagonisms are those between *Ent. bursa* and *Ent. caudatum*, when the former engulfs the latter and also induces the latter to form caudal spines (Poljansky and Strelkow, 1938), and between *Ophryoscolex* spp. and *Epidinium* spp. (Eadie, 1967).

E. ESTABLISHMENT OF CILIATE PROTOZOA IN YOUNG RUMINANTS

There is no resistant phase in the life cycle of rumen protozoa (Strelkow *et al.*, 1933) and ruminants remained free of protozoa for life provided that they had no contact with faunated ruminants (Becker and Hsiung, 1929; Eadie, 1962a). Rumen contents of young ruminants tended to be acid until they started grazing and this prevented the establishment of protozoa (Hungate, 1966). However, if the pH of the rumen contents was maintained above 6·5 ciliate protozoa could appear in lambs and calves kept with the dam by only 9 days after birth and be universally present after 3 weeks (Eadie, 1962a). *Entodinium* spp. became established first, followed by the larger entodiniomorphid protozoa and then the holotrich protozoa, although in calves *Isotricha* spp. became established earlier than in lambs (Eadie, 1962a). The same result was obtained regardless of whether the lamb or calf was kept with the dam or artificially inoculated. Once established, the protozoal populations were maintained provided that the pH of the rumen did not fall below 5·0 (Purser and Moir, 1959). If the pH did fall due to over-feeding of a starch-rich ration, the *Entodinium* spp. and *Isotricha* spp. were least sensitive to the change (Eadie, 1962a).

In the author's experience, lambs kept on pasture at Babraham under normal farm conditions sometimes take 3–6 months to obtain their full adult protozoal population. In three successive years (1973–1975) 10/20 lambs after 2–4 months, 17/61 after 3–5 months and 17/72 after 2–4 months contained no large entodiniomorphid protozoa (G. Coleman and P. Kemp, unpublished work). Two 5-year-old breeding ewes out of a flock of 28 also contained no large protozoa. B-type protozoa tended to become established before those of type A.

Comparison of the protozoal types in lambs (3–5 months old) and their dams showed no evidence for the transfer of large protozoa from a ewe to her own lamb because the protozoal populations were different in 22 out of 34 pairs (Table 1). Even among twin lambs there were examples in which each lamb had a different protozoal population which also differed from that in the dam (Table 2) (G. Coleman and P. Kemp, unpublished work). This suggests that the lambs pick up these large protozoa at random from any

TABLE 1

The type of protozoal population present in ewes and their lambs

Population type in ewe[a]	Population type in lamb[a,b]	Number found
B	B	10
B	A	6
B	0	3
A	A	2
A	B	8
A	0	2
0	0	0
0	A	1
0	B	2

[a] A, A-type population; B, B-type population; 0, no characteristic large protozoa.
[b] At age 3–5 months.

TABLE 2

The type of protozoal population in individual ewes and their twin lambs

Population type in ewe[a]	Population types in lambs[a,b]
B	B,B
B	A,B
B	A,A
B	0,0
A	A,B
0	A,B
?	0,B

[a] A, A-type population; B, B-type population; 0, no characteristic large protozoa.
[b] At age 3–5 months.

member of the flock. Some mature ewes have no large protozoa, which suggests either that the chances of infection are low and that some animals can avoid it or that there is some factor, possibly in the saliva, that prevents infection by certain protozoa in certain animals. This latter possibility is believed to be the more likely because theoretically it would require only one *Polyplastron multivesiculatum* to enter the rumen of every sheep for all the B-type protozoa to have disappeared years ago. As this has not occurred some mechanism must exist to prevent infection in normal animals.

F. UTILIZATION OF PROTOZOA BY THE HOST

To calculate what proportion of the nitrogen entering the duodenum is derived from protozoa, it is necessary to consider (a) the proportion of the rumen nitrogen present in protozoa and (b) whether the protozoa leave the rumen at the same rate as the bacteria and the liquid phase.

Various methods have been used to calculate the contributions of bacteria and protozoa to the total rumen nitrogen including direct measurement (Hungate *et al.*, 1971), incorporation of ^{15}N and separation of the micro-organisms (Pilgrim *et al.*, 1970) and use of diaminopimelic acid and amino-ethylphosphonate which occur specifically in bacterial cell walls and protozoal phospholipids respectively (Ibrahim and Ingalls, 1972). On conventional and semi-purified diets, between 20 and 60% (58%—Hungate *et al.*, 1971; 44·5% and 50·5%—Ibrahim and Ingalls, 1972; 20%—Pilgrim *et al.*, 1970; 33%—Weller *et al.*, 1958) of the mirobial nitrogen was present in protozoa.

Initially all workers assumed that all fractions in the rumen contents passed into the omasum together and that the proportion of protozoal nitrogen in rumen contents gave a measure of the importance of protozoa to the host animal (Ibrahim and Ingalls, 1972). However, Hungate *et al.* (1971), on the basis of measurements of carbon and nitrogen recoveries and the rate of passage of a marker, polyethylene glycol (PEG), out of the rumen, concluded that only half of rumen nitrogen left the rumen with the marker. It was calculated that PEG turnover rate was 2·27 total rumen volumes per day, microbial turnover rate 1·25 volumes per day and protozoal turnover rate 0·69 volumes per day, showing that the protozoa left the rumen more slowly than other microorganisms. Bird *et al.* (1978) showed that the density of protozoa in omasal fluid was only 8–10% of that in the rumen. Nolan (1975), by use of ^{15}N, and Weller and Pilgrim (1974), by direct observation of protozoa leaving the rumen, obtained similar results, the latter showing that the protozoa left the rumen at only 20% of the rate of a soluble marker and that less than 2% of the dietary nitrogen could be protozoal. However, no measurement was made of passage of bacteria and samples were taken only twice a day so it is possible, in view of the diurnal variation in the numbers of protozoa, that the protozoa could all pass into the omasum together at another time.

The protozoa (*Epidinium* spp.—Bauchop and Clarke, 1976; *Isotricha* spp. —Orpin and Letcher, 1978) and to a lesser extent the bacteria (Akin, 1976; Latham *et al.*, 1978a, b) may be retained in the rumen because of their attachment to food particles especially as there is often, at the top of the rumen, a solid 'crust' of plant material which tends to be retained in the rumen. Leng (1976) assumed that because the protozoa may not pass on with the remainder of the rumen contents they must die and be lysed, but this need not occur. Weller and Pilgrim (1974) and Czerkawski and Breckenridge (1977, 1978) showed that satisfactory growth of rumen protozoa in continuous cultures was obtained only if there was a large 'dead' space where the protozoa could become sequestered. Under these conditions the population density in the free liquid could remain constant with a protozoal generation time greater than that in the liquid phase.

Rumen protozoa were more completely digested than bacteria by rats (McNaught *et al.*, 1954; Bergen *et al.*, 1968), presumably because, unlike the bacteria, they were completely destroyed by acid conditions in the stomach (Smith, 1969). As conditions are similar in the abomasum the same increased digestibility may obtain in the normal host.

G. BLOAT

Apart from simple bloat caused by lack of roughage in the ration, there are two other kinds of bloat, legume bloat and feedlot bloat. The exact causes of these two forms of bloat are not known although the genetic composition of the animal (McIntosh and Cockrem, 1977), the nature of the ration (Jones and Lyttleton, 1969) and the rumen microorganisms, including protozoa (Bartley *et al.*, 1975), have all been suggested as possible causative agents.

1. *Legume bloat*

This occurs when animals are fed on red clover and is caused by the build up of a stable foam (Reid *et al.*, 1975) produced by the trapping of fermentation gases in protein derived from plant leaves, saliva and holotrich protozoa (Bartley and Bassett, 1961). As will be shown below, these protozoa tend to burst when fed an excess of free sugar and Clarke (1965) suggested that the drop in numbers of protozoa 2 h after feeding fresh clover, which is rich in free sugars, was due to this. Proteins from holotrich protozoa form very rigid foams of high compressive strength and may act as a stabilizing agent for rumen foams caused by plant and salivary proteins (Jones and Lyttleton, 1972). Attempts were therefore made to control legume bloat by killing the holotrich protozoa. Clarke (1966) and Clarke *et al.* (1969) used dimetridazole (an anti-protozoal agent) and obtained protection from bloat until the protozoal population had recovered. Davis and Essig (1972) also used copper sulphate, dioctyl sodium sulphosuccinate and a non-ionic surfactant (poloxalene) to reduce the protozoal numbers and successfully treat bloat and concluded that the protozoa were important in legume bloat. However, all the agents used had other effects on the animals and it is difficult to be certain that protozoa are an important factor in bloat.

2. *Feedlot bloat*

This occurs in the U.S.A. when beef cattle, fed previously on roughage, are gradually given increasing amounts of a 'high grain' finishing ration for 4–5 months. As with legume bloat, the cause is the formation of stable foams but in this case the foaming agents all appeared to be of microbial origin (Bartley *et al.*, 1975). Protozoa have again been implicated and Mishra (1964) found that protozoal population density and the degree of bloat were inversely related. It is possible that the large numbers of protozoa resulting from some high-grain rations might engulf both the starch and bacteria present and prevent the latter utilizing the former with the production of large amounts of the slime that gives rise to stable foams. This subject has been reviewed by Clarke and Reid (1974).

III. Cultivation of Ciliate Protozoa *In Vitro*

The rumen provides a warm (39°C) anaerobic environment normally deficient in readily metabolizable compounds such as sugars and amino

acids but rich in particulate matter such as starch grains, cellulose, chloroplasts and bacteria. All successful attempts to culture these protozoa have aimed to simulate the condition *in vivo*. The holotrich protozoa have proved easy to keep alive for short periods but very difficult to culture for long periods, whereas many of the entodiniomorphid protozoa have been grown for periods of at least 2 years without difficulty.

A. ENTODINIOMORPHID PROTOZOA

1. *Growth conditions*

These protozoa were first grown successfully by Hungate (1942, 1943) who used a NaCl-rich medium rendered oxygen-free by the passage of N_2/CO_2 (95 : 5, v/v) with cellulose or starch as substrates but without the addition of reducing agents. It proved difficult to repeat this work (Sugden, 1953; Oxford, 1958) but *Eremoplastron bovis* was grown for 5 months (Clarke, 1963), *Ophryoscolex purkynei* for 32 months (Mah, 1964), *Epidinium ecaudatum* for 6 months (Gutierrez and Davis, 1962) and *Entodinium simplex* for more than 40 days (Jarvis and Hungate, 1968), by adding reducing material to the medium in order to keep the redox potential down. In addition, Einszporn (1961) and Michalowski (1975) grew *Ent. longinucleatum* and mixed protozoa respectively on sterile rumen fluid plus starch and hay. Although all the protozoa mentioned above were grown on media deficient in readily metabolizable substrates, Hino *et al.* (1973a, b, c) successfully grew mixed *Entodinium* spp. for many months on media containing egg protein, peptone, yeast extract and glucose in addition to starch.

Over the past 20 years the present author has made an extensive study of the cultivation of these protozoa (Coleman, 1958, 1960, 1969a, 1978a; Coleman *et al.*, 1972, 1976, 1977; Owen and Coleman, 1976). The protozoa can be divided into two groups depending on whether the optimum partial pressure of CO_2 in the gas phase is 5% (for *Ent. caudatum*) or 100% (e.g., for *Ent. simplex*). The media contained principally potassium phosphate, and dissolved oxygen was removed by vigorous bubbling with the appropriate gas. The redox potential was reduced by the addition of cysteine and sometimes prepared fresh rumen fluid. The protozoa can be divided into two further groups (independently of the first two groups) depending on whether they utilize cellulose, when the cultures must be fed daily on ground dried grass, or starch, when they must be fed on ground wheat and a little dried grass. Clone cultures were initiated by inoculating individual protozoa into the appropriate medium. Experimental details are given by Coleman (1978a). Minimum generation times for the growth of protozoa *in vitro* varied from 6 to 38 h.

All the protozoal cultures contained living bacteria and no attempt was made to keep the cultures sterile. As the protozoa live by engulfing particulate matter, the rationale behind the method used is to provide them regularly with small amounts of food in such a way that all is taken up by the protozoa at once, leaving little for bacterial attack. The limited bacterial growth that

occurred on the sugars liberated from the protozoa during digestion of the food (Coleman, 1969b) was sufficient to remove any trace of dissolved oxygen introduced during the feeding procedure and to maintain a low redox potential in the medium. Unlike Hino *et al.* (1973a, b, c), the present author has always found that continuous addition of soluble nutrients encouraged heavy bacterial growth and depressed protozoal growth.

2. *Requirement for other protozoa*

Polyplastron multivesiculatum (Coleman *et al.*, 1972) and *Entodinium bursa* (Coleman *et al.*, 1977) will grow readily *in vitro* only in the presence of *Epidinium* spp. and *Ent. caudatum* respectively and it is essential to add these protozoa daily with the other food.

3. *Morphological changes*

During the first 6 months' growth *in vitro* rumen ciliates that have caudal spines when isolated from the rumen, tended partially or completely to lose these spines. This loss was not normally reversible except with *Entodinium caudatum* where the spines reappeared after a few weeks' growth in the presence of *Ent. bursa* (Coleman *et al.*, 1977). With *Ophryoscolex caudatus*, the change occurred after 2–4 weeks in culture, with the main caudal spine becoming shorter and twisted and the body shorter and fatter. It was also accompanied by a physiological change because cultures often died at this time (Coleman, 1978a).

4. *Life of cultures*

Except for *Entodinium caudatum* and *Ent. simplex* most entodiniomorphid protozoa grew for only $1\frac{1}{2}$–2 years *in vitro* (Coleman, 1978a) before there was a 'loss of vigour' and the cultures gradually died. The cause is unknown but the process could be reversed by inoculation into a defaunated rumen and re-isolation of the protozoa after 6 days. It is possible that some rejuvenating process such as conjugation can occur only in the rumen.

5. *Synthetic media*

Most of the work on the cultivation of entodiniomorphid protozoa has been done with natural food materials but Broad and Dawson (1976) showed that the commercial rice starch, rumen fluid and dried grass used in cultures could be replaced by purified rice starch, choline and β-sitosterol, although the generation time was longer than in conventional media. Hino *et al.* (1973c), using a complex semi-synthetic medium, also reported that mixed *Entodinium* spp. required β-sitosterol and campesterol.

6. *Continuous culture*

Rumen entodiniomorphid protozoa have not been successfully grown in a conventional continuous culture apparatus (Rufener *et al.*, 1963). Various

reasons have been suggested including (a) protozoa cannot feed on starch grains while being artificially stirred (G. Coleman, unpublished work), (b) toxic end-products accumulate (Abe and Kumeno, 1973) and (c) the protozoa require a 'dead' space in the culture vessel in which they can become sequestered (Weller and Pilgrim, 1974). Abe and Kumeno (1973) overcame the problems by dialysing to remove toxic end-products and obtained protozoal division every 2 days. Weller and Pilgrim (1974) and Czerkawski and Breckenridge (1977) devised similar cylindrical fermentors which contained a perforated hollow piston that occupied approximately 70% of the volume of the vessel. The piston, which moved slowly up and down, contained nylon bags of food material into which the protozoa could swim. Under these conditions protozoal populations in the effluent, similar to those found in the rumen, could be maintained at dilution rates of the bulk liquid of 0·34–0·96 volumes per day. However, the concentration of protozoa in the solid matter in the bags was over 20 times that in the effluent (Czerkawski and Breckenridge, 1978). Hoover *et al.* (1976) designed a simple apparatus that selectively retained solid matter in the culture vessel and also obtained satisfactory protozoal growth, especially at higher dilution rates.

7. *Axenic and monoxenic cultures*

It has proved difficult to render rumen ciliates bacteria-free because (a) the protozoa all have endoplasmic vesicles containing bacteria which could be removed only by prolonged treatment with antibiotic and (b) it is difficult to sterilize the food materials because these will not support protozoal growth after autoclaving or ethylene oxide treatment (Jarvis and Hungate, 1968) or dry heat (Coleman, 1960). Coleman (1962) produced almost bacteria-free *Entodinium caudatum* by treatment with four antibiotics, but the protozoa were almost moribund. More recently Jarvis (1974) claimed to have washed *Ent. biconcavum* free of bacteria although the protozoa were not broken to liberate intracellular bacteria before incubation in bacteriological culture media.

Hino and Kametaka (1977) solved the sterilization problems by irradiating the starch with ^{60}Co and autoclaving their plant material anaerobically. These substrates supported the growth of *Ent. caudatum* and by means of combined treatment of the protozoa with antibiotics and a suspension of *Escherichia coli* or other bacteria it was possible to prepare and grow the protozoa in the presence of just the one bacterium. These authors also maintained *Ent. caudatum* in declining numbers in the presence of dead *Escherichia coli* for 22 days.

B. HOLOTRICH PROTOZOA

Isotricha spp. and *Dasytricha ruminantium* are relatively easy to keep alive for up to 4 weeks *in vitro* in media equilibrated with CO_2 and containing glucose as source of carbohydrate (Sugden and Oxford, 1952; Gutierrez, 1955; Purser and Weiser, 1963). The most characteristic feature of these

protozoa is the rapidity with which they assimilate glucose and convert it into amylopectin. This makes the protozoa very dense and it is comparatively easy to separate them from other ciliates by differential settling of a suspension under gravity (Heald *et al.*, 1952; Eadie and Oxford, 1957). However, it is probable that holotrich protozoa densely filled with storage material in this way are unable to divide easily; in a ruminant fed only once daily, *D. ruminantium* divided only 20 h after feeding (Purser, 1961). Clarke and Hungate (1966) used this information to devise a feeding regime for cultures *in vitro* in which sucrose was fed to protozoa for two periods of 2 h each day. After each feed the protozoa were transferred to sugar-free medium. By use of this technique and media (containing serum albumin or protozoal extract), so highly reduced that methanogenic bacteria developed, these authors maintained *D. ruminantium* alive for over 60 days at population densities up to 1300 ml^{-1}. *Isotricha* spp. were also maintained at low population densities for up to 50 days under the same conditions.

IV. Structure of Entodiniomorphid Protozoa

These protozoa vary in size from 25×11 μm (*Entodinium exiguum*—Dogiel, 1927) to 205×123 μm (*Polyplastron multivesiculatum*—Coleman *et al.*, 1972) and are covered by a relatively tough pellicle except at the posterior end where there is an anus and the anterior end where there is an extrudable peristome. This is U-shaped in transverse section and is partially covered by a band of cilia which propel the protozoa (Coleman and Hall, 1971). The cilia usually beat together in groups, called syncilia, containing 100–200 cilia (Noirot-Timothée, 1960). One end of the ciliary band extends down one side of the inside of the U (the oesophagus) and is used to propel particles such as bacteria and starch grains towards an area of naked cytoplasm at the bottom where food vacuoles form (Coleman and Hall, 1971). In all genera except *Entodinium* there is also a second band of cilia situated on the dorsal side of the protozoa either at the anterior end or displaced posteriorly. Under unfavourable conditions the peristome can be retracted, the outer lips coming together over the top apparently to seal up the protozoon from the medium.

Internally these protozoa are divided into ectoplasm and endoplasm by a boundary layer and there is evidence (Coleman, 1967b, 1969b) that the external pellicle is freely permeable to solutes whereas the boundary layer is not. The protozoal ectoplasm contains (except in *Entodinium* and *Diplodinium* spp.) so-called 'skeletal plates'—groups of amylopectin granules arranged in patterns characteristic of different genera (Dogiel, 1927). The individual granules are of an irregular shape that enables them to fit together to form the complete plate without large spaces in between. These plates, which stain readily with iodine when full of starch, are the last storage area to lose their starch on starvation and the first to be refilled on feeding starved protozoa. The ectoplasm also contains spherical or more cylindrical polysaccharide granules with a smooth outline. These are commonly found packed together at the extremities of the cell and are storage organelles like

the skeletal plates. Entodiniomorphid protozoa all contain one or more 'contractile' vacuoles in the ectoplasm but there is doubt as to whether they ever contract (Hungate, 1966), although MacLennan (1933) measured a pulsatory cycle of from 1 to 60 min or longer in media of low osmotic pressure. The macro- and micro-nuclei are also present in the ectoplasm.

The endoplasm contains particles engulfed by the protozoon which normally include starch grains, cellulose fibres, chloroplasts and bacteria. They are digested in vesicles and any indigestible material is extruded via the anus. Many species of rumen ciliates are selective in what they engulf but *Ent. caudatum* will take up any particle that is small enough (Coleman and Hall, 1969). All rumen ciliates grown *in vitro* or *in vivo* contain bacteria apparently living in vesicles in the endoplasm. Many also have bacteria (identified as *Streptococcus bovis* and *Ruminococcus albus* by Imai and Ogimoto, 1978) attached to the outside of the pellicle when isolated from the rumen, although the author has never seen any attached to *Ent. caudatum* grown *in vitro* (Coleman and Hall, 1974).

The various genera of entodiniomorphid protozoa are separated on the basis of size, number of ciliary bands, presence, number and distribution of skeletal plates and macronuclear shape. Species have been delineated on the basis of the number and distribution of caudal spines, but both Hungate (1966) and the present author consider these to be poor criteria as single protozoa can give rise to cultures containing individuals with different spination and because (in, e.g., *Ent. caudatum*) the characteristic caudal spine is present only in the presence of *Ent. bursa*.

V. Metabolism of Individual Species of Entodiniomorphid Protozoa

Many of the earlier studies on rumen ciliates were made with mixed protozoal preparations and it is difficult therefore to be certain which species were responsible for the effects observed. If there are no comparable studies on single species, the results obtained will be referred to under *Ent. caudatum*. Information on the uptake and digestion of mixed rumen bacteria by all the protozoa examined by the author is given in Table 3.

A. *Entodinium caudatum*

Entodinium caudatum has been cultured by the author for almost 20 years and much of the work on the metabolism of these protozoa has been made with these cultures. On isolation from the growth medium *Ent. caudatum* contained approximately 16 *Klebsiella aerogenes*, 8 *Proteus mirabilis*, 1 *Butyrivibrio* sp. and 1 *Bacteroides* sp. (White, 1969) although the actual numbers depended on the time since the protozoa were last fed. Protozoa isolated from the rumen also contained similar numbers of different intracellular bacteria (Coleman and White, 1970). Any results obtained on the metabolism of, e.g., sugars must therefore take account of the presence of these bacteria which have been shown to take up low-molecular-weight compounds from the medium (Coleman, 1978c; Coleman and Hall, 1974).

TABLE 3

The uptake and digestion of mixed rumen bacteria by rumen ciliate protozoa

Protozoon	Grown *in vivo* or *in vitro*[a]	Relative size[b]	Bacteria engulfed[c]		Volume cleared of bacteria[d]		Relative digestion[e]
			Maximum	At 10^9 bacteria per ml	Maximum	At 10^9 bacteria per ml	
Entodinium caudatum	T	3·3	2700	950	1·5	1·0	0·08–0·3
Ent. caudatum	V		1070	130	0·18	0·14	0–1·5
Ent. caudatum - spined	V	5·9	4070	340	0·40	0·38	0
Ent. longinucleatum	T	5·6	35000	1220	1·43	1·40	
Ent. simplex[f]	V	1·0	450	190	0·28	0·20	1·5
Ent. bursa	T	12·7	21000	1600	1·76	1·63	1·2
Ent. bursa	V		7500	1080	0·83	0·98	0
Epidinium ecaudatum caudatum (ovine)	T	18	10100	3600	8·7	6·8	0
Ep. ecaudatum caudatum (ovine)	V		6500	770	0·89	0·84	0
Ep. ecaudatum caudatum (bovine)	T		16000	5600	23	4·7	0
Ep. ecaudatum tricaudatum	T		5000	3200	6·0	2·6	0
Polyplastron multivesiculatum (− *Epidinium* spp.)	T	48	97000	8900	15	10	0
P. multivesiculatum (+ *Epidinium* spp.)	T	113	83000	12000	17	13	0
Eudiplodinium maggii	T	29	68000	6200	6·9	7·2	0–6
Eremoplastron bovis	T	7·9	4500	905	0·83	0·125	0
Er. bovis	V		980	380	0·24	0·24	0
Ophryoscolex caudatus	T	47	100000	21200	30	28	0·3
Ostracodinium obtusum bilobum	T	14·9	10600	2180	0·23	0·22	
Os. obtusum bilobum	V		1220	124	0·15	0·12	0·4
Enoploplastron triloricatum	V	8·7		1200[g]			2·8

[a] T, *in vitro;* V, *in vivo.*

[b] Relative to that of *Ent. simplex.*

[c] Per protozoon per hour, determined over 3–4 h.

[d] $\times 10^{-6}$ μm^3 per protozoon per hour, determined over 3–4 h.

[e] Amount of bacterial carbon free in the medium after 3–4 h divided by the amount of bacterial carbon in the protozoa at that time.

[f] A small protozoa fraction from a normal A-type rumen population. It was principally *Ent. simplex.*

[g] At $1{\cdot}8 \times 10^9$ ml^{-1}.

1. *Metabolism of carbohydrates*

Entodinium caudatum normally feeds on starch grains which are engulfed within a few seconds after addition to a protozoal culture. However, it is difficult to do experiments on the production of metabolites from added carbohydrates because all healthy rumen ciliates contain much reserve amylopectin and on starvation tend to die as soon as the reserves are exhausted. Intracellular starch was fermented by *Ent. caudatum* with the production of principally acetic acid, butyric acid and CO_2 with small amounts of lactic acid and propionic acid (Abou Akkada and Howard, 1960). Although starch was readily utilized by *Ent. caudatum* and digested to glucose and maltose (Abou Akkada and Howard, 1960; Coleman, 1969b), glucose did not stimulate the rate of gas production by washed suspensions of protozoa. However, as ^{14}C from [^{14}C]glucose was taken up into the pool, the protozoal polysaccharide and intracellular bacteria, it might be expected to stimulate the rate of gas production. The reason why it did not is that maltose and glucose inhibit the action of the amylase and maltase respectively that form them from starch. Any increase in glucose concentration in the pool due to uptake of external glucose would therefore have tended to inhibit the production of glucose from maltose. The concentration of glucose in the pool therefore tended to remain constant. Further, as the glucose concentration was much higher than the K_m value for the hexokinase present, any small increase in the glucose concentration would not have increased the rate of formation of glucose-6-phosphate (Coleman, 1969b).

Glucose like most other low-molecular-weight compounds was taken up by washed suspensions of *Ent. caudatum* by an active and a passive process. The latter, which predominated at high external glucose concentrations, represented a trapping of some of the external medium in a compartment of the cell from which it could not be removed by washing in salt solution at room temperature. As the amount of material trapped was increased in the presence of the metabolic inhibitor iodoacetate, it is possible that the medium containing the [^{14}C]glucose was trapped in a structure such as the contractile vacuole, the rate of emptying of which was reduced at room temperature and inhibited by iodoacetate (Coleman, 1969b). The glucose taken up by the passive process was probably not available for further metabolism and the rate of synthesis of protozoal polysaccharide was proportional to the rate of active uptake. Although glucose taken up by either process was retained in a place from which it could not be removed by washing, approximately 40% of the organism (probably the ectoplasm) was freely permeable to glucose. Active uptake is believed to be into the endoplasm.

Although there was a starch phosphorylase present in *Ent. caudatum*, the major route of starch utilization was by hydrolysis to maltose and then glucose followed by phosphorylation of the latter by adenosine triphosphate (ATP) to glucose-6-phosphate. This method of utilizing starch appears to be wasteful as from 10% (with growing protozoa) to 55% (with resting protozoa) of the starch-glucose was liberated into the medium as maltose and glucose (Coleman, 1969b). There is little evidence about the pathway of

glucose-6-phosphate utilization but it is presumed to be by glycolysis. Aldolase was present (C. Simpson and G. Coleman, unpublished work) but the only direct evidence is the observation that on incubation of *Ent. caudatum* with $^{14}CO_2$ the glucose of the protozoal polysaccharide was labelled in the 3 and 4 positions. This would be expected if glycolysis were reversed.

It is noteworthy that the protozoal reserve polysaccharide is an amylopectin with a branch length of 19 units rather than a glycogen as might be expected in an animal (Eadie *et al.*, 1963).

Ent. caudatum contained no other carbohydrase or pectinase (Abou Akkada and Howard, 1960, 1961) apart from those necessary to utilize starch, although other *Entodinium* spp. contained a wide range of these enzymes (Bailey and Clarke, 1963b) and it has been suggested that *Ent. caudatum* is an atypical member of the genus. Bonhomme (1968) and Bonhomme-Florentin (1975a) showed that *Entodinium* spp. contained some cellulase activity although substrates containing cellulose would not support the life of *Ent. caudatum* (Coleman, 1960). The activity of mixed *Entodinium* spp. (mostly *Ent. simplex*) against cellulose regenerated by phosphoric acid was only 0·5% of that of *Eremoplastron bovis*, a recognized cellulolytic protozoon (G. Coleman, unpublished work). The cellulolytic activity of *Entodinium* spp. is therefore probably unimportant compared with that of other protozoa.

2. *Metabolism of bacteria*

The most important source of nitrogenous compounds for the synthesis of protozoal protein and nucleic acid was bacteria (Coleman, 1964b, 1967a; Onodera and Kandatsu, 1969) which were engulfed and then killed and digested in vesicles in the cytoplasm (Coleman and Hall, 1969, 1972). *Entodinium caudatum* grown *in vitro* engulfed any particle small enough to pass down the oesophagus (Coleman and Hall, 1969) and took up a mixture of bacteria from the medium in the proportion in which they were present showing no preference or aversion for any bacterium. With *Escherichia coli* as test bacterium, the maximum rate of uptake was 200 bacteria per protozoon per minute (Coleman, 1964b). However, the rate at which different bacteria were killed and digested was very variable, the half-life of a bacterium after engulfment ranging from a few minutes to several hours (Coleman, 1964b, 1967b). *Esch. coli* was killed rapidly and in resting protozoa at least half of the bacterial carbon appeared as low-molecular-weight compounds in the medium, the first products appearing after 1 h.

Studies in the electron microscope showed that all the *Esch. coli* cell was digested with the steady loss of cell contents until only the two lipopolysaccharide membranes of the cell envelope remained (Coleman and Hall, 1972). Other Gram-negative bacteria were digested in a similar way. Among the Gram-positive bacteria the cell walls of *Bacillus megaterium* were highly sensitive to attack and disappeared rapidly, whereas those of *Streptococcus faecalis* and *Staphylococcus aureus* were much more resistant and persisted

after the cell contents had disappeared. The undigested remnants of the bacteria were extruded through the anus.

More recent work with *Ent. caudatum* and mixed *Entodinium* spp. grown *in vivo* (Coleman and Sandford, 1979a) suggested that the high rate of uptake of *Esch. coli* by cultured *Ent. caudatum* is not typical and that values of 10–40 bacteria per protozoon per hour for the engulfment of rumen bacteria are more realistic. Rumen-grown protozoa also showed a preference for bacteria of rumen origin and digested some rapidly. As two of these were cellulolytic bacteria (*Butyrivibrio fibrisolvens* and *Ruminococcus flavefaciens*) it is possible that *Entodinium* spp. may tend to remove these bacteria selectively from the rumen.

Although *Ent. caudatum* has the characteristic caudal spine when found in a normal sheep or cow, all metabolic studies have been made on the spineless form because it has been grown *in vitro* or *in vivo* in the absence of *Ent. bursa*. It has recently proved possible to grow the spined form *in vivo* in the presence of *Ent. bursa* and to prepare pure suspensions. The spined form (omitting the spine) was 1·8 times larger than the spineless form and took up individual bacterial species 1·9–2·4 times as rapidly, showing that the two forms were equally active per unit volume. However, the spined form digested bacteria more rapidly. The amount of starch inside the protozoa influenced the rate of uptake of bacteria, which could be depressed by over 60% in the presence of an excess of starch grains. It is interesting that the uptake of starch grains was very rapid and was complete within a few seconds, whereas the uptake of bacteria was progressive for several hours. Starvation of the protozoa initially increased the rate of bacterial uptake but this was depressed again if starch was withheld too long. Starvation also tended to increase the amount of bacterial carbon released into the medium (Coleman, 1975a; Coleman and Sandford, 1979a).

Washed suspensions of *Ent. caudatum* grown *in vitro* (Coleman and Laurie, 1974b) or *in vivo* (Abou Akkada and Howard, 1962; Onodera and Kandatsu, 1970b) released proteinaceous material, amino acids, ammonia and pipecolic (pipecolinic) acid (Onodera and Kandatsu, 1969) into the medium during incubation *in vitro* in the absence of added substrates. Possibly this in part represented the digestion of bacteria engulfed before the protozoa were harvested.

3. *Metabolism of protein and amino acids*

The amino acids of bacterial protein were incorporated efficiently (25%) into protozoal protein even by resting protozoa (Coleman, 1967b). There was little catabolism or interconversion of most of the amino acids but some were liberated into the medium free or as their *N*-acetyl or *N*-formyl derivatives (Coleman, 1967b). There was limited oxidative deamination of leucine, isoleucine and valine with the formation of CO_2 and isovaleric, α-methylbutyric or isobutyric acids respectively (Coleman, 1967b; Wakita and Hoshino, 1975). The rate of uptake of bacteria was such that the supply of bacterial amino acids would allow for division every 6–9 h compared with

minimum generation times of 5½ h *in vivo* (Warner, 1962) or 23 h *in vitro* (Coleman, 1960). *Entodinium caudatum* also engulfed particles of protein (Coleman, 1964b) but utilized soluble protein much less efficiently (Onodera and Kandatsu, 1970a).

Free ^{14}C-labelled amino acids were taken up by *Ent. caudatum* actively and passively. They were incorporated into protozoal protein without conversion into other amino acids or catabolism except to the extent mentioned above (Coleman, 1967a), although Onodera *et al.* (1977) found that δ-amino valeric acid was formed from proline by mixed rumen ciliates and extracts of mixed ciliates dethiomethylated methionine (Merricks and Salisbury, 1974). However, the rate of uptake of amino acids was low and could allow division of the protozoa only every 140 h if they were the only source of amino acids. Onodera and Kandatsu (1968) found that after allowing for the release of amino acids by protozoa there was a net uptake of only glutamic acid, tryptophane, lysine and histidine by mixed rumen ciliates.

Although there is little evidence for the synthesis of protein from carbohydrate (Coleman, 1969b), Harmeyer and Hekimoglu (1968) and Harmeyer (1967) found that the second carbon atom of acetate and CO_2-carbon were incorporated into protozoal amino acids. Of greater importance is the finding that free and mucopeptide-bound diaminopimelic acid could be decarboxylated to give lysine (Onodera and Kandatsu, 1974; Onodera *et al.*, 1974).

Protein synthesis in cell-free preparations of rumen protozoa has been reported by Chesters (1968) and Hoshino and Sugiyama (1974). Bonhomme-Florentin (1975b) showed that mixed rumen ciliates hydrolyse urea.

4. *Nucleic acid metabolism*

Free adenine, guanine and uracil are taken up by *Entodinium caudatum* and incorporated into nucleic acid. There is limited interconversion of adenine and guanine but up to 30% of the uracil is converted into cytosine. No evidence exists for either the incorporation of free [^{14}C]ribose into nucleic acid, although ^{14}C appears in protozoal polysaccharide, or the synthesis of ribose from glucose or starch. However, [^{32}P]phosphate was incorporated into protozoal nucleic acid (Coleman, 1968).

The most important source of nucleic acid constituents for protozoal nucleic acid synthesis was bacteria, although with resting suspensions the incorporation of the bases was less efficient than that of the amino acids, more being lost into the medium. Experiments using bacterial nucleic acid labelled in different constituents showed that nucleotides were transferred intact from bacterial to protozoal nucleic acid. The ribose for protozoal nucleic acid was therefore derived from bacterial nucleic acid. Nucleotides were also taken up more rapidly than free bases or nucleosides from the medium (Coleman, 1968; G. Coleman, unpublished work).

Adenine and guanine either added free to the medium or liberated from bacterial nucleic acid were converted into hypoxanthine and xanthine respectively and then the latter was oxidized to the former. Free uracil and

thymine were reduced to their dihydro derivatives (Coleman, 1968). This last reaction cannot be quantitatively important in the rumen as McAllen and Smith (1973) found that uracil tended to accumulate as an end-product of nucleic acid metabolism.

5. *Choline and ethanolamine*

As shown above, choline was essential for the growth of *Entodinium caudatum* and was normally supplied in the commercial rice starch used for the maintenance of the cultures (Coleman, 1960), although it was also present in chloroplasts added in dried grass, which were engulfed by the protozoa (Hall *et al.*, 1974). The choline could not be replaced by ethanolamine or by its mono- or di-methyl derivatives (Broad and Dawson, 1976).

Free choline was taken up rapidly at a rate that appeared to be the maximum possible for soluble compounds by *Ent. caudatum* (Broad and Dawson, 1975, 1976) and was converted equally rapidly into phosphorylcholine by the action of choline kinase which was widely distributed in the cell. No free choline was found inside the cell even when uptake was measured at 10°C. The uptake mechanism was substrate-saturable and the apparent K_m for uptake was the same as that for the phosphorylation of choline by cell-free preparations. The uptake of choline was also inhibited by hemicholinium-3 and the degree of inhibition by a given concentration was similar to that of choline kinase by the same concentration of inhibitor (Bygrave and Dawson, 1976). It has therefore been suggested that even if choline kinase is not part of the uptake reaction, it does provide a very efficient trapping mechanism for the choline as the cell is not permeable to phosphorylcholine. Although choline was incorporated into phospholipid the rate of uptake was not increased by the concomitant uptake of particulate matter under which conditions membrane synthesis might be expected to be rapid (Broad and Dawson, 1975).

The phosphorylcholine was converted into cytidine diphosphorylcholine (CDP-choline) by the action of choline phosphate cytidylyl transferase in the soluble fraction of the cell. During the uptake of [^{14}C]choline, ^{14}C-labelled CDP-choline was not normally found as it was rapidly converted into phosphatidylcholine by CDP-choline,1,2diacylglycerolcholinephosphotransferase, found principally in membrane fractions. Choline phosphate cytidylyl transferase was probably the rate-limiting enzyme for the conversion of choline into phosphatidylcholine (Bygrave and Dawson, 1976).

Ethanolamine was taken up much more slowly than choline but was also converted into CDP-ethanolamine and phosphatidylethanolamine. No evidence has been obtained for the methylation of ethanolamine to form choline or of base exchange (Bygrave and Dawson, 1976). Phosphatidylethanolamine was used intact for the synthesis of ceramide phosphorylethanolamine (Broad and Dawson, 1973, 1975).

Inorganic phosphate was also taken up from the medium and incorporated into phosphatidylcholine and phosphatidylethanolamine. However, the highest rate of incorporation was into phosphatidylinositol which turned

over more rapidly than choline or ethanolamine phospholipids (Broad and Dawson, 1975).

6. *Aminoethanephosphonic acid*

This compound ($NH_2 . CH_2 . CH_2 . P(OH)_2$) is characterized by the presence of a carbon–phosphorus bond and occurs as plasmalogen and diglyceride derivatives; it was first isolated by Horiguchi and Kandatsu (1959). It accounts for 20% of the phospholipid phosphorus in *Entodinium caudatum* (Broad and Dawson, 1975) and has proved to be a valuable marker as it occurs in the rumen only in the protozoa and can be used to estimate the amount of protozoal nitrogen present (Ibrahim and Ingalls, 1972). Little is known about the biosynthesis of this compound, except that it was derived from a three-carbon glycolytic intermediate but not from phosphatidylethanolamine (Broad and Dawson, 1975; Coleman *et al.*, 1971). There was more aminoethylphosphonate in *Isotricha* spp. than in *Entodinium* spp. (Abou Akkada *et al.*, 1968).

7. N(*2-Hydroxyethyl*)*alanine*

This compound ($HO . CH_2 . CH_2 . NH . CH(CH_3) . COOH$) was first isolated bound to phosphatidic acid in the phospholipid of *Entodinium caudatum* by Kemp and Dawson (1969a, b). This phosphoglyceride occurred only in anaerobic protozoa where it appeared to replace phosphatidylserine. Phosphatidylhydroxyethyl alanine was synthesized directly from phosphatidylethanolamine by adding to the amino group a carboxyethyl group derived from a three-carbon glycolytic intermediate (Coleman *et al.*, 1971; Broad and Dawson, 1975).

8. *Lipid*

Entodinium caudatum engulfed any particles, including olive oil droplets, that were small enough to pass down the oesophagus (Coleman and Hall, 1969; Hall *et al.*, 1974). *In vivo*, almost all of the dietary lipid is presented to the protozoa in particulate form. In animals on pasture this will be as chloroplasts which contain most of the linolenic acid in grass. Unfortunately, very few studies on lipid metabolism have been made with pure suspensions of protozoa and all the results quoted below have been obtained with mixed preparations and must be interpreted in this light.

(a) *Hydrolysis.* Olive oil droplets engulfed by *Ent. caudatum* can allegedly be digested (Warner, cited by Prins, 1977), although there is no evidence to support this claim. Clarke and Hawke (1970) found that the organisms responsible for the hydrolysis of triglyceride in the rumen were associated with the particulate matter and that homogenization released the activity. This activity could be associated with the protozoa as these would sediment with the particles and be broken by homogenization. Latham *et al.* (1972) also found lipolytic activity in the protozoal fraction of bovine rumen contents; in animals on high and low roughage rations 29% and 35% respectively

of the added triglyceride was hydrolysed in 8 h, whereas the bacterial fraction hydrolysed 88% and 6% respectively. This apparently means that on the low roughage (high starch) ration a much larger proportion of lipolysis was carried out by the protozoa but the protozoal fraction used in the second case might contain starch grains that could be contaminated with possibly lipolytic bacteria. Although the author believes that it is highly likely that intact *Entodinium* spp. can hydrolyse lipids, the evidence is poor and there is no information on the possible role of intracellular bacteria.

(b) *Hydrogenation.* Shorland *et al.* (1957) first showed that unsaturated fatty acids were hydrogenated on incubation with rumen contents and Garton *et al.* (1961) and Hawke and Silcock (1969) found that hydrolysis of the lipid had to occur before hydrogenation took place. Evidence on the role of protozoa is not satisfactory. Experiments on faunated and defaunated animals showed that the former contained more oleic acid and less linoleic and linolenic acids in their plasma lipids than the latter, suggesting that protozoa were responsible in part for the hydrogenation of fatty acids (Klopfenstein *et al.*, 1966; Lough, 1968; Abaza *et al.*, 1975). However, no evidence was available about the species of protozoa involved. Abaza *et al.* (1975), using change in iodine number as criterion, found that entodiniomorphid protozoa (but not holotrich protozoa) were able to hydrogenate fatty acids either added free or as various oils to washed suspensions of the protozoa. However, protozoa could desaturate their own fatty acids. Williams and Dinusson (1973) examined the rumen digesta of faunated and protozoa-free calves and found a higher proportion of saturated fatty acids in faunated animals, although extensive biohydrogenation of the ration fatty acids had occurred in both groups of animals. The entodiniomorphid protozoa also contained a higher proportion of saturated acids than the holotrich protozoa or bacteria. Chalupa and Kutches (1968) examined the hydrogenation of [^{14}C]linoleic acid by a washed preparation of entodiniomorphid protozoa in the presence and absence of penicillin and streptomycin and showed that the protozoa hydrogenated the substrate to stearic and octadecenoic acids. The antibiotics decreased the bacterial population in the preparation by over 90% and increased the amount of substrate metabolized. They also increased the proportion of *trans* isomer in the [^{14}C]octadecenoic acid formed. It was therefore considered unlikely that intracellular bacteria were involved in the hydrogenation process. In these experiments up to 90% of the ^{14}C could be associated with protozoa, suggesting that the protozoa took up the fatty acid before reducing it and releasing it into the medium, although possibly the fatty acid was bound to the outside of the organism.

In contrast, Harfoot *et al.* (1973), who incubated linoleic acid with whole rumen contents and then determined the distribution of reduction products between various fractions, found stearic acid associated with plant material rather than the protozoal fraction. These authors believed hydrogenation to be an extracellular process and that the protozoa were relatively unimportant. Similarly, Dawson and Kemp (1969) showed that the rate of hydrogenation of oleic and linoleic acids by whole rumen contents was the same before and

after defaunation and suggested that protozoa were comparatively unimportant in the process. However, the bacterial population density increases on defaunation (Kurihara *et al.*, 1968) and it is possible that the number of hydrogenating bacteria may increase in response to the increase in the levels of unsaturated fatty acids following defaunation.

(c) *Synthesis of fatty acids.* Fatty acids are synthesized from propionate, butyrate and isoleucine by mixed rumen protozoa. They produce monoene by direct desaturation of saturated acids but cannot produce polyunsaturated fatty acids (Emmanuel, 1974). Although protozoa can metabolize fatty acids, high concentrations in the ration are toxic and the numbers of protozoa in the rumen decline (Czerkawski, 1973; Henderson *et al.*, 1977).

9. *Sterols*

Sterols are essential for the growth of *Entodinium* spp. Broad and Dawson (1976) showed that rumen fluid and dried grass in the culture medium for *Ent. caudatum* (Coleman, 1960) could be replaced by β-sitosterol, whereas Hino *et al.* (1973b) found that this sterol was ineffective for mixed *Entodinium* spp. unless campesterol was also added. The major sterols in a rumen protozoal preparation containing 80% *Entodinium* spp. were stigmastanol (60–66%), campestanol (13–15%) and cholestanol (11–17%) (Hino and Kametaka, 1975), which may have been derived from sterols such as β-sitosterol. During incubation of protozoa in sterol-free medium, the total amount of protozoal sterol decreased while the amount of cholestanol increased: the reason is unknown (Hino and Kametaka, 1975).

10. *Survival of intracellular bacteria*

When harvested from the growth medium, *Entodinium caudatum* contained variable numbers of two principal bacteria, *Klebsiella aerogenes* and *Proteus mirabilis* in the proportion of 2 : 1 (White, 1969). The numbers present decreased with the time since the protozoa were last fed, but they never completely disappeared even on prolonged incubation of the protozoa in sterile medium in the absence of substrate. The two bacteria were present in the growth medium and had apparently colonized the protozoa. The cell envelope of *P. mirabilis* was always resistant to digestion (Coleman, 1967b), whereas that of *K. aerogenes* was sensitive to digestion unless covered by a capsule that the bacterium synthesized from glucose under anaerobic conditions (Coleman, 1975b, 1978c). It has been suggested that *K. aerogenes*, at least, synthesized capsule from the glucose and maltose liberated into the protozoal pool during digestion of starch by *Ent. caudatum* (Coleman, 1969b) and that the survival of the bacterium depended on whether or not it could synthesize capsule faster than the protozoal enzymes could digest it (Coleman, 1975b). This theory is supported by the observation that after incubation for 5 h with [^{3}H]glucose, dead labelled bacteria were present inside the protozoon (Coleman and Hall, 1974). This showed that bacteria alive at the beginning of the experiment had been killed before it finished.

11. *Protozoal compartments and rates of clearance of soluble and particulate materials*

The volume of *Entodinium caudatum* was determined from the number of protozoa in a packed pad as $4{\cdot}7 \times 10^4$ μm^3 (Coleman, 1967a). Of the volume of such a pad of well-fed protozoa, 86% was impermeable to *Escherichia coli* at 20°C, approximately 50% impermeable to low-molecular-weight compounds at 20°C and 25% impermeable to low-molecular-weight compounds in the presence of a detergent that could destroy permeability barriers (Coleman, 1967a, 1968, 1969b). These results were interpreted as showing that $100-86=14\%$ of the pad was interstitial fluid (i.e. the volume that a bacterium can penetrate in presence of inactive protozoa), 25% was solid material in the protozoa, $100-(25+14)=61\%$ was liquid space in the protozoa and $100-(50+14)=36\%$ was space that could be penetrated by low-molecular-weight compounds in normal inactive protozoa. There is evidence (Coleman, 1969b) that this last was the ectoplasm. In well-fed protozoa 50–80% of the solid material was starch; as this could decrease to nothing in starved protozoa, the amount of solid matter could be as low as 5% and the total amount of liquid in the protozoa correspondly larger. Another compartment in the cell was that in which external medium is trapped in the presence of iodoacetate (see above, Section V.A.1). It occupied 11% of the volume of the actual protozoon (9·5% of the packed pad) and was presumed to be metabolically part of the endoplasm because, as mentioned above, the ectoplasm was considered to be freely permeable. The volume of fluid in the endoplasm was therefore 100 − (interstitial fluid = 14) − (solid matter = 5 to 25) − (volume of ectoplasm = 36) − (volume in which medium was trapped in presence of iodoacetate = 9·5) = 15·5 to 35·5% of the packed pad.

The soluble compounds metabolized most rapidly by *Ent. caudatum* were glycine (converted into *N*-acetyl glycine: Coleman, 1964a), thymine (converted into dihydrothymine: Coleman, 1968) and choline (taken up: Broad and Dawson, 1975), all the substrate in $8{\cdot}7 \times 10^4$ μm^3, 11×10^4 μm^3 and $8{\cdot}6 \times 10^4$ μm^3 respectively being attacked by one protozoon each hour. If it is assumed that all the substrate in a volume of medium is metabolized in one "passage" through the protozoon, then these very similar volumes (approximately 10×10^4 μm^3) must represent the rate at which medium passed in and out of the protozoon. If this is a maximum value, it is therefore not unexpected that the rate is not increased by, for example, increased use of choline for membrane synthesis. It is not known where these reactions take place or even if the ectoplasm is as freely permeable in active protozoa at 39°C as it is in inactive protozoa at 20°C. However, if it assumed that this medium must penetrate into the endoplasm then $10 \times 10^4 \div$ (maximum volume of endoplasm $= 0{\cdot}355 \times 4{\cdot}7 \times 10^4) = 6{\cdot}0$ endoplasm volumes of medium are metabolized each hour; thus all the substrate in the medium would be metabolized in 10 minutes within the endoplasm. These rates of metabolism are slow compared with those at which bacteria were cleared from the medium, $2{\cdot}9 \times 10^6$ μm^3 and $1{\cdot}8 \times 10^6$ μm^3 of medium being cleared hourly of *Esch. coli* from an infinitely dilute suspension and a population of 1×10^9

bacteria ml^{-1} respectively. As bacteria are removed by passage of medium in and out of the oesophagus, $2{\cdot}9 \times 10^6 \div (4{\cdot}7 \times 0{\cdot}86 \times 10^4) \div 60 = 1{\cdot}2$ body volumes (0·74 at 1×10^9 bacteria ml^{-1}) of medium can be cleared of bacteria each minute.

B. *ENTODINIUM SIMPLEX*

1. *Growth* in vitro

Entodinium simplex differs from *Ent. caudatum* as its preferred source of starch was ground wheat and it used rice starch only in the presence of bran (Coleman, 1969a). In the author's experience the addition of rumen fluid was essential for growth *in vitro*, although Jarvis and Hungate (1968) found it inhibited their established cultures. They found an extract of rumen protozoa to be stimulatory but agreed with the present author that autoclaving substrates before addition to the growth medium was detrimental to the protozoa.

Ent. simplex, the smallest rumen ciliate (40×25 μm) that has been studied in detail, is also the most numerous, accounting for 70–80% of the numbers of protozoa in a sheep's rumen (Coleman, 1972).

2. *Uptake and digestion of bacteria*

Three items have been measured to compare the rate of uptake of different bacteria by this and other rumen ciliates. Firstly, the maximum number of bacteria engulfed per hour from an infinitely concentrated bacterial suspension, secondly the maximum volume of bacteria engulfed under the same conditions (as it is amount of space inside the protozoa which is limiting) and lastly, the maximum volume of medium cleared of bacteria from an infinitely dilute suspension where the protozoa have to find and catch their prey from a large volume of medium.

With *Entodinium simplex* grown *in vitro* there was a forty-fold variation between bacterial species in the maximum number of bacteria engulfed, a six-fold variation in the maximum volume of bacteria engulfed and a 3·3-fold variation in the maximum volume of medium cleared of bacteria (Coleman, 1972). As the least variation was in the maximum volume of medium cleared, it was suggested that the protozoa may always pass the same volume of medium in and out of the oesophagus and that differences in the rate of uptake of bacteria may depend on the rate at which bacteria can be removed from this stream of medium. It is interesting that the efficiency of removal of *Proteus mirabilis* was almost independent of the number present, whereas that of *Butyrivibrio fibrisolvens* decreased steadily with increasing population density. Comparison of the maximum number and volume of bacteria engulfed showed that *P. mirabilis* and *Klebsiella aerogenes*, present in the growth medium, were engulfed more rapidly than the other bacteria including those isolated from the rumen. The only bacterium taken up poorly was *Bacillus megaterium*, which may have been too large to be readily engulfed by a smail protozoon (Coleman, 1972).

As with *Ent. caudatum*, there was considerable variation in the rate of killing and digestion of engulfed bacteria, *Escherichia coli* and *K. aerogenes* being killed rapidly whereas *P. mirabilis* and *Serratia marcescens* were resistant to killing and digestion. The rate of killing was not related to Gram reaction or cell shape and there was wide variation even among the Enterobacteriaceae. During the digestion of *Esch. coli*, *Ent. simplex* released soluble products into the medium more rapidly than *Ent. caudatum*, over 40% of the engulfed bacteria being digested and the products released into the medium in 100 minutes (Coleman, 1972).

3. *Amino acid and protein metabolism*

Entodinium simplex did not extensively catabolize either free or bacterial amino acids, less being broken down than was incorporated by the protozoa. Within this limited breakdown glutamic acid, leucine, isoleucine and valine were degraded most rapidly with preferential loss of carbon atom 1 (Coleman, 1972).

4. *Carbohydrate metabolism*

No evidence has been obtained for the synthesis of amino acids from starch grains or glucose in protozoa incubated in the presence of antibiotics, although some carbon from carbohydrate was incorporated into soluble protein in their absence. As *Klebsiella aerogenes*, one of the intracellular bacteria, can synthesize all its amino acids from glucose, it is possible for starch to be hydrolysed to glucose by the protozoa, for the glucose to be used to synthesize bacterial protein and then for the bacteria to be killed and digested with the utilization of bacterial amino acids for protozoal protein synthesis. However, the importance of this process is not known (Coleman, 1972).

5. *Lipid metabolism*

Entodinium simplex concentrated fatty acids from the medium (Gutierrez *et al.*, 1962) and the endogenous rate of gas production by the protozoa was stimulated by tributyrin, myristate, linoleate, oleate and other fatty acids but not by glycerol (Williams *et al.*, 1963).

C. *ENTODINIUM LONGINUCLEATUM*

1. *Growth* in vitro

Entodinium longinucleatum grew on starch grains and ground dried grass in medium containing 10% prepared fresh rumen fluid. It was difficult to maintain at high population densities and has been cultivated *in vitro* for only 15 months although it could grow well with a mean generation time of 1·8 days (Owen and Coleman, 1976; Coleman, 1978a).

2. *Metabolism*

Entodinium longinucleatum engulfed all the bacteria tested, except for *Bacteroides ruminicola* and a *Pseudomonas* sp., but showed a preference for *Klebsiella aerogenes* and *Proteus mirabilis* present in the growth medium. Some bacteria were digested with release of soluble materials into the medium. There was no appreciable synthesis of protozoal protein from starch. Although free amino acids were taken up from the medium, bacterial protein was the principal source of amino acids for protozoal protein synthesis (Owen and Coleman, 1977).

D. *ENTODINIUM BURSA*

1. *Engulfment and digestion of* Entodinium caudatum

Entodinium bursa (syn. *Ent. vorax*) differs from the other *Entodinium* spp. discussed above as, both *in vivo* and *in vitro*, it specifically engulfed the spineless form of *Ent. caudatum* (Poljansky and Strelkow, 1938; Coleman *et al.*, 1977). *Ent. caudatum* will apparently grow indefinitely both *in vivo* and *in vitro* without forming characteristic caudal spines. However, on introducing *Ent. bursa* into a rumen containing only *Ent. caudatum*, small caudal spines appeared on the latter after 6 days and completely developed spines after 8–20 days (Poljansky and Strelkow, 1938).

On incubation of a washed suspension of the spineless form of *Ent. caudatum* with a suspension of *Ent. bursa*, the latter engulfed the former at a rate of 1·5–2·2 *Ent. caudatum* per *Ent. bursa* per hour. *Ent. caudatum* fixed with glutaraldehyde were engulfed but not digested, whereas spined *Ent. caudatum* were engulfed much more slowly suggesting that the caudal spine is a good defence mechanism especially as the *Ent. caudatum* were engulfed posterior end first. *Ent. caudatum* also possessed a second defence mechanism in that it increased in size in the presence of *Ent. bursa* until it was too large to be engulfed (Coleman *et al.*, 1977).

The author has also grown *Ent. caudatum* and *Ent. bursa* together in the rumen of a sheep; there tended to be a cyclic rise and fall in the population density of the two protozoa. The maximum number of one coincided with the minimum number of the other, suggesting that the *Ent. caudatum*, which all had spines of various sizes, became progressively more and then less attractive prey and that the numbers of *Ent. bursa* rose when there were large numbers of attractive prey which they consumed and fell when there were few such prey.

After engulfment into a vesicle *Ent. caudatum* became rounded and its pellicle was slowly digested. The surface membrane disappeared first followed by the epiplasm which was broken down to comma-shaped pieces. The internal structure of the *Ent. caudatum* then broke down and particles such as bacteria and starch grains were taken up into the *Ent. bursa* cytoplasm. Finally the vesicles disappeared (Coleman and Hall, 1979). Experiments on the fate of starch and protein in *Ent. caudatum* showed that approximately equal amounts of these components were retained by *Ent. bursa* and released into the medium (G. Coleman, unpublished work).

2. *Growth* in vitro

Entodinium bursa grew *in vitro* only in the presence of *Ent. caudatum* and was normally cultured by the daily addition of starch grains, dried grass and some *Ent. caudatum* culture. The amount of the culture must be carefully controlled to prevent the formation of spines on the *Ent. caudatum* that are not engulfed. *Ent. bursa* could also be grown by addition of small inocula to a culture of *Ent. caudatum* under which conditions the lowest mean generation time was 6 h and 2·2 *Ent. caudatum* were engulfed by each *Ent. bursa* per hour (Coleman, 1978a; Coleman *et al.*, 1977). Why *Ent. caudatum* were essential for the growth of *Ent. bursa* is not known; they could not be replaced by *Ent. simplex* or *Ent. longinucleatum.*

3. *Metabolism of bacteria*

All bacteria that have been tested, including mixed rumen bacteria, were engulfed by *Entodinium bursa* (Table 3); however, it is not known if bacterial amino acids were utilized.

E. *EPIDINIUM ECAUDATUM CAUDATUM*

1. *Nomenclature*

There is unfortunately confusion in the earlier literature in the naming of both the genus and the various species of what are now known as *Epidinium* spp. Kofoid and MacLennan (1933) list up to eight names by which each species has been known. The author has followed the classification of Dogiel (1927) but even here there is difficulty because two forms of *Epidinium ecaudatum caudatum* occur respectively in sheep and cows (Coleman *et al.*, 1972).

2. *Surface bacteria*

Epidinium spp. grown *in vitro* or *in vivo* were characterized by the presence of at least two types of bacteria attached to the outside of the pellicle (Coleman and Hall, 1974; Imai and Ogimoto, 1978). On *Ep. ecaudatum caudatum* (ovine) grown *in vitro* these have been designated A, a small probably Gram-positive coccus with fimbriae and uniformly osmiophilic contents and B, a larger probably Gram-negative coccus which does not stain uniformly (Coleman and Hall, 1974). Type B was also found in the cytoplasm. In experiments on the incorporation of ^{3}H-labelled soluble compounds, type B bacteria attached to the pellicle were always more heavily labelled than the same bacteria in the cytoplasm or type A bacteria (Coleman and Hall, 1974). Imai and Ogimoto (1978), using fluorescein isothiocyanate-labelled antisera, demonstrated the presence of *Streptococcus bovis* and *Ruminococcus albus* on *Epidinium* spp. isolated from the rumen.

3. *Growth* in vitro

Epidinium ecaudatum caudatum (ovine and bovine) and *Ep. ecaudatum tricaudatum* grew best when fed daily with ground wheat and dried grass (Coleman *et al.*, 1972) but *Ep. ecaudatum caudatum* (ovine) would also grow at a low population density on powdered dried grass alone, suggesting that it may be cellulolytic (Coleman *et al.*, 1976). The characteristic caudal spine(s) was (were) lost after some months in culture (Clarke, 1963; Coleman, 1978a). Like other rumen ciliates, *Epidinium* spp. grew *in vitro* for only about 2 years; after about 1 year in culture they suffered "loss of vigour" and grew progressively more poorly until they died. This process could be reversed by inoculating the cultured protozoa into the defaunated rumen of a sheep and reisolating the protozoa as soon as sufficient were available. The reisolated protozoa survived for a further 2 years.

4. *Engulfment of bacteria*

The behaviour of *Epidinium ecaudatum caudatum* (ovine) cultured *in vitro* was unusual compared with that of other rumen ciliates studied as there was considerable variation between species in the maximum number of bacteria engulfed (260–120 000 protozoon^{-1} h^{-1}) and the maximum volume of medium cleared of bacteria ($0{\cdot}1 \times 10^6$–16×10^6 μm^3 protozoon^{-1} h^{-1}); the rate of uptake of most bacteria was also much less per unit volume of protozoon (Coleman and Laurie, 1974a). With most single bacterial species the rate of uptake was similar to that with the much smaller *Entodinium caudatum*. There was no uniform pattern of preference by any of the three *Epidinium* spp. for bacteria isolated from any particular source although *Proteus mirabilis* was taken up most rapidly. The volume of medium cleared of bacteria by *Ep. ecaudatum caudatum* (ovine) was almost independent of the bacterial population density which meant that at infinitely high bacterial population density, the number engulfed was very high. In contrast, the number of yeast taken up from a suspension of *Saccharomyces fragilis* was almost independent of the yeast population density suggesting that the protozoa could remove only a certain number of yeasts per minute regardless of the number present. However, the behaviour with most prey organisms lay between these two extremes. *Ep. ecaudatum tricaudatum* engulfed bacteria more slowly than did the two forms of *Ep. ecaudatum caudatum* (Coleman and Laurie, 1974a). Mixed rumen bacteria (^{35}S-labelled) were taken up more rapidly than suspensions of pure cultures of bacteria (Table 3). Although there was doubt as to the significance of uptake rates obtained with mixed rumen bacteria, recent results (Coleman and Sandford, 1979a) suggested that they did give a valid estimate of the number engulfed provided that $^{35}SO_4^{2-}$, [^{14}C]guanine or [^{14}C]isoleucine were used to label the bacteria.Washed suspensions of *Ep. ecaudatum caudatum* (ovine) grown *in vivo* differed from those cultured *in vitro* in that they took up most bacteria much more rapidly and at a rate that was comparable per unit volume with that obtained with other protozoa. However, they were more selective than those grown *in vitro*

and engulfed *Escherichia coli* and *Klebsiella aerogenes* only slowly (Coleman and Sandford, 1979a).

Unlike *Entodinium* spp., well-fed *Ep. ecaudatum caudatum* (ovine) grown *in vivo* or *in vitro* did not digest mixed rumen bacteria or many other bacteria with the release of soluble digestion products into the medium, although digestion products were present inside the cell. However, starved *Epidinium* spp. took up bacteria more slowly than well-fed protozoa and released soluble digestion products into the medium suggesting that, provided sufficient energy was available, *Epidinium* spp. were more economical with bacterial digestion products than were *Entodinium* spp. (Coleman and Sandford, 1979a).

The very variable rate of uptake between different bacterial species by *Ep. ecaudatum caudatum* (ovine) grown *in vitro* may be associated with the nature of the outer surface of the bacteria because on sonication of the various pure bacterial suspensions the differences in rates of uptake between species were greatly reduced (Coleman and Laurie, 1974a).

Ep. ecaudatum caudatum (ovine and bovine) and *Ep. ecaudatum tricaudatum*, like other rumen ciliates, slowly released whole cell contents, not apparently by massive cell lysis, into the medium, but differed from other ciliates in also releasing a lytic enzyme. This enzyme resembled lysozyme in the pattern of bacteria that it attacked and produced rapid lysis of, for example, *Bacillus megaterium*; 20–80 times as much soluble bacterial carbon was present in the medium as in the protozoon after incubation for 5–60 minutes. These bacterial digestion products were then taken up by the protozoon (Coleman and Laurie, 1974b; Coleman and Hall, 1974).

5. *Metabolism of protein and amino acids*

Free amino acids were taken up 3–25 times more rapidly by *Epidinium ecaudatum caudatum* (ovine) than by *Entodinium caudatum*. These amino acids were incorporated into protozoal protein and the bacteria (especially B) attached to the pellicle and present in the cytoplasm. It is likely that the medium did not have free access to the intracellular bacteria as incorporation of amino acids into the bacteria increased markedly on disruption of the protozoa. The amino acids were all catabolized to a limited extent with the production of volatile fatty acids and CO_2 which probably came from carbon atom 1. Inside the protozoa some amino acids were incorporated unchanged into protozoal protein, whereas others, e.g. aspartic acid and serine, were extensively converted into other amino acids (Coleman 1978c; Coleman and Laurie, 1974a).

6. *Metabolism of carbohydrates*

Epidinium ecaudatum caudatum could synthesize limited amounts of certain amino acids, e.g. alanine, aspartic acid, glutamic acid, lysine and serine, from starch grains but obtained less than 1% of its amino acid requirements from this source. As intracellular bacteria also synthesize amino acids from starch

these could be available to the protozoon after the death and digestion of the bacteria.

Ingestion of starch stimulated gas production by *Epidinium* spp. (Gutierrez and Davis, 1962). The properties of an amylase, a specific maltase and an isomaltase have been described by Bailey (1958) and Bailey and Howard (1963a, b). *Ep. ecaudatum caudatum* also contained an α-galactosidase that specifically hydrolysed galactosyl galactosyl glycerol and may be important in the hydrolysis of mono- and di-galactosyl diglycerides present in chloroplasts (Bailey and Howard, 1963a). No cellulase activity and only occasional cellobiase activity was described by Bailey (1958) and this agrees with the finding of Coleman *et al.* (1976) that this protozoon grew only poorly on dried grass alone. Starch was degraded initially to maltose which then underwent hydrolysis to glucose or phosphorolysis to glucose-1-phosphate which was converted into glucose-6-phosphate (Coleman and Laurie, 1976). Extracts of *Epidinium* spp. also hydrolysed a range of plant hemicelluloses (Bailey *et al.*, 1962; Bailey and Gaillard, 1965).

[^{14}C]Glucose was taken up by *Ep. ecaudatum caudatum* (ovine) and incorporated into protozoal and bacterial polysaccharide. ^{14}C was also incorporated into a limited number of amino acids. The uptake of glucose by this protozoon was unusual in that the amount taken up depended on the square of the glucose concentration. The reason for this is unknown, although it was incorporation into the protozoal polysaccharide rather than other cell fractions that showed this relationship (Coleman and Laurie, 1976). This has been confirmed by autoradiography (Coleman and Hall, 1974). Glucose was probably taken up by the protozoa as such into a pool and then rapidly converted in the presence of ATP into glucose-6-phosphate on passage into a second pool where it was dephosphorylated back to glucose. This second pool contained most of the free sugars and their derivatives that came from the degradation of starch. Glucose for glycolysis and the synthesis of protozoal polysaccharide was derived from this second pool (Coleman and Laurie, 1976).

7. *Degradation of plant tissues*

When plant material entered the rumen, *Epidinium* spp. became attached to the damaged regions (Bauchop and Clarke, 1976). In studies using leaf sections, Amos and Akin (1978) showed that the mesophyll and portions of the parenchyma bundle sheath of cool-season but not warm-season forage grasses were degraded by *Epidinium* spp. As there was a loss in dry weight even in the presence of streptomycin, it was suggested that the protozoa actively digested the plant material. This could be the result of either the action of enzymes known to be released by the protozoa, or the removal of tiny pieces of plant material by engulfment. The role of other protozoa in the degradation of plant tissues is not clear as both groups of authors used rumen contents rich in *Epidinium* spp. It is possible that one role of *Epidinium in vivo* is to break up plant tissue and make it more available to bacterial attack. Yoder *et al.* (1966), also using rumen contents containing *Epidinium*,

found a synergistic action of protozoa and bacteria in the digestion of cellulose.

8. *Sources of amino acids for protein synthesis*

To divide once every 24 h *Epidinium ecaudatum caudatum* (ovine) grown *in vitro* must synthesize 600 pg of protein h^{-1}. As rumen bacteria can supply only 37 to 150 pg, free amino acids 50 pg and synthesis from starch 1 pg, the remainder must come from another source. As this protozoon attacks plant material, plant protein may supply the shortfall (Coleman and Laurie, 1974a).

9. *Metabolism of purines and pyrimidines*

Adenine and guanine were converted initially into hypoxanthine and xanthine respectively. Hypoxanthine was then converted into xanthine which was metabolized, possibly via uric acid and allantoic acid, to CO_2. The purine and pyrimidine bases were also incorporated into protozoal nucleic acid with some interconversion of adenine and guanine and of uracil and cytosine.

10. *Metabolism of lipids*

Epidinium ecaudatum caudatum metabolized the lipid in engulfed chloroplasts by reducing triene to diene and monoene to stearic acid but hydrogenated diene to monoene only slowly (Wright, 1959). Wright (1961) also found that protozoal extracts hydrolysed tributyrin but not triolein or olive oil and produced evidence, on the basis of the action of penicillin in depressing lipolysis under these conditions, that the protozoa (principally *Epidinium* spp.) were responsible for 30–40% of the activity in the rumen.

F. *POLYPLASTRON MULTIVESICULATUM*

Polyplastron multivesiculatum occurs in three different sizes depending on the growth conditions. The largest organisms (205×123 μm) were found when the protozoon was growing in the presence of (and engulfing) *Epidinium*, *Diplodinium*, or other middle-sized protozoa either *in vivo* or *in vitro*. Protozoa in the middle size range (175×127 μm) occurred during growth in the rumen in the absence of *Epidinium*—i.e. in a rumen with a normal A-type protozoal population. The smallest *P. multivesiculatum* (123×92 μm) were found during growth *in vitro* in the absence of other protozoa.

1. *Relationship with other protozoa* in vivo

When small numbers of *Polyplastron multivesiculatum* were introduced into the rumen of a sheep containing a normal B-type population all these latter protozoa disappeared in 7–11 days, the *Epidinium* spp. going before *Eudiplodinium maggii* (Eadie, 1962b, 1967). However, in some calves the B-type

population persisted in spite of inoculations with *P. multivesiculatum* and in one animal *P. multivesiculatum* and *Eu. maggii* were found together for a time. In this animal *Eu. maggii* increased markedly in size, a response that has been observed in other potential prey protozoa in the presence of predators. The predation by *P. multivesiculatum* involved only the characteristic B-type protozoa (*Epidinium*, *Eudiplodinium*, *Eremoplastron* and *Ostracodinium* spp.), *Entodinium* and the holotrich protozoa not being involved in any population changeover. In contrast to *P. multivesiculatum* grown *in vitro*, *P. multivesiculatum* grown *in vivo* in the absence of *Epidinium* began to engulf these protozoa as soon as they were put together (Eadie, 1967). Starvation of *P. multivesiculatum* abolished its ability to engulf *Epidinium*, although at an earlier stage, when food was becoming less easy to find, cannibalism occurred (Eadie, 1967).

2. *Growth* in vitro *and engulfment of protozoa*

When grown in medium rich in potassium phosphate, *Polyplastron multivesiculatum* had an obligate requirement for certain other protozoa (Coleman *et al.*, 1972). These included *Eudiplodinium maggii*, *Diplodinium monacanthum* and *Epidinium* spp. of which *Ep. ecaudatum caudatum* gave the best growth and induced the best development of the large form of *P. multivesiculatum*. *Entodinium* spp., *Diploplastron affine* and *Ophryoscolex caudatus* did not support growth. It is not known why there is this specificity, although many of the prey protozoa are covered with bacteria whereas *Entodinium* spp. are not and it is possible that *P. multivesiculatum* "recognizes" prey protozoa as bacteria.

P. multivesiculatum (smallest form) grew poorly in the absence of other protozoa on media rich in sodium chloride (Coleman *et al.*, 1972). If these protozoa were then inoculated into a culture of *Ep. ecaudatum caudatum* (ovine) it took 3–4 days to "recognize" the prey but after this lag it grew with a mean generation time of 14 h and engulfed two *Epidinium* per day. The maximum rate of engulfment was 10 *Epidinium* per protozoon per day.

The *Epidinium* were engulfed posterior end first and were initially digested only slowly with the gradual formation of holes in the epiplasm. Thereafter they disintegrated rapidly until only a large almost empty vesicle containing fragments of membrane remained in the *P. multivesiculatum*. The cytoplasm of *P. multivesiculatum* grown under these conditions was unique among the rumen ciliates in containing irregularly shaped, heavily staining bodies which may have been pieces of cytoplasm from engulfed *Epidinium* (Coleman and Hall, 1978).

3. *Metabolism of bacteria*

Polyplastron multivesiculatum grown *in vitro* with or without *Epidinium* engulfed a wide range of bacteria, showing a preference for *Proteus mirabilis* which occurred in the growth medium rather than for bacteria that occurred in the rumen or mixed rumen bacteria (Table 3), which could supply only 25%

of the amino acid requirement for division once a day (Coleman and Laurie, 1977). As with other ciliates, the effect of change in bacterial population density on uptake varied from that with *P. mirabilis*, which was proportional to bacterial population density, to that with *Butyrivibrio fibrisolvens*, when uptake was almost independent of density. Protozoa grown on *Epidinium* engulfed more bacteria than those grown in their absence. Only *P. mirabilis* was digested with the release of low-molecular-weight compounds into the medium (Coleman and Laurie, 1977).

4. *Metabolism of amino acids*

Free amino acids were taken up rapidly by *Polyplastron multivesiculatum* and could supply all the amino acids required for division once a day. The protozoa have limited ability to interconvert amino acids (Coleman and Laurie, 1977).

5. *Metabolism of carbohydrates*

Polyplastron multivesiculatum contained a very active amylase and a maltase. It could also hydrolyse cellulose, cellobiose, polygalacturonic acid and pentosan (Abou Akkada *et al.*, 1963). However, Coleman and Laurie (1977) found that starch probably underwent phosphorylysis to glucose-1-phosphate which was isomerized to glucose-6-phosphate. This protozoon could also synthesize certain amino acids (alanine, aspartic acid, glutamic acid and serine) from starch, but this is unlikely to be an important source of amino acids for growth.

Free glucose was also taken up by *P. multivesiculatum* initially as glucose-6-phosphate and then incorporated into the polysaccharide of the protozoon and its intracellular bacteria. All protein amino acids contain glucose carbon but the rate of synthesis from 0·1 mM glucose would supply only 10% of the requirements for division once a day (Coleman and Laurie, 1977).

6. *Metabolism of purines and pyrimidines*

Adenine and guanine were taken up into protozoal nucleic acid as such and were also interconverted. In the medium, both gave rise to hypoxanthine and xanthine which was subsequently metabolized in such a way that carbon atom 8 gave CO_2. Uracil was partially converted into cytosine by the protozoa and was metabolized so that carbon atom 2 gave rise to CO_2 (Coleman and Laurie, 1977).

G. *EUDIPLODINIUM MAGGII*

1. *Growth* in vitro

Eudiplodinium maggii is a cellulolytic protozoon that was grown in the presence of bacteria on powdered dried grass as sole source of organic

material (Hungate, 1943; Coleman *et al.*, 1976). The size of this ciliate varies considerably and the author has grown isolates ranging from 88×46 μm to 156×84 μm.

2. *Digestion of cellulose*

Hungate (1943) first showed that extracts of *Eudiplodinium maggii* produced reducing sugars from cellulose and that intact starved protozoa synthesized reserve polysaccharide from cellulose. There was, however, some doubt as to whether intracellular bacteria were responsible for the formation of the cellulase. Recently Coleman (1978b) has shown by incubation with antibiotics that 70% of the cellulase inside the protozoa was soluble and not of bacterial origin, although a bacterial enzyme was probably present in washed protozoal suspensions.

[^{14}C]Cellulose was digested to [^{14}C]cellobiose and [^{14}C]glucose, neither of which was released into the medium; both were utilized for the synthesis of protozoal polysaccharide, fermentation to principally acetic and butyric acids and, to a very limited extent, protein synthesis. If this were the sole source of amino acids, division would occur only every 346 days (Coleman, 1978b). The optimum pH of the cellulase was 6·0 and, as Weineck (1934) showed that the endoplasm of the protozoa was acid, the enzyme would be active *in vivo*.

3. *Metabolism of starch and glucose*

As in other rumen ciliates, starch was digested readily to maltose and glucose which were released into the medium. Starch was also fermented to volatile fatty acids and used like cellulose to a very limited extent for the synthesis of protein.

Glucose was taken up by *Eudiplodinium maggii* principally into protozoal and bacterial polysaccharides, but was also used for protein synthesis at a rate that would allow division every 1200 h. Disruption of the protozoa markedly increased the incorporation of glucose into the bacterial fraction, suggesting that with intact protozoa many of the bacteria were not freely in contact with the external medium and were presumably inside the protozoon (Coleman, 1978b).

4. *Metabolism of bacteria and amino acids*

There was considerable variation in the rate at which different bacterial species were engulfed by *Eudiplodinium maggii*; *Bacteriodes ruminicola* and *Klebsiella aerogenes* were not engulfed and *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens* and *Streptococcus bovis* were taken up rapidly—although only the first two of these were digested with the release of soluble materials into the medium (Coleman and Sandford, 1979b). Half of the amino acids required for growth could be derived from the uptake of mixed rumen bacteria. Free amino acids were also taken up by *E. maggii* and could supply the other half of the amino acids required for growth.

5. *Metabolism of nucleic acid components*

Adenine and guanine were taken up as such into protozoal nucleic acid and were interconverted to a limited extent. They were degraded in the medium to hypoxanthine and xanthine respectively which were then further degraded in such a way that carbon atom 8 was converted into a volatile compound. Experiments to compare the uptake of free bases, nucleosides and nucleotides showed that uridine 5′-monophosphate was taken up more rapidly and utilized much more efficiently than uridine or uracil. The uracil portion of these molecules was taken up as such into nucleic acid and also partially converted into cytosine. In the medium uridine 5′-monophosphate was degraded initially to uridine and then to uracil which was partially reduced to dihydrouracil. Ribose was also taken up by the protozoa and converted into glucose; none was incorporated into nucleic acid as such.

It is interesting that, whereas *Eudiplodinium maggii* cleared bacteria and uridine 5′-monophosphate from the medium at comparable rates, *Entodinium caudatum* removed bacteria ten times more efficiently than any soluble compound so far tested.

H. *EREMOPLASTRON BOVIS*

Eremoplastron bovis (also known as *Eudiplodinium neglectum*) was first shown by Hungate (1942) to contain a cellulase and a cellobiase. This was confirmed by Bailey and Clarke (1963a) who also showed the presence of α-amylase and xylanase, and that cellodextrins were hydrolysed by stepwise removal of cellobiose. *Er. bovis* has also been cultured by Hungate (1943) and by Coleman (1978a).

There is little evidence about the engulfment of individual bacterial species by cultured *Er. bovis* although mixed rumen bacteria were taken up (Table 3). Washed suspensions of *Er. bovis* grown as a single species in the rumen did not engulf *Escherichia coli* or *Klebsiella aerogenes* and engulfed cellulolytic bacteria only slowly. However, certain other bacteria were taken up rapidly and possibly *Er. bovis* tended selectively to remove non-cellulolytic bacteria (Coleman and Sandford, 1979a).

I. *METADINIUM MEDIUM*

Metadinium medium, a large entodiniomorphid protozoon (also known as *Eudiplodinium medium*) was isolated from the buffalo and grown *in vitro* by Naga and El-Shazly (1968) with a mean generation time, during short-term experiments, of 2 h—considerably lower than values reported for any other rumen protozoon. In contrast Coleman (1978a) found the same organism isolated from a cow difficult to grow and could only maintain it for 6 months at a population density of 2 ml^{-1}.

M. medium engulfed cellulose particles but was apparently unable to utilize them (Naga and El-Shazly, 1968; Westphal, 1934). It did not use sugars, pectin, inulin or levan but metabolized starch fractions readily with the formation of reducing sugars and probably acetic and formic acids, although it was difficult to distinguish the metabolism of exogenous substrate

from that of endogenous reserves. This production of large amounts of formic acid was unusual among rumen ciliates.

M. medium liberated free amino acid into the medium during incubation in the absence of added substrates; the amount was increased in the presence of certain proteins and further increased by the addition of amylopectin (Naga and El-Shazly, 1968).

J. *OPHRYOSCOLEX* SPECIES

Ophryoscolex purkynei has been cultured by Mah (1964) at a population density of 700–1000 ml^{-1} for 32 months and *O. caudatus* at a density of 50 ml^{-1} for 2 years by Coleman (1978a); both authors used ground wheat and dried grass as substrates. Spination of both species varied considerably in culture, *O. caudatus* "losing" its characteristic long caudal spine after only a few weeks and *O. purkynei* coming to resemble *O. inerminis*.

O. purkynei did not ferment soluble sugars, cellulose or protein with the production of gas. Starch was actively fermented to acetic, butyric and lactic acids and pectin was demethylated and degraded to oligogalacturonides, although these products were not utilized by the protozoon (Mah and Hungate, 1965; Williams *et al.*, 1961).

O. caudatus engulfed bacteria including a large *Streptococcus* (Mah, 1964), mixed rumen bacteria (Table 3) at the same rate per unit volume as other protozoa, and some pure suspensions of rumen and non-rumen bacteria (Coleman, 1975a). Mixed rumen bacteria at least were digested with the release of low-molecular-weight compounds into the medium. Mah and Hungate (1965) found that ammonia was produced by *O. purkynei* in the presence of certain proteins but this could have resulted from secondary attack on free amino acids. These authors also calculated that if this protozoon passed over into the omasum at the concentration at which it was present in the rumen of their animals, it could supply 11% of the nitrogen requirements of the host.

VI. METABOLISM OF HOLOTRICH PROTOZOA

These protozoa, which belong to the order Trichostomatida and the family Isotrichidae, are represented by three species: *Isotricha intestinalis*, *I. prostoma* and *Dasytricha ruminantium*. They are uniformly covered in cilia and have a mouth which is characteristically situated. Related species that occur rarely in the rumen are *Buetschlia parva* (Clarke, 1964; Dehority, 1970), *Charon* (=*Charonina*?) *ventriculi* (Jameson, 1925; Wolska, 1967), *C. equi* (Schumacher, 1915; Clarke, 1964) and *Blepharocorys bovis* (Dogiel, 1926); nothing is known about their metabolism.

A. METABOLISM OF CARBOHYDRATES

1. Isotricha *spp.*

Much of the early work was carried out with mixed *Isotricha* spp. (Heald and Oxford, 1953) but recently Prins and Van Hoven (1977) studied the

metabolism of *I. prostoma* alone. This protozoon took up glucose from concentrated solutions with the formation of amylopectin but, contrary to the widely held belief (Hungate, 1966), did not continue to do so until it burst. The maximum amount of amylopectin formed was constant over a wide range of glucose concentrations, but when the pH fell below 5, bursting did occur suggesting that it was caused by accumulation of acidic end products. The net formation or utilization of amylopectin depended on the external glucose concentration and at a certain critical concentration (20 mM) neither breakdown nor synthesis occurred, although the glucose was fermented. The carbohydrates most rapidly fermented were fructose, sucrose, glucose, raffinose and pectin and the principal products were storage amylopectin, H_2, CO_2, acetic, butyric and lactic acids.

2. Dasytricha ruminantium

This protozoon fermented cellobiose, galactose, raffinose, glucose, fructose, maltose and sucrose rapidly and pectin more slowly. The fermentation products from exogenous glucose and endogenous amylopectin were H_2, CO_2, acetic, butyric and lactic acids (L from amylopectin and D from high concentrations of glucose), amylopectin (from exogenous sugars) and, under some conditions, formic acid (Van Hoven and Prins, 1977). These authors produced evidence that, as with *Isotricha prostoma*, there was a maximum amount of amylopectin that could be synthesized and that rupture of the cells occurred only because of the intracellular accumulation of lactic acid. However, Williams and Harfoot (1976) disagreed and believed amylopectin synthesis to be uncontrolled and to be the cause of the bursting of the cells. *Isotricha* spp. also engulfed intact starch grains.

Of the glucose taken up by *D. ruminantium*, 3–5% was converted into protein (Williams and Harfoot, 1976) and depending on the glucose concentration this could supply sufficient amino acids to allow the protozoa to divide every 16–100 h. Acetate carbon was also incorporated to a limited extent into intracellular amylopectin (Williams and Harfoot, 1976), and into amino acids (principally alanine, aspartic acid, glutamic acid, leucine and lysine: Williams and Harfoot, 1976; Harmeyer and Hekimoglu, 1968) and released as CO_2. However, it is not known if there is a net synthesis of amino acids from acetate.

B. ENGULFMENT OF BACTERIA

Comparatively little is known about the metabolism of bacteria by holotrich protozoa. Suspensions of mixed *Isotricha* spp. engulfed *Escherichia coli* at a rate of 3000 bacteria per protozoon per hour for 24 h. The bacteria were rapidly killed and digested with release of soluble compounds into the medium (Wallis and Coleman, 1967). If the amino acids retained by the protozoa were utilized for protein synthesis, it would allow division every 120 h. Gutierrez (1958) showed, by isolation of bacteria from *I. prostoma* that had been incubated with mixed rumen bacteria, that the protozoon

selectively engulfed certain unidentified bacteria. Unfortunately this technique would tend to select those bacteria that were engulfed and digested only slowly rather than those that were digested rapidly. *Dasytricha ruminantium* has been observed to engulf selectively certain small cocci and stimulation of growth was observed on the addition to protozoal cultures of this bacterium and a rod isolated from the protozoon (Gutierrez and Hungate, 1957).

C. METABOLISM OF AMINO ACIDS AND PROTEIN

Although Harmeyer (1971a) showed no net uptake of ^{12}C-labelled amino acids by mixed *Isotricha* spp., Wallis and Coleman (1967) found that ^{14}C-labelled amino acids were incorporated into protozoal protein without conversion into other amino acids. Washed suspensions of *Isotricha* spp. also liberated free amino acids and other nitrogenous compounds into the medium but this may represent digestion of previously engulfed proteinaceous material (Heald and Oxford, 1953; Harmeyer, 1971b).

^{14}C from $^{14}CO_2$ was also incorporated into alanine, aspartic acid, glutamic acid, histidine and threonine by *Isotricha* spp. (Harmeyer, 1965).

D. METABOLISM OF LIPIDS

There is little evidence about the ability of holotrich protozoa to hydrolyse lipids. Girard and Hawke (1978) found little hydrolysis of phosphatidylcholine when this was incubated with suspensions of *Isotricha* spp. but, on further addition of bacteria to the mixture, rapid hydrolysis occurred. It was therefore suggested that bacterial and plant, rather than protozoal, lipases were important in the rumen. However, tributyrin stimulated gas and volatile fatty acid production by both *I. intestinalis* and *I. prostoma*, suggesting that hydrolysis of this trigylceride had occurred (Williams *et al.*, 1963; Gutierrez *et al.*, 1962).

Holotrich protozoa took up free fatty acids (Gutierrez *et al.*, 1962; Williams *et al.*, 1963; Girard and Hawke, 1978). Linoleic acid was taken up as free fatty acid and also incorporated into phosphatidylcholine but only the level of the former was decreased on the addition of bacteria (Girard and Hawke, 1978).

Mixed *Isotricha* spp. hydrogenated linoleic or oleic acids only in the presence of bacteria (Girard and Hawke ,1978; Chalupa and Kutches, 1968). However, Williams *et al.* (1963), using *I. intestinalis*, and Gutierrez *et al.* (1962), using *I. prostoma*, found hydrogenation of oleic acid to stearic acid, although there was no evidence that this was not due to bacteria. Katz and Keeney (1967) found little stearic acid in holotrich protozoa, but this may not be significant evidence against hydrogenation as Abaza *et al.* (1975) showed that rumen protozoa could desaturate their own fatty acids.

E. ATTACHMENT TO PLANT PARTICLES

Isotricha intestinalis and *I. prostoma* both showed chemotaxis towards, and attached themselves to, particulate materials from which sucrose, glucose and fructose diffused provided that soluble protein was present (Orpin and

Letcher, 1978). Attachment occurred by means of a longitudinal ridge on the anterior dorsolateral surface of the protozoa (Orpin and Hall, 1977). As *Isotricha* spp. take up soluble sugars this behaviour may be advantageous to the protozoon in its quest for food and partially explain the retention of protozoa in the rumen.

VII. Parasites of Rumen Protozoa

Lubinsky (1955a) described *Sphaerita hoari*, a chytrid fungus, in a number of entodiniomorphid protozoa, principally *Eremoplastron bovis*, from domesticated ruminants in India. Although present in 19% of *Er. bovis*, examined, the fungus appeared to do little harm to the host. Lubinsky (1955b) also described *Sagittospora cameroni* and closely related species parasitizing *Eudiplodinium maggii* and other entodiniomorphid protozoa from a goat in India. The parasite invariably killed the host protozoon but was found in only 1% of the rumens examined and then in only 2% of the protozoa. Jírovec (1933) also described *Sphaerita* spp. from *Entodinium simplex*. Bretschneider and Van Vorstenbosch (1964) described rods in the ectoplasm of *Entodinium* sp. from cattle and organisms that could have been *Rickettsia* in the macronucleus of *Isotricha intestinalis*.

VIII. Role of Protozoa in the Normal Rumen

A. ENGULFMENT OF STARCH

Oxford (1955) first proposed, and Krogh (1959) provided evidence, that one function of entodiniomorphid protozoa was to remove starch grains from the fluid phase in the rumen and make them unavailable for bacterial attack. Inside the protozoa the starch was fermented comparatively slowly to acetic and butyric acids which were absorbed through the rumen wall rather than being more rapidly fermented by bacteria to lactic acid which was absorbed only slowly (Williams and McKenzie, 1965). As the protozoal population took some time to increase in response to an increase in the proportion of starch in the ration, this system for the removal of starch broke down if the amount of starch in the ration was increased too rapidly. As rumen protozoa engulfed bacteria as well as starch, Mackie *et al.* (1978) suggested that protozoa controlled rumen fermentation by taking up both enzyme and substrate. They provided evidence that, if a small excess of starch was fed, lactic acid production increased but that bacteria utilizing lactic acid then also increased and removed the lactic acid, allowing the protozoa to regain control of fermentation.

B. DIGESTION OF BACTERIA

Coleman (1975a), in speculations based on (a) the known bacterial and protozoal population densities (10^9–10^{10} and 2×10^6 ml^{-1} respectively) in the rumen of animals on a restricted high-grain ration and (b) the rate at which

Entodinium caudatum grown *in vitro* engulfed bacteria (50 bacteria per protozoon per minute), calculated that 10^8 bacteria per ml could be engulfed by the protozoa each minute. Half of the cellular constituents of the bacteria (45 g per sheep per day) would be released into the bulk fluid, principally as amino acids. These amino acids would then be rapidly fermented by the remaining bacteria with the formation of more bacteria, ammonia and volatile fatty acids. This would account for the known action of protozoa in increasing the level of these compounds in the rumen (Abou Akkada and El-Shazly, 1964) and decreasing the bacterial population density (Kurihara *et al.*, 1968). Recently Coleman and Sandford (1979a) studied the rate of uptake and digestion of pure cultures and mixed rumen bacteria by single protozoal species and mixed protozoa of the same genus; they concluded that a value of 10 bacteria per *Entodinium* per minute was more realistic, so that 2×10^7 bacteria per ml would be engulfed each minute.

On a more normal ration of 800 g hay and 100 g oats (Coleman and Sandford, 1979a), or wheaten and lucerne chaff (Warner, 1962), $0{\cdot}2$–$0{\cdot}5 \times 10^6$ *Entodinium* per ml were present and it was estimated that, at 10^9 bacteria per ml, $4{\cdot}5 \times 10^6$ bacteria per ml (0·45%) were engulfed and $2{\cdot}7 \times 10^6$ bacteria per ml (0·27%) digested each minute. Coleman (1975a) calculated that this represented 2·4 g of bacteria per rumen per day. At 10^{10} bacteria per ml, 0·20% would be engulfed and 0·13% digested. This means that the recycling rate of the bacteria would be 3·7 h at 10^9 bacteria per ml or 8·3 h at 10^{10} bacteria per ml. Jarvis (1968) investigated the rate of disappearance of, and release of radioactivity from, *Streptococcus bovis* added to the rumen of a cow fed on alfalfa and found that, depending on the conditions, 0·6 to 22% (average 5%) of the bacteria (2×10^8 ml^{-1}) were digested by $4{\cdot}3 \times 10^4$ mixed protozoa (mainly *Entodinium* spp.) each hour. Thus at this low population density (in terms of the total rumen bacterial population), assuming average release, 230 bacteria were destroyed per protozoon per hour. As the rate of engulfment of bacteria increased almost linearly with bacterial population density below 10^9 bacteria per ml (Coleman, 1964b), Jarvis's results showed that, at this level, approximately 19 bacteria per protozoon per minute were destroyed. Although this is somewhat higher than the present author's results, Jarvis's protozoal preparation contained larger protozoa which took up bacteria more rapidly than *Entodinium*.

Cottle *et al.* (1978) used ^{15}N to investigate the recycling of bacterial nitrogen and considered that in a sheep with 10^6 protozoa per ml in the rumen, in which 2·2 g bacterial N and 1·3 g protozoal N left the rumen each day, 6 g of nitrogen (=approx. 36 g protein) were recycled between the bacteria and protozoa each day. This is lower than the original value of 45 g derived by Coleman (1975a) and higher than the values of 2·4 g (at 10^9 bacteria per ml) and 11·7 g (at 10^{10} bacteria per ml) calculated from the values of Coleman and Sandford (1979a).

There is now a little evidence (Coleman and Sandford, 1979a; Kurihara *et al.*, 1978) that protozoa may have a selective effect on the bacterial flora, with *Entodinium* spp. tending to remove cellulolytic bacteria.

References

Abaza, M. A., Abou Akkada, A. R. and El-Shazly, K. (1975). Effect of rumen protozoa on dietary lipid in sheep. *Journal of Agricultural Science*, **85**, 135–143.

Abe, M. and Kumeno, F. (1973). *In vitro* simulation of rumen fermentation: apparatus and effects of dilution rate and continuous dialysis on fermentation and protozoal population. *Journal of Animal Science*, **36**, 941–948.

Abe, M., Shibui, H., Iriki, T. and Kumeno, F. (1973). Relation between diet and protozoal population in the rumen. *British Journal of Nutrition*, **29**, 197–202.

Abou Akkada, A. R. and El-Shazly, K. (1964). Effect of absence of ciliate protozoa from the rumen on microbial activity and growth of lambs. *Applied Microbiology*, **12**, 384–390.

Abou Akkada, A. R. and El-Shazly, K. (1965). Effect of presence or absence of rumen ciliate protozoa on some blood components, nitrogen retention and digestibility of food constituents in lambs. *Journal of Agricultural Science*, **64**, 251–255.

Abou Akkada, A. R. and Howard, B. H. (1960). The biochemistry of rumen protozoa. 3. The carbohydrate metabolism of *Entodinium*. *Biochemical Journal*, **76**, 445–451.

Abou Akkada, A. R. and Howard, B. H. (1961). The biochemistry of rumen protozoa. 4. The decomposition of pectic substances. *Biochemical Journal*, **78**, 512–517.

Abou Akkada, A. R. and Howard, B. H. (1962). The biochemistry of rumen protozoa. 5. The nitrogen metabolism of *Entodinium*. *Biochemical Journal*, **82**, 313–320.

Abou Akkada, A. R., Eadie, J. M. and Howard, B. H. (1963). The biochemistry of rumen protozoa. 7. The carbohydrases of *Polyplastron multivesiculatum* (Dogiel and Fedorowa). *Biochemical Journal*, **89**, 268–272.

Abou Akkada, A. R., Messmer, D. A., Fina, L. R. and Bartley, E. E. (1968). Distribution of 2-aminoethylphosphonic acid among rumen microorganisms. *Journal of Dairy Science*, **51**, 78–81.

Akin, D. E. (1976). Ultrastructure of rumen bacterial attachment to forage cell walls. *Applied and Environmental Microbiology*, **31**, 562–568.

Amos, H. E. and Akin, D. E. (1978). Rumen protozoal degradation of structurally intact forage tissues. *Applied and Environmental Microbiology*, **36**, 513–522.

Bailey, R. W. (1958). Bloat in cattle. X. The carbohydrases of the cattle rumen ciliate *Epidinium ecaudatum* Crawley isolated from cows fed on red clover (*Trifolium pratense* L.). *New Zealand Journal of Agricultural Research*, **1**, 825–833.

Bailey, R. W. and Clarke, R. T. J. (1963a). Carbohydrase activity of rumen *Entodinium* species from sheep on a starch-free diet. *Nature* (*London*), **198**, 787.

Bailey, R. W. and Clarke, R. T. J. (1963b). Carbohydrases of the rumen oligotrich *Eremoplastron bovis*. *Nature* (*London*), **199**, 1291–1292.

Bailey, R. W. and Gaillard, B. D. E. (1965). Carbohydrases of the rumen ciliate *Epidinium ecaudatum* (Crawley). Hydrolysis of plant hemicellulose fractions and β-linked glucose polymers. *Biochemical Journal*, **95**, 758–766.

Bailey, R. W. and Howard, B. H. (1963a). The biochemistry of rumen protozoa. 6. The maltases of *Dasytricha ruminantium*, *Epidinium ecaudatum* (Crawley) and *Entodinium caudatum*. *Biochemical Journal*, **86**, 446–452.

Bailey, R. W. and Howard, B. H. (1963b). Carbohydrases of the rumen ciliate *Epidinium ecaudatum* (Crawley). 2. α-Galactosidase and isomaltase. *Biochemical Journal*, **87**, 146–151.

Bailey, R. W., Clarke, R. T. J. and Wright, D. E. (1962). Carbohydrases of the rumen ciliate *Epidinium ecaudatum* (Crawley). Action on plant hemicellulose. *Biochemical Journal*, **83**, 517–523.

Bartley, E. E. and Bassette, R. (1961). Bloat in cattle, III. Composition of foam in legume bloat. *Journal of Dairy Science*, **44**, 1365–1366.

Bartley, E. E., Meyer, R. M. and Fina, L. R. (1975). Feedlot or grain bloat. *In* "Digestion and Metabolism in the Ruminant" (I. W. McDonald and A. E. R. Warner, eds.), pp. 551–562. University of New England Publishing Unit, Armidale.

Bauchop, T. and Clarke, R. T. J. (1976). Attachment of the ciliate *Epidinium* Crawley to plant fragments in the sheep rumen. *Applied and Environmental Microbiology*, **32**, 417–422.

Becker, E. R. and Everett, R. C. (1930). Comparative growths of normal and infusoria-free lambs. *American Journal of Hygiene*, **11**, 362–370.

Becker, E. R. and Hsiung, T. S. (1929). The method by which ruminants acquire their fauna of infusoria and remarks concerning experiments on the host specificity of these protozoa. *Proceedings of the National Academy of Sciences, New York*, **15**, 684–690.

Becker, E. R., Schultz, J. A. and Emmerson, M. A. (1929). Experiments on the physiological relationships between the stomach infusoria of ruminants and their hosts, with a bibliography. *Iowa State College Journal of Science*, **4**, 215–251.

Bergen, W. G., Purser, D. B. and Cline, J. H. (1968). Determination of limiting amino acids of rumen-isolated microbial proteins fed to rats. *Journal of Dairy Science*, **51**, 1698–1700.

Bird, S., Baigent, D. R., Dixon, R. and Leng, R. A. (1978). Ruminal protozoa and growth in lambs. *Proceedings of the Australian Society for Animal Production*, **12**, 137.

Bonhomme, A. (1968). Mise en évidence de l'action cellulolytique des ciliés oligotriches du genre *Entodinium*. *Protistologica*, **4**, 61–66.

Bonhomme-Florentin, A. (1975a). Activité cellulolytique des ciliés entodiniomorphes. *Journal of Protozoology*, **22**, 447–451.

Bonhomme-Florentin, A. (1975b). Ureolytic activity of endocommensal Ciliata Entodiniomorpha of ruminants. *Protistologica*, **11**, 325–329.

Borhami, B. E. A., El-Shazly, K., Abou Akkada, A. R. and Ahmed, I. A. (1967). Effect of early establishment of ciliate protozoa in the rumen on microbial activity and growth of early weaned buffalo calves. *Journal of Dairy Science*, **50**, 1654–1660.

Boyne, A. W., Eadie, J. M. and Raitt, K. (1957). The development and testing of a method of counting rumen ciliate protozoa. *Journal of General Microbiology*, **17**, 414–423.

Bretschneider, L. H. and Van Vorstenbosch, C. J. A. H. V. (1964). Das Vorkommen von intraplasmatischen und intranuklearen Mikroorganismen in einigen Pansenciliaten. *Proceedings Koninklijke Nederlandse Akademie van Wetenschappen*, Series C, **67**, 313–319.

Broad, T. E. and Dawson, R. M. C. (1973). Formation of ceramide phosphorylethanolamine from phosphatidylethanolamine in the rumen protozoon *Entodinium caudatum*. *Biochemical Journal*, **134**, 659–662.

Broad, T. E. and Dawson, R. M. C. (1975). Phospholipid biosynthesis in the anaerobic protozoon *Entodinium caudatum*. *Biochemical Journal*, **146**, 317–328.

Broad, T. E. and Dawson, R. M. C. (1976). Role of choline in the nutrition of the rumen protozoon *Entodinium caudatum*. *Journal of General Microbiology*, **92**, 391–397.

Bygrave, F. L. and Dawson, R. M. C. (1976). Phosphatidylcholine biosynthesis and choline transport in the anaerobic protozoon *Entodinium caudatum*. *Biochemical Journal*, **160**, 481–490.

Chalmers, M. I., Davidson, J., Eadie, J. M. and Gill, J. C. (1968). Some comparisons of performance of lambs with and without rumen ciliate protozoa. *Proceedings of the Nutrition Society*, **27**, 29A.

Chalupa, W. A. and Kutches, A. J. (1968). Biohydrogenation of linoleic-1-^{14}C-acid by rumen protozoa. *Journal of Animal Science*, **27**, 1502–1508.

Chesters, J. K. (1968). Cell-free protein synthesis by rumen protozoa. *Journal of Protozoology*, **15**, 509–512.

Christiansen, W. C., Kawashima, R. and Burroughs, W. (1965). Influence of protozoa upon rumen acid production and liveweight gains in lambs. *Journal of Animal Science*, **24**, 730–734.

Clarke, R. T. J. (1963). The cultivation of some rumen oligotrich protozoa. *Journal of General Microbiology*, **33**, 401–408.

Clarke, R. T. J. (1964). Ciliates of the rumen of domestic cattle (*Bos taurus* L.). *New Zealand Journal of Agricultural Research*, **7**, 248–257.

Clarke, R. T. J. (1965). Diurnal variation in the numbers of rumen ciliate protozoa in cattle. *New Zealand Journal of Agricultural Research*, **8**, 1–9.

Clarke, R. T. J. (1966). Bloat in cattle. XXVII. Preliminary observations with the anti-protozoal agent 1,2-dimethyl-5-nitroimidazole, for the prevention of bloat. *New Zealand Journal of Agricultural Research*, **9**, 437–443.

Clarke, D. G. and Hawke, J. C. (1970). Studies on rumen metabolism. 6. *In vitro* hydrolysis of triglyceride and isolation of a lipolytic fraction. *Journal of the Science of Food and Agriculture*, **21**, 446–452.

Clarke, R. T. J. and Hungate, R. E. (1966). Culture of the rumen holotrich ciliate *Dasytricha ruminantium* Schuberg. *Applied Microbiology*, **14**, 340–345.

Clarke, R. T. J. and Reid, C. S. W. (1974). Foamy bloat of cattle. A review. *Journal of Dairy Science*, **57**, 753–785.

Clarke, R. T. J., Reid, C. S. W. and Young, P. W. (1969). Attempts to prevent legume bloat in dry and lactating cows by partial or complete elimination of the rumen holotrich protozoa with dimetridazole. *New Zealand Journal of Agricultural Research*, **12**, 446–466.

Coleman, G. S. (1958). Maintenance of oligotrich protozoa from the sheep rumen *in vitro*. *Nature* (*London*), **182**, 1104–1105.

Coleman, G. S. (1960). The cultivation of sheep rumen oligotrich protozoa *in vitro*. *Journal of General Microbiology*, **22**, 555–563.

Coleman, G. S. (1962). The preparation and survival of almost bacteria-free suspensions of *Entodinium caudatum*. *Journal of General Microbiology*, **28**, 271–281.

Coleman, G. S. (1964a). The metabolism of ^{14}C-glycine and ^{14}C-bicarbonate by washed suspensions of the rumen ciliate *Entodinium caudatum*. *Journal of General Microbiology*, **35**, 91–103.

Coleman, G. S. (1964b). The metabolism of *Escherichia coli* and other bacteria by *Entodinium caudatum*. *Journal of General Microbiology*, **37**, 209–223.

Coleman, G. S. (1967a). The metabolism of free amino acids by washed suspensions of the rumen ciliate *Entodinium caudatum*. *Journal of General Microbiology*, **47**, 433–447.

Coleman, G. S. (1967b). The metabolism of the amino acids of *Escherichia coli* and other bacteria by the rumen ciliate *Entodinium caudatum*. *Journal of General Microbiology*, **47**, 449–464.

Coleman, G. S. (1968). The metabolism of bacterial nucleic acid and of free components of nucleic acid by the rumen ciliate *Entodinium caudatum*. *Journal of General Microbiology*, **54**, 83–96.

Coleman, G. S. (1969a). The cultivation of the rumen ciliate *Entodinium simplex*. *Journal of General Microbiology*, **57**, 81–90.

Coleman, G. S. (1969b). The metabolism of starch, maltose, glucose and some other sugars by the rumen ciliate *Entodinium caudatum*. *Journal of Genera Microbiology*, **57**, 303–332.

Coleman, G. S. (1972). The metabolism of starch, glucose, amino acids, purines, pyrimidines and bacteria by the rumen ciliate *Entodinium simplex*. *Journal of General Microbiology*, **71**, 117–131.

Coleman, G. S. (1975a). The interrelationship between rumen ciliate protozoa and bacteria. *In* "Digestion and Metabolism in the Ruminant" (I. W. McDonald and A. C. I. Warner, eds), pp. 149–164. University of New England Publishing Unit, Armidale.

Coleman, G. S. (1975b). The role of bacteria in the metabolism of rumen entodiniomorphid protozoa. *In* "Symbiosis" (D. H. Jennings and D. L. Lee, eds.), *Symposia of the Society for Experimental Biology*, vol. 29, pp. 533–558. Cambridge University Press, Cambridge.

Coleman, G. S. (1978a). Rumen entodiniomorphid protozoa. *In* "Methods of Cultivating Parasites *in vitro*" (A. E. R. Taylor and J. R. Baker, eds.), pp. 39–54. Academic Press, London.

Coleman, G. S. (1978b). The metabolism of cellulose, glucose and starch by the rumen ciliate protozoon *Eudiplodinium maggii*. *Journal of General Microbiology*, **107**, 359–366.

Coleman, G. S. (1978c). Methods for the study of the metabolism of rumen ciliate protozoa and their closely associated bacteria. *In* "Techniques for the study of mixed populations" (D. W. Lovelock and R. Davies, eds.), pp. 143–163. Academic Press, London.

Coleman, G. S. and Hall, F. J. (1969). Electron microscopy of the rumen ciliate *Entodinium caudatum*, with special reference to the engulfment of bacteria and other particulate matter. *Tissue and Cell*, **1**, 607–618.

Coleman, G. S. and Hall, F. J. (1971). A study in the light and electron microscope of the extruded peristome and related structures of the rumen ciliate *Entodinium caudatum*. *Tissue and Cell*, **3**, 371–380.

Coleman, G. S. and Hall, F. J. (1972). Fine structural studies on the digestion of bacterial species in the rumen ciliate, *Entodinium caudatum*. *Tissue and Cell*, **4**, 37–48.

Coleman, G. S. and Hall, F. J. (1974). The metabolism of *Epidinium ecaudatum caudatum* and *Entodinium caudatum* as shown by autoradiography in the electron microscope. *Journal of General Microbiology*, **85**, 265–273.

Coleman, G. S. and Hall, F. J. (1978). Digestion of *Epidinium ecaudatum caudatum* by the rumen ciliate *Polyplastron multivesiculatum* as shown by studies in the electron microscope. *Society for General Microbiology Quarterly*, **6**, 29–30.

Coleman, G. S. and Hall, F. J. (1979). Digestion of *Entodinium caudatum* by the rumen ciliate *Entodinium bursa* as shown by studies in the electron microscope. *Society for General Microbiology Quarterly*, **6**, 81–82.

Coleman, G. S. and Laurie, J. I. (1974a). The metabolism of starch, glucose, amino acids, purines, pyrimidines and bacteria by three *Epidinium* spp. isolated from the rumen. *Journal of General Microbiology*, **85**, 244–256.

Coleman, G. S. and Laurie, J. I. (1974b). The utilization of *Bacillus megaterium* and the release of a lytic enzyme by three *Epidinium* spp. isolated from the rumen. *Journal of General Microbiology*, **85**, 257–264.

Coleman, G. S. and Laurie, J. I. (1976). The uptake and metabolism of glucose, maltose and starch by the rumen ciliate *Epidinium ecaudatum caudatum*. *Journal of General Microbiology*, **95**, 364–374.

Coleman, G. S. and Laurie, J. I. (1977). The metabolism of starch, glucose, amino acids, purines, pyrimidines and bacteria by the rumen ciliate *Polyplastron multivesiculatum*. *Journal of General Microbiology*, **98**, 29–37.

Coleman, G. S. and Sandford, D. C. (1979a). The engulfment and digestion of mixed rumen bacteria and individual bacterial species by single and mixed species of rumen ciliate protozoa grown *in vivo*. *Journal of Agricultural Science* (*Cambridge*), **92**, 729–742.

Coleman, G. S. and Sandford, D. C. (1979b). The uptake and utilization of bacteria, amino acids and nucleic acid components by the rumen ciliate *Eudiplodinium maggii*. *Journal of Applied Bacteriology*, **47**, 409–419.

Coleman, G. S. and White, R. W. (1970). Re-establishment of *Entodinium caudatum*, cultured *in vitro*, in the rumen of a defaunated sheep. *Journal of General Microbiology*, **62**, 265–266.

Coleman, G. S., Kemp, P. and Dawson, R. M. C. (1971). The catabolism of phosphatidylethanolamine by the rumen protozoon *Entodinium caudatum* and its conversion into the *N*-(1-carboxyethyl) derivative. *Biochemical Journal*, **123**, 97–104.

Coleman, G. S., Davies, J. I. and Cash, M. A. (1972). The cultivation of the rumen ciliates *Epidinium ecaudatum caudatum* and *Polyplastron multivesiculatum in vitro*. *Journal of General Microbiology*, **73**, 509–521.

Coleman, G. S., Laurie, J. I., Bailey, J. E. and Holdgate, S. A. (1976). The cultivation of cellulolytic protozoa isolated from the rumen. *Journal of General Microbiology*, **95**, 144–150.

Coleman, G. S., Laurie, J. I. and Bailey, J. E. (1977). The cultivation of the rumen ciliate *Entodinium bursa* in the presence of *Entodinium caudatum*. *Journal of General Microbiology*, **101**, 253–258.

Cottle, D. J., Nolan, J. V. and Leng, R. A. (1978). Turnover of protozoa and bacteria in the rumen of sheep. *Proceedings of the Australian Society of Animal Production*, **12**, 138.

Czerkawski, J. W. (1973). Effect of linseed oil fatty acids and linseed oil on rumen fermentation in sheep. *Journal of Agricultural Science* (*Cambridge*) **81**, 517–531.

Czerkawski, J. W. and Breckenridge, G. (1977). Design and development of a long-term rumen simulation technique (Rusitec). *British Journal of Nutrition*, **38**, 371–384.

Czerkawski, J. W. and Breckenridge, G. (1978). Use of the rumen simulation technique (Rusitec) to study the distribution of microbial matter in the solid and liquid phases of the reaction mixture: sequestration of microorganisms. *Proceedings of the Nutrition Society*, **37**, 70A.

Davis, J. D. and Essig, H. W. (1972). Comparison of three bloat-preventing compounds for cattle grazing clover. *Canadian Journal of Animal Science*, **52**, 329–335.

Dawson, R. M. C. and Kemp, P. (1969). The effect of defaunation on the phospholipids and on the hydrogenation of unsaturated fatty acids in the rumen. *Biochemical Journal*, **115**, 351–352.

Dehority, B. A. (1970). Occurrence of the ciliate protozoa *Bütschlia parva* Schuberg in the rumen of the ovine. *Applied Microbiology*, **19**, 179–181.

Dehority, B. A. and Purser, D. B. (1970). Factors affecting the establishment and numbers of holotrich protozoa in the ovine rumen. *Journal of Animal Science*, **30**, 445–449.

De Vuyst, A., Vanbelle, M., Jossart, J. M. and Baguette, A. (1975). The effect of methionine hydroxyanalogue supplementation of the diet on the concentration of ciliate protozoa in the rumen of sheep. *Zeitshrift für Tierphysiologie, Tiernährung und Futtermittelkunde*, **35**, 316–321.

Dogiel, V. A. (1926). Une nouvelle espèce du genre Blepharocorys, *B. bovis* n.sp. habitant l'estomac du boeuf. *Annales de Parasitologie Humaine et Comparée*, **4**, 61–64.

Dogiel, V. A. (1927). Monographie der familie Ophryoscolecidae. *Archiv für Protistenkunde*, **59**, 1–288.

Eadie, J. M. (1962a). The development of rumen microbial populations in lambs and calves under various conditions of management. *Journal of General Microbiology*, **29**, 563–578.

Eadie, J. M. (1962b). Interrelationships between certain rumen ciliate protozoa. *Journal of General Microbiology*, **29**, 579–588.

Eadie, J. M. (1967). Studies on the ecology of certain rumen ciliate protozoa. *Journal of General Microbiology*, **49**, 175–194.

Eadie, J. M. and Gill, J. C. (1971). The effect of the absence of rumen ciliate protozoa on growing lambs fed on a roughage-concentrate diet. *British Journal of Nutrition*, **26**, 155–167.

Eadie, J. M. and Hobson, P. N. (1962). Effect of the presence or absence of rumen ciliate protozoa on the total rumen bacterial count in lambs. *Nature (London)*, **193**, 503–505.

Eadie, J. M. and Mann, S. O. (1970). Development of the rumen microbial population: high starch diets and instability. *In* "Physiology of Digestion and Metabolism in the Ruminant" (A. T. Phillipson, ed.), pp. 335–347. Oriel Press, Newcastle-upon-Tyne.

Eadie, J. M. and Oxford, A. E. (1957). A safe and simple procedure for the removal of holotrich ciliates from the rumen of adult fistulated sheep. *Nature (London)*, **179**, 485.

Eadie, J. M., Manners, D. J. and Stark, J. R. (1963). The molecular structure of a reserve polysaccharide from *Entodinium caudatum*. *Biochemical Journal*, **89**, 91P–92P.

Eadie, J. M., Hobson, P. N. and Mann, S. O. (1967). A note on some comparisons between the rumen contents of barley-fed steers and that of young calves also fed on a high concentrate ration. *Animal Production*, **9**, 247–250.

Eadie, J. M., Hyldegaard-Jensen, J., Mann, S. O., Reid, R. S. and Whitelaw, F. G. (1970). Observations on the microbiology and biochemistry of the rumen in cattle given different quantities of a pelleted barley ration. *British Journal of Nutrition*, **24**, 157–177.

Einszporn, T. (1961). Effect of proteins on the population size of *Entodinium longinucleatum* Dogiel in culture. *Acta Parasitologica Polonica*, **9**, 193–210.

Emmanuel, B. (1974). On the origin of rumen protozoan fatty acids. *Biochimica et Biophysica Acta*, **337**, 404–413.

Garton, G. A., Lough, A. K. and Vioque, E. (1961). Glyceride hydrolysis and glycerol fermentation by sheep rumen contents. *Journal of General Microbiology*, **25**, 215–225.

Giesecke, D. (1970). Comparative microbiology of the alimentary tract. *In* "Physiology of Digestion and Metabolism in the Ruminant" (A. T. Phillipson, ed.), pp. 306–318. Oriel Press, Newcastle-upon-Tyne.

Girard, V. and Hawke, J. C. (1978). The role of holotrichs in the metabolism of dietary linoleic acid in the rumen. *Biochimica et Biophysica Acta*, **528**, 17–27.

Gruby, D. and Delafond, H. M. O. (1843). Recherches sur des animalcules se dévellopant en grand nombre dans l'estomac et dans les intestins pendant la digestion des animaux herbivores et carnivores. *Compte rendu hebdomadaire des séances de l'Académie des sciences, Paris*, **17**, 1304–1308.

Gutierrez, J. (1955). Experiments on the culture and physiology of holotrichs from the bovine rumen. *Biochemical Journal*, **60**, 516–522.

Gutierrez, J. (1958). Observations of bacterial feeding by the rumen ciliate *Isotricha prostoma*. *Journal of Protozoology*, **5**, 122–126.

Gutierrez, J. and Davis, R. E. (1962). Culture and metabolism of the rumen ciliate *Epidinium ecaudatum* Crawley. *Applied Microbiology*, **10**, 305–308.

Gutierrez, J. and Hungate, R. E. (1957). Interrelationship between certain bacteria and the rumen ciliate *Dasytricha ruminantium*. *Science* (*Washington*), **126**, 511.

Gutierrez, J., Williams, P. P., Davis, R. E. and Warwick, E. J. (1962). Lipid metabolism of rumen ciliates and bacteria. 1. Uptake of fatty acids by *Isotricha prostoma* and *Entodinium simplex*. *Applied Microbiology*, **10**, 548–551.

Hall, F. J., West, J. and Coleman, G. S. (1974). Fine structural studies on the digestion of chloroplasts in the rumen ciliate *Entodinium caudatum*. *Tissue and Cell*, **6**, 243–253.

Harfoot, C. G., Noble, R. C. and Moore, J. H. (1973). Food particles as a site for biohydrogenation of unsaturated fatty acids in the rumen. *Biochemical Journal*, **132**, 829–842.

Harmeyer, J. (1965). Die Fixierung von CO_2 in Aminosäuren durch isolierte Pansenprotozoenarten (*Isotricha prostoma* und *Isotricha intestinalis*). *Zentralblatt für Veterinärmedizin*, Reihe A, **12**, 9–17.

Harmeyer, J. (1967). Untersuchungen über den Stickstoffwechsel von Pansenprotozoen (*Entodinium*) mittels ^{14}C-Karbonatinkorporation. *Journal of Protozoology*, **14**, 376–378.

Harmeyer, J. (1971a). Der Aminosäurenstoffwechsel isolierter Pansenprotozoenarten (*Isotricha prostoma* und *I. intestinalis*). 1 Mitteilung. Untersuchungen über den Abbau von Aminosäuren. *Zeitschrift für Tierphysiologie, Tiernährung und Futtermittelkunde*, **28**, 65–75.

Harmeyer, J. (1971b). Der Aminosäurenstoffwechsel isolierter Pansenprotozoenarten (*Isotricha prostoma* und *I. intestinalis*). 2 Mitteilung. Exkretion von Aminosäuren. *Zeitschrift für Tierphysiologie, Tiernährung und Futtermittelkunde*, **28**, 75–85.

Harmeyer, J. and Hekimoglu, H. (1968). Acetatinkorporation durch isolierte Pansenprotozoenarten. *Zentralblatt für Veterinärmedizin*, Reihe A, **15**, 242–254.

Hawke, J. C. and Silcock, W. R. (1969). Lipolysis and hydrogenation in the rumen. *Biochemical Journal*, **112**, 131–132.

Heald, P. J. and Oxford, A. E. (1953). Fermentation of soluble sugars by anaerobic holotrich ciliate protozoa of the genera *Isotricha* and *Dasytricha*. *Biochemical Journal*, **53**, 506–512.

Heald, P. J., Oxford, A. E. and Sugden, B. (1952). A convenient method for preparing massive suspensions of virtually bacteria-free ciliate protozoa of the genera *Isotricha* and *Dasytricha* for manometric studies. *Nature* (*London*), **169**, 1055–1056.

Henderson, C., Stewart, C. S. and Hine, R. S. (1977). The effect of added tallow on the rumen digestion rate and microbial populations of sheep fed dried grass. *Proceedings of the Nutrition Society*, **36**, 148A.

Hino, T. and Kametaka, M. (1974). Effects of diets on the number of protozoa in the rumen, with special reference to the effect of purified diets and sterol in diets. *Japanese Journal of Zootechnical Science*, **45**, 223–232.

Hino, T. and Kametaka, M. (1975). Sterols in rumen oligotrich protozoa. *Japanese Journal of Zootechnical Science*, **46**, 693–705.

Hino, T. and Kametaka, M. (1977). Gnotobiotic and axenic culture of a rumen protozoon *Entodinium caudatum*. *Journal of General and Applied Microbiology*, **23**, 37–48.

Hino, T., Kametaka, M. and Kandatsu, M. (1973a). The cultivation of rumen oligotrich protozoa. 1. Factors affecting the life of *Entodinium*. *Journal of General and Applied Microbiology*, **19**, 305–315.

Hino, T., Kametaka, M. and Kandatsu, M. (1973b). The cultivation of rumen oligotrich protozoa. 2. Growth of entodinia *in vitro*. *Journal of General and Applied Microbiology*, **19**, 325–337.

Hino, T., Kametaka, M. and Kandatsu, M. (1973c). The cultivation of rumen oligotrich protozoa. 3. White clover factors which stimulate the growth of entodinia. *Journal of General and Applied Microbiology*, **19**, 397–413.

Hoover, W. H., Crooker, B. A. and Sniffen, C. J. (1976). Effect of differential solid-liquid removal rates on protozoa numbers in continuous cultures of rumen contents. *Journal of Animal Science*, **43**, 528–534.

Horiguchi, M. and Kandatsu, M. (1959). Isolation of 2-aminoethane phosphonic acid from rumen protozoa. *Nature (London)*, **184**, 901–902.

Hoshino, S. and Sugiyama, S.-I. (1974). Protein synthesis in a cell-free preparation from rumen protozoa. *Comparative Biochemistry and Physiology*, **48B**, 39–45.

Hungate, R. E. (1942). The culture of *Eudiplodinium neglectum* with experiments on the digestion of cellulose. *Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts*, **83**, 303–319.

Hungate, R. E. (1943). Further experiments on cellulose digestion by protozoa in the rumen of cattle. *Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts*, **84**, 157–163.

Hungate, R. E. (1966). "The Rumen and its Microbes". Academic Press, London and New York.

Hungate, R. E., Reichl, J. and Prins, R. (1971). Parameters of rumen fermentation in a continuously fed sheep: evidence of a microbial rumination pool. *Applied Microbiology*, **22**, 1104–1113.

Ibrahim, E. A. and Ingalls, J. R. (1972). Microbial protein biosynthesis in the rumen. *Journal of Dairy Science*, **55**, 971–978.

Ibrahim, E. A., Ingalls, J. R. and Stanger, N. E. (1970). Effect of dietary diethyl-stilboestrol on populations of ciliate protozoa in dairy cattle. *Canadian Journal of Animal Science*, **50**, 101–106.

Imai, S. and Ogimoto, K. (1978). Scanning electron and fluorescent microscopic studies on the attachment of spherical bacteria to ciliate protozoa in the ovine rumen. *Japanese Journal of Veterinary Science*, **40**, 9–19.

Jameson, A. P. (1925). A new ciliate *Charon ventriculi* n.g. n.sp., from the stomach of ruminants. *Parasitology*, **17**, 403–405.

Jarvis, B. D. W. (1968). Lysis of viable rumen bacteria in bovine rumen fluid. *Applied Microbiology*, **16**, 714–723.

Jarvis, B. D. W. (1974). A method of obtaining axenic rumen ciliate protozoa. *Journal of General and Applied Microbiology*, **20**, 385–387.

Jarvis, B. D. W. and Hungate, R. E. (1968). Factors influencing agnotobiotic cultures of the rumen ciliate *Entodinium simplex*. *Applied Microbiology*, **16**, 1044–1052.

Jírovec, D. (1933). Beobachtungen über die Fauna des Rindespansens. *Zeitschrift für Parasitenkunde*, **5**, 584–591.

Jones, W. T. and Lyttleton, J. W. (1969). Bloat in cattle. XXIX. The foaming properties of clover proteins. *New Zealand Journal of Agricultural Research*, **12**, 31–46.

Jones, W. T. and Lyttleton, J. W. (1972). Bloat in cattle. XXXVII. The foaming properties of bovine salivary secretions and protozoal proteins. *New Zealand Journal of Agricultural Research*, **15**, 506–511.

Katz, I. and Keeney, M. (1967). The lipids of some rumen holotrich protozoa. *Biochimica et Biophysica Acta*, **144**, 102–112.

Kemp, P. and Dawson, R. M. C. (1969a). Isolation of a new phospholipid, phosphatidyl-*N*-(2-hydroxyethyl)-alanine from rumen protozoa. *Biochemical Journal*, **113**, 555–558.

Kemp, P. and Dawson, R. M. C. (1969b). Characterization of *N*-(2-hydroxyethyl)-alanine as a component of a new phospholipid isolated from rumen protozoa. *Biochimica et Biphysica Acta*, **176**, 678–679.

Klopfenstein, T. J., Purser, D. B. and Tyznik, W. T. (1964). Influence of aureomycin on rumen metabolism and blood metabolites. *Journal of Animal Science*, **23**, 490–495.

Klopfenstein, T. J., Purser, D. B. and Tyznik, W. J. (1966). Effects of defaunation on feed digestibility, rumen metabolism and blood metabolites. *Journal of Animal Science*, **25**, 765–773.

Kofoid, C. A. and MacLennan, R. F. (1933). Ciliates from *Bos indicus* Linn. III. *Epidinium* Crawley, *Epiplastron* gen. nov., and *Ophryoscolex* Stein. *University of California Publications in Zoology*, **39**, 1–33.

Krogh, N. (1959). Studies on alterations in the rumen fluid of sheep, especially concerning the microbial composition, when readily available carbohydrates are added to the food. 1. Sucrose. *Acta Veterinaria Scandinavica*, **1**, 74–97.

Kurihara, Y., Eadie, J. M., Hobson, P. N. and Mann, S. O. (1968). Relationship between bacteria and ciliate protozoa in the sheep rumen. *Journal of General Microbiology*, **51**, 267–288.

Kurihara, Y., Takechi, T. and Shibata, F. (1978). Relationship between bacteria and ciliate protozoa in the rumen of a sheep fed a purified diet. *Journal of Agricultural Science* (*Cambridge*), **90**, 373–382.

Latham, M. J., Storry, J. E. and Sharpe, M. E. (1972). Effect of low-roughage diets on the microflora and lipid metabolism in the rumen. *Applied Microbiology*, **24**, 871–877.

Latham, M. J., Brooker, B. E., Pettipher, G. L. and Harris, P. J. (1978a). *Ruminococcus flavefaciens* cell coat and adhesion to cotton cellulose and to cell walls of perennial ryegrass (*Lolium perenne*). *Applied and Environmental Microbiology*, **35**, 156–165.

Latham, M. J., Brooker, B. E., Pettipher, G. L. and Harris, P. J. (1978b). Adhesion of *Bacteroides succinogenes* in pure culture and in the presence of *Ruminococcus flavefaciens* to cell walls in leaves of perennial ryegrass (*Lolium perenne*). *Applied and Environmental Microbiology*, **35**, 1166–1173.

Leng, R. A. (1976). Factors influencing net protein production by the rumen microbiota. *Reviews in Rural Science*, **2**, 85–91.

Lough, A. K. (1968). Component fatty acids of plasma lipids of lambs with and without rumen ciliate protozoa. *Proceedings of the Nutrition Society*, **27**, 30A.

Lubinsky, G. (1955a). On some parasites of parasitic protozoa. I. *Sphaerita hoari* sp. n.—a chytrid parasitizing *Eremoplastron bovis*. *Canadian Journal of Microbiology*, **1**, 440–450.

Lubinsky, G. (1955b). On some parasites of parasitic protozoa. II. *Sagittospora cameroni* gen. n., sp.n.—a phycomycete parasitizing Ophryoscolecidae. *Canadian Journal of Microbiology*, **1**, 675–684.

Mackie, R. I., Gilchrist, F. M. C., Robberts, A. M., Hannah, P. E. and Schwartz, H. M. (1978). Microbiological and chemical changes in the rumen during stepwise adaptation of sheep to high concentrate diets. *Journal of Agricultural Science* (*Cambridge*), **90**, 241–254.

MacLennan, R. F. (1933). The pulsatory cycle of the contractile vacuoles of Ophryoscolecidae, ciliates from the stomach of cattle. *University of California Publications in Zoology*, **39**, 205–250.

Mah, R. A. (1964). Factors influencing the *in vitro* culture of the rumen ciliate *Ophryoscolex purkynei* (Stein). *Journal of Protozoology*, **11**, 546–552.

Mah, R. A. and Hungate, R. E. (1965). Physiological studies on the rumen ciliate, *Ophryoscolex purkynei* (Stein). *Journal of Protozoology*, **12**, 131–136.

Males, J. R. and Purser, D. B. (1970). Relationship between rumen ammonia levels and the microbial population and volatile fatty acid proportions in faunated and defaunated sheep. *Applied Microbiology*, **19**, 485–490.

McAllan, A. B. and Smith, R. H. (1973). Degradation of nucleic acid derivatives by rumen bacteria *in vitro*. *British Journal of Nutrition*, **29**, 467–474.

McIntosh, J. T. and Cockrem, F. R. M. (1977). Genetics of the susceptibility to bloat in cattle. II. Preliminary results from saliva samples from cows of high and low susceptibility. *New Zealand Journal of Agricultural Research*, **20**, 263–268.

McNaught, M. L., Owen, E. C., Henry, K. M. and Kon, S. K. (1954). The utilization of non-protein nitrogen in the bovine rumen. 8. The nutritive value of the proteins of preparations of dried rumen bacteria, rumen protozoa and brewers' yeast for rats. *Biochemical Journal*, **56**, 151–156.

Merricks, D. L. and Salisbury, R. L. (1974). Involvement of vitamin B_6 in the dethiomethylation of methionine by rumen microorganisms. *Applied Microbiology*, **28**, 106–111.

Michalowski, T. (1975). The effects of certain feedingstuffs on rumen ciliate protozoa *in vitro*. *Journal of Agricultural Science* (*Cambridge*), **85**, 151–158.

Michalowski, T. (1977). Diurnal changes in concentration of rumen ciliates and in occurrence of dividing forms in water buffalo (*Bubalus bubalus*) fed once daily. *Applied and Environmental Microbiology*, **33**, 802–804.

Michalowski, T. and Muszynski, P. (1978). Diurnal variations in numbers of ciliate protozoa in the rumen of sheep fed once and twice daily. *Journal of Agricultural Science* (*Cambridge*), **90**, 1–5.

Mishra, B. D. (1964). Ph.D. Thesis, Kansas State University—quoted by Bartley *et al.* (1975).

Moir, R. J. and Somers, M. (1956). A factor influencing the protozoal population in sheep. *Nature* (*London*), **178**, 1472.

Naga, M. A. and El-Shazly, K. (1968). The metabolic characterization of the ciliate protozoon *Eudiplodinium medium* from the rumen of buffalo. *Journal of General Microbiology*, **53**, 305–315.

Noirot-Timothée, C. (1960). Étude d'une familie dés ciliés: les "Ophryoscolecidae". Structures et ultrastructures. *Annales des Science Naturelles, Zoologie et biologie animale*, 12^{e} *Serie*, **11**, 533–718.

Nolan, J. V. (1975). Quantitative models of nitrogen metabolism in sheep. *In* "Digestion and Metabolism in the Ruminant" (I. W. McDonald and A. C. I. Warner, eds.), pp. 416–431. University of New England Publishing Unit, Armidale.

Onodera, R. and Kandatsu, M. (1968). Amino acids and protein metabolism of rumen ciliate protozoa. 1. Consumption of amino acids. *Japanese Journal of Zootechnical Science*, **39**, 206–211.

Onodera, R. and Kandatsu, M. (1969). Occurrence of L-(−)pipecolic acid in the culture medium of rumen ciliate protozoa. *Agricultural and Biological Chemistry*, **33**, 113–115.

Onodera, R. and Kandatsu, M. (1970a). Amino acid and protein metabolism of rumen ciliate protozoa. IV. Metabolism of casein. *Japanese Journal of Zootechnical Science*, **41**, 307–313.

Onodera, R. and Kandatsu, M. (1970b). Amino acid and protein metabolism of rumen ciliate protozoa. VI. Endogenous nitrogenous compounds of rumen ciliates. *Japanese Journal of Zootechnical Science*, **41**, 349–357.

Onodera, R. and Kandatsu, M. (1974). Formation of lysine from α,ε-diaminopimelic acid and negligible synthesis of lysine from some other precursors by rumen ciliate protozoa. *Agricultural and Biological Chemistry*, **38**, 913–920.

Onodera, R., Shinjo, T. and Kandatsu, M. (1974). Formation of lysine from α-ε-diaminopimelic acid contained in rumen bacterial cell walls by rumen ciliate protozoa. *Agricultural and Biological Chemistry*, **38**, 921–926.

Onodera, R., Tsutsumi, W. and Kandatsu, M. (1977). Formation of δ-aminovaleric acid from proline, ornithine and arginine by rumen ciliate protozoa. *Agricultural and Biological Chemistry*, **41**, 2169–2176.

Orpin, C. G. and Hall, F. J. (1977). Attachment of the rumen holotrich protozoon, *Isotricha intestinalis* to grass particles. *Proceedings of the Society for General Microbiology*, **4**, 82–83.

Orpin, C. G. and Letcher, A. J. (1978). Some factors controlling the attachment of rumen holotrich protozoa *Isotricha intestinalis* and *I. prostoma* to plant particles *in vitro*. *Journal of General Microbiology*, **106**, 33–40.

Owen, R. W. and Coleman, G. S. (1976). The cultivation of the rumen ciliate *Entodinium longinucleatum*. *Journal of Applied Bacteriology*, **41**, 341–344.

Owen, R. W. and Coleman, G. S. (1977). The uptake and utilization of bacteria, amino acids and carbohydrates by the rumen ciliate *Entodinium longinucleatum* in relation to the sources of amino acids for protein synthesis. *Journal of Applied Bacteriology*, **43**, 67–74.

Oxford, A. E. (1955). The bacteriology and protozoology of ruminant digestion. *Journal of the Science of Food and Agriculture*, **6**, 413–418.

Oxford, A. E. (1958). Bloat in cattle. IX. Some observations on the culture of the cattle rumen ciliate *Epidinium ecaudatum* Crawley occurring in quantity in cows fed on red clover (*Trifolium pratense* L.). *New Zealand Journal of Agricultural Research*, **1**, 809–824.

Pilgrim, A. F., Gray, F. V., Weller, R. A. and Belling, C. B. (1970). Synthesis of microbial protein from ammonia in the sheep's rumen and the proportion of dietary nitrogen converted into microbial nitrogen. *British Journal of Nutrition*, **24**, 589–598.

Poljansky, G. and Strelkow, A. (1938). Étude expérimentale sur la variabilité de quelques Ophryoscolécidés. *Archives de Zoologie Expérimentale et Générale*, **80**, 1–123.

Pounden, W. D. and Hibbs, J. W. (1950). The development of calves raised without protozoa and certain other characteristic rumen microorganisms. *Journal of Dairy Science*, **33**, 639–644.

Prins, R. A. (1977). Biochemical activities of gut micro-organisms. *In* "Microbial Ecology of the Gut" (R. T. J. Clarke and T. Bauchop, eds.), pp. 73–183. Academic Press, London and New York.

Prins, R. A. and Van Hoven, W. (1977). Carbohydrate fermentation by the rumen ciliate *Isotricha prostoma*. *Protistologica*, **13**, 549–556.

Purser, D. B. (1961). A diurnal cycle for holotrich protozoa of the rumen. *Nature (London)*, **190**, 831–832.

Purser, D. B. and Moir, R. J. (1959). Ruminal flora studies in the sheep. IX. The effect of pH on the ciliate population of the rumen *in vivo*. *Australian Journal of Agricultural Research*, **10**, 555–564.

Purser, D. B. and Weiser, H. H. (1963). Influence of time of addition of antibiotic on the *in vitro* life of rumen holotrich protozoa. *Nature (London)*, **200**, 290.

Purser, D. B., Klopfenstein, T. J. and Cline, J. H. (1965). The influence of tylosin and aureomycin upon rumen metabolism and the microbial population. *Journal of Animal Science*, **24**, 1039–1044.

Reid, C. S. W., Clarke, R. T. J., Cockrem, F. R. M., Jones, W. T., McIntosh, J. T. and Wright, D. E. (1975). Physiological and genetical aspects of pasture (legume) bloat. *In* "Digestion and Metabolism in the Ruminant" (I. W. McDonald and A. C. I. Warner, eds.), pp. 524–536. University of New England Publishing Unit, Armidale.

Rufener, W. H. Jr., Nelson, W. O. and Wolin, M. J. (1963). Maintenance of rumen microbial population in continuous culture. *Applied Microbiology*, **11**, 196–201.

Schumacher, I. C. (1915). On *Blepharocorys equi* sp. nov., a new ciliate from the caecum of the horse. *University of California Publications in Zoology*, **16**, 95–106.

Schwartz, H. M. and Gilchrist, F. M. C. (1975). Microbial interactions with the diet and the host animal. *In* "Digestion and Metabolism in the Ruminant" (I. W. McDonald and A. C. I. Warner, eds.), pp. 165–179. University of New England Publishing Unit, Armidale.

Sénaud, J., Jouany, J.-P., Grain, T. and de Puytorac, P. (1973). Dynamique d'une population de *Polyplastron multivesiculatum* (Cilié, Oligotriche) en equilibre dans le rumen de mouton. *Comptes Rendus de l'Académie des Sciences de Paris*, **277**, 197–200.

Shorland, F. B., Weenink, R. O., Johns, A. T. and McDonald, I. R. C. (1957). The effect of sheep-rumen contents on unsaturated acids. *Biochemical Journal*, **67**, 328.

Smith, R. H. (1969). Nitrogen metabolism and the rumen. *Journal of Dairy Research*, **36**, 313–331.

Strelkow, A., Poljansky, G. and Issakowa-Keo, M. (1933). Ueber die Infektionswege der im Pansen und in der Haube der Wiederkaüer befindlichen Infusorien. *Archiv für Tierernährung und Tierzucht*, **9**, 679–697.

Sugden, B. (1953). The cultivation and metabolism of oligotrich protozoa from the sheep's rumen. *Journal of General Microbiology*, **9**, 44–53.

Sugden, B. and Oxford, A. E. (1952). Cultural studies with holotrich ciliate protozoa of the sheep's rumen. *Journal of General Microbiology*, **7**, 145–153.

Van der Wath, J. G. and Myburgh, S. J. (1941). Studies on the alimentary tract of merino sheep in South Africa. VI. The role of infusoria in ruminal digestion with some remarks on ruminal bacteria. *Onderstepoort Journal of Veterinary Science*, **17**, 61–85.

Van Hoven, W. and Prins, R. A. (1977). Carbohydrate fermentation by the rumen ciliate *Dasytricha ruminantium*. *Protistologica*, **13**, 599–606.

Wakita, M. and Hoshino, S. (1975). A branched chain amino acid aminotransferase from the rumen ciliate genus *Entodinium*. *Journal of Protozoology*, **22**, 281–285.

Wallis, O. C. and Coleman, G. S. (1967). Incorporation of ^{14}C-labelled components of *Escherichia coli* and of amino acids by *Isotricha intestinalis* and *Isotricha prostoma* from the sheep rumen. *Journal of General Microbiology*, **49**, 315–323.

Warner, A. C. I. (1962). Some factors influencing the rumen microbial population. *Journal of General Microbiology*, **28**, 129–146.

Warner, A. C. I. (1966a). Diurnal changes in the concentrations of micro-organisms in the rumens of sheep fed limited diets once daily. *Journal of General Microbiology*, **45**, 213–235.

Warner, A. C. I. (1966b). Periodic changes in the concentration of micro-organisms in the rumen of sheep fed a limited ration every three hours. *Journal of General Microbiology*, **45**, 237–241.

Warner, A. C. I. (1966c). Diurnal changes in the concentration of micro-organisms in the rumens of sheep fed to appetite in pens or at pasture. *Journal of General Microbiology*, **45**, 243–251.

Weineck, E. (1934). Die Celluloseverdauung bei den Ciliaten des Wiederkäuermagens. *Archiv für Protistenkunde*, **82**, 169–202.

Weller, R. A. and Pilgrim, A. F. (1974). Passage of protozoa and volatile fatty acids from the rumen of the sheep and from a continuous *in vitro* fermentation system. *British Journal of Nutrition*, **32**, 341–351.

Weller, R. A., Gray, F. V. and Pilgrim, A. F. (1958). The conversion of plant nitrogen to microbial nitrogen in the rumen of the sheep. *British Journal of Nutrition*, **12**, 421–429.

Westphal, A. (1934). Studien über Ophryoscoleciden in der Kultur. *Zeitschrift für Parasitenkunde*, **18**, 71–117.

White, R. W. (1969). Viable bacteria inside the rumen ciliate *Entodinium caudatum*. *Journal of General Microbioolgy*, **56**, 403–408.

Williams, A. G. and Harfoot, C. G. (1976). Factors affecting the uptake and metabolism of soluble carbohydrates by the rumen ciliate *Dasytricha ruminantium* isolated from ovine rumen contents by filtration. *Journal of General Microbiology*, **96**, 125–136.

Williams, P. P. and Dinusson, W. E. (1973). Ruminal volatile fatty acid concentrations and weight gains of calves reared with and without ruminal ciliated protozoa. *Journal of Animal Science*, **36**, 588–591.

Williams, P. P., Davis, R. E., Doetsch, R. N. and Gutierrez, J. (1961). Physiological studies of the rumen ciliate *Ophryoscolex caudatus* Eberlein. *Applied Microbiology*, **9**, 405–409.

Williams, P. P., Gutierrez, J. and Davis, R. E. (1963). Lipid metabolism of rumen ciliates and bacteria. 2. Uptake of fatty acids and lipid analysis of *Isotricha intestinalis* and rumen bacteria with further information on *Entodinium simplex*. *Applied Microbiology*, **11**, 260–264.

Williams, V. J. and McKenzie, D. D. S. (1965). The absorption of lactic acid from the reticulo-rumen of the sheep. *Australian Journal of Biological Sciences*, **18**, 917–934.

Wolska, M. (1967). Study on the family Blepharocorythidae Hsiung. II. *Charonina ventriculi* (Jameson). *Acta Protozoologica*, **4**, 279–283.

Wright, D. E. (1959). Hydrogenation of lipids by rumen protozoa. *Nature* (*London*), **184**, 875–876.

Wright, D. E. (1961). Bloat in cattle. XX. Lipase activity of rumen microorganisms. *New Zealand Journal of Agricultural Research*, **4**, 216–223.

Yoder, R. D., Trenkle, A. and Burroughs, W. (1966). Influence of rumen protozoa and bacteria upon cellulose digestion *in vitro*. *Journal of Animal Science*, **25**, 609–612.

Youssef, F. G. and Allen, D. M. (1968). Part played by ciliate protozoa in rumen function. *Nature* (*London*), **217**, 777–778.

Numerical Analysis of Enzyme Polymorphism: A New Approach to the Epidemiology and Taxonomy of Trypanosomes of the Subgenus *Trypanozoon*

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I. Introduction

The subgenus *Trypanozoon* contains several kinds of trypanosome which are morphologically indistinguishable but differ in their behaviour (see Table 1). At various times specific or subspecific status has been accorded to each kind (see Hoare, 1972), but, in recent years, those cyclically transmitted by tsetse in Africa have been separated into subspecies of *Trypanosoma brucei*, while the others, transmitted non-cyclically outside the African tsetse belts, have retained separate species status. However, although such new terminology may be more correct taxonomically, the practical problems of distinguishing the trypanosomes still remain.

Enzyme electrophoresis is being used on an increasingly wide scale for the intrinsic characterization (Lumsden, 1974) of trypanosomes (Kilgour *et al.*, 1975; Godfrey and Kilgour, 1976; Kilgour and Godfrey, 1973, 1977; Miles *et al.*, 1977, 1978; Gibson *et al.*, 1978; Baker *et al.*, 1978; Godfrey, 1979; Letch, 1979). However, as the number of enzyme systems being used for identification grows, comparison of stocks* becomes increasingly difficult and intricate, particularly when an enzyme occurs in a multibanded form after electrophoresis. Accordingly, after using 12 enzyme systems to characterize 160 *Trypanozoon* stocks, the results were analysed by computer to establish the degree of relatedness of each of the 59 zymodemes† encountered. The analysis is presented here.

II. Characterization Methods

A. Enzyme electrophoresis

1. *Principles and applications*

Since cell proteins are gradually modified by genetic mutation, it is possible to assess the relatedness of organisms by comparing the structure of their homologous proteins. In particular, enzymes are chosen for study since they are easily identified by their substrate specificity. If the different molecular structures of enzymes are associated with different charge, they may be separated in an electric field by electrophoresis; when the matrix for electrophoresis is a molecular-sieving medium such as starch gel, separation by size also occurs.

Enzyme electrophoresis has been widely used in the characterization of parasitic and non-parasitic protozoa, e.g. *Paramecium* (Tait, 1969), *Tetrahymena* (Borden *et al.*, 1973), *Entamoeba* (Reeves and Bischoff, 1968; Sargeaunt *et al.*, 1978), *Plasmodium* (Carter, 1970; Carter and Voller, 1975), *Leishmania* (Chance, 1979), *Eimeria* (Shirley and Rollinson, 1979) and *Trypanosoma* (see Section I). Some of this work has merely demonstrated that enzyme polymorphism occurs in different strains of the protozoa examined,

* Stock, a population derived by serial passage *in vivo* and/or *in vitro* from a primary isolation, without any implication of homogeneity or characterization (WHO, 1978).

† Zymodeme, a population with a unique combination of enzyme patterns (WHO, 1978).

TABLE 1

Distinguishing behavioural features of subgenus Trypanozoon *trypanosomes*[a]

Trypanosome	Mammalian hosts	Disease	Distribution	Transmission	Morphology
T. brucei or T. b. brucei	Not man; wild and domestic animals	Nagana, characterized by anaemia, oedema, emaciation	Tropical Africa	By intermediate host (*Glossina*) in saliva containing infective metacyclic trypanosomes; also to carnivores via fresh, infected carcases	Pleomorphic
T. gambiense or *T. b. gambiense*	Man	Typically chronic human trypanosomiasis = gambian[b] sleeping sickness	West and Central tropical Africa		
T. rhodesiense or *T. b. rhodesiense*	Man, wild and domestic animals	Typically acute human trypanosomiasis = rhodesian[b] sleeping sickness	East tropical Africa		
T. evansi	Camels, equines, dogs and other wild and domestic animals, including vampire bats	Surra, El Debab, Murrina, etc., but same symptoms as Nagana	North Africa, Middle East, Asia, Latin America	By bloodsucking flies, e.g. *Tabanus*, *Stomoxys*, on contaminated proboscis; also via vampire bats and infected carcases	Typically monomorphic
T. equiperdum	Equines	Dourine, characterized by oedematous swellings and skin plaques	Cosmopolitan	During coitus	Typically monomorphic

[a] After Hoare (1972).

[b] Since "rhodesian" and "gambian" do not refer to geographical areas, lower case initial letters are used (after Ormerod, 1967).

without correlating this variation with epidemiological or other biological data.

However, Carter and Voller (1975), by examining the electrophoretic patterns of three enzymes of *Plasmodium falciparum* from African malaria patients, showed that the organisms formed a genetic continuum from East to West Africa, although differences in serology and drug resistance were known to exist. Godfrey and Kilgour (1976) separated *T. b. gambiense* from *T. b. brucei* and *T. b. rhodesiense* by its characteristic aminotransferase pattern. Miles *et al.* (1977) identified two distinct zymodemes of *T. cruzi*, the causative organism of Chagas' disease, circulating in independent sylvatic and domestic (including man) cycles in Brazil; it was later shown that at least three enzymically distinct types of *T. cruzi* occurred in man in Brazil, one of which was that found in the sylvatic cycle described previously (Miles *et al.*, 1978). Sargeaunt *et al.* (1978) differentiated the invasive form of *Entamoeba histolytica*—that causing clinical amoebiasis—from the non-invasive form by electrophoresis of two enzymes; altogether four electrophoretically distinct groups of *E. histolytica* were identified. Al-Taqi and Evans (1978) identified *Leishmania tropica* of both the rural (*L. t. major*) and urban (*L. t. minor*) nosodemes in Kuwait by comparing their enzyme patterns with those of known stocks, while Peters *et al.* (1977) used buoyant density of deoxyribonucleic acid (DNA) and serotype in addition to enzyme patterns to identify possibly man-infective *Leishmania* strains from sandflies and wild mammals.

Enzyme electrophoresis is thus becoming invaluable for epidemiological studies of a variety of parasitic protozoa; the technique allows many samples and many characters to be compared quickly and easily, since each enzyme band is a separate character.

2. *Possible misinterpretations and pitfalls*

The demonstration of structural differences among isofunctional enzymes by electrophoresis depends largely on the molecules having different electrical charges; therefore a substitution of one amino acid for another with an equal charge will not be detected. Harris and Hopkinson (1976) estimated that only a third of possible amino acid substitutions would result in alteration of electrophoretic mobility. Thus two enzymes are not necessarily structurally identical because their electrophoretic mobilities are the same, and consequently two samples are not necessarily from related organisms because they have the same electrophoretic pattern for one enzyme. However, it is reasonable to assume that the more enzymes that are electrophoretically identical between two samples, the greater is the likelihood that the two samples are from related organisms; moreover the greater the number of identities, the closer is the relationship.

Multiple enzyme forms may arise primarily from multiple genetic foci or alleles, or, secondarily, from post-translational changes (Harris and Hopkinson, 1976). Such secondary alterations include enzyme aggregates, enzymes with additional carbohydrate components, conformational isomers,

enzymes bound with coenzymes or non-specifically bound to other cell constituents and enzymes that are the products of digestion by proteolytic enzymes or reaction with other chemicals. Only some of these changes occur in the living cell; others may be artifacts produced by isolation procedures and storage. Thus, in a comparative study, it is important to be aware that electrophoretically distinct enzymes could arise haphazardly, rather than as the products of different genes; on the other hand, structural changes occurring secondarily will depend on the basic amino acid skeleton of the original enzyme, and thus still provide information about the genotype.

For the purpose of this study it was immaterial whether or not the enzyme bands that appeared on the gel truly represented enzymes that occurred *in vivo*. As long as the enzyme results were consistent and repeatable they were considered to be significant, since preparation procedures were uniform. Similarly, the names assigned to these enzymes may not reflect their true biological specificities, since they are based on staining procedures for human enzymes; however, as long as the standard conditions for enzyme action are described, the results and comparisons drawn will remain valid, even if the enzyme nomenclature changes in the future (Miles *et al.*, 1977).

Besides these rather theoretical points, the practical problems of interpretation should be considered. No difficulty arises when comparing enzyme bands that are distinct and well stained, but sometimes bands are diffuse, faint or merge with each other. Probably, if an enzyme often produces poor results on electrophoresis, it is better not used at all in comparative studies. Particular electrophoretic patterns are not always exactly identical on different occasions; perhaps the range of variation possible in a single sample should be assessed before comparison with others is attempted. This is particularly important in judging how great the distance between two bands on a zymogram must be to constitute a distinct difference in enzyme mobility.

In summary, the possible pitfalls and misinterpretations by no means invalidate the findings of enzyme electrophoresis, although it is necessary to recognize the presumptions that are made.

B. OTHER METHODS

Electrophoresis results, although useful, should not be considered in isolation from other criteria. For subgenus *Trypanozoon* there was already a large body of data available, concerning man-infectivity and human plasma resistance, with which the electrophoretic data could be correlated; the value of these data is discussed below, quoting the specific or subspecific terminology used by the previous authors.

Few of the *Trypanozoon* stocks examined here had been typed antigenically and so this potentially valuable method of characterization (see Gray, 1972; Goedbloed *et al.*, 1973; Paris *et al.*, 1976; van Meirvenne *et al.*, 1977) is not considered. Nor is the measurement of DNA buoyant density because, dontrary to earlier findings (Newton and Burnett, 1972), no consistent cifference between *Trypanozoon* trypanosomes has been substantiated (D. C. Barker and B. A. Newton, personal communication).

1. *Infectivity to man*

By the experimental inoculation of volunteers, it was shown that several domestic and wild mammals were naturally infected with human trypanosomes (Denecke, 1941; Heisch *et al.*, 1958; Onyango *et al.*, 1966; Geigy *et al.*, 1975) and that cyclical passage for many years through other animals did not render *T. rhodesiense* non-infective to man (Willett and Fairbairn, 1955).

However, owing to the difficulties in obtaining volunteers for such experiments, usually only one or two could be inoculated with each trypanosome stock, although it was shown that even a known *T. rhodesiense* stock often failed to infect volunteers (Fairbairn and Burtt, 1946; Willett and Fairbairn, 1955). Thus a trypanosome stock that fails to infect one or two volunteers may infect others at later attempts. Moreover, the demonstration of infectivity is not conclusive. The promptness of chemotherapy once trypanosomes are seen in the volunteer's blood masks the possibility of a naturally resolving infection with *T. b. brucei*; such a fleeting infection has been observed (van Hoof, 1947). Unfortunately the only absolute proof that a particular trypanosome stock is either *T. b. gambiense* or *T. b. rhodesiense* is not simply infectivity but that it causes sleeping sickness with central nervous system (CNS) involvement in man. Such testing is out of the question because of the risks and the toxicity of the drugs used for treatment; thus infectivity remains the only criterion that can be examined.

Experiments to test the infectivity to man of virulent trypanosomes like *T. b. rhodesiense* are relatively safe to conduct because of the rapid onset of parasitaemia and illness produced. But with the typically slow-growing *T. b. gambiense*, infection may be difficult to detect and invasion of the CNS may occur insidiously, rendering such experimentation very dangerous.

Thus, considering the inconclusive nature of man-infectivity experiments, the great ethical and practical difficulties involved seem hardly worth surmounting.

2. *Blood incubation infectivity test (BIIT)*

The BIIT was originally designed to differentiate *T. brucei* from *T. rhodesiense* without recourse to human experimentation (Rickman and Robson, 1970). The test was based on the earlier observations of other workers that human blood or plasma had a trypanocidal effect on *T. brucei* but not *T. rhodesiense*, which remained infective to laboratory animals (see Targett and Wilson, 1973). With this technique, Rickman and Robson (1970) showed that all 13 stocks of *T. rhodesiense* used were BITT positive, i.e. they retained their infectivity to rats after incubation with human blood, and that all but one of the *T. brucei* (man-tested) stocks used were BIIT negative, i.e. they lost their infectivity to rats after incubation with human blood; the behaviour of the other *T. brucei* stock was equivocal, with one positive and one negative BIIT result.

The BIIT appeared to be a useful tool in the search for animal reservoir hosts of *T. b. rhodesiense* and was widely used. Geigy *et al.* (1971) and Mwambu and Mayende (1971) isolated BIIT-positive trypanosomes from

hyena, lion, waterbuck, hartebeest and cattle in the Musoma District, Tanzania.* Robson *et al.* (1972) identified cattle, sheep and reedbuck as reservoir hosts of *T. rhodesiense* in the Lambwe Valley, Kenya, and one BIIT-positive stock from a cow was later shown to be infective to a volunteer.

However, increasing numbers of BIIT-equivocal stocks were found and workers began to examine the BIIT more closely.

Targett and Wilson (1973) thought that passage through abnormal hosts might cause loss of resistance to human blood, since only five of nine laboratory stocks of *T. b. rhodesiense* were consistently BIIT positive. A similar result was obtained by Fairbairn (1933a), who found that, although most of the 64 *T. rhodesiense* stocks used were resistant to human serum, this resistance was a variable quality that disappeared and reappeared with passage.

Remarkably inconsistent BIIT results were obtained using mixed infections of known plasma-resistant and plasma-sensitive trypanosomes (Rickman and Robson, 1974; Geigy *et al.*, 1975); this probably explained the equivocal BIIT results produced by some fresh isolates from wild animals which might not have been pure *T. b. rhodesiense* (Baker and McConnell, 1973; Rickman and Robson, 1974). Geigy *et al.* (1975) found that a proportion of less than 20% plasma-resistant trypanosomes could not be detected in the BIIT.

Hawking (1973, 1976a, b) proposed that a spectrum or continuum of serum resistance should be expected, since any trypanosome stock may be composed of a mixture of serum-resistant and serum-sensitive individuals; his concern was to decide where the line should be drawn between stocks potentially pathogenic and those harmless to man. Therefore he rigorously standardized the test and gave it a statistical basis by using groups of five mice instead of a single rat (Hawking, 1973). Non-specific killing of trypanosomes during incubation, as reported by Rickman and Robson (1970) and Targett and Wilson (1973), was reduced by minimizing incubation time, a practical proposition since the main trypanocidal effect of human plasma is exerted *in vivo* (Hawking, 1973; Geigy *et al.*, 1975). Hawking (1976a) proposed that a stock should be designated 'sensitive' if all trypanosomes lost their infectivity to rodents after exposure to human plasma and 'highly resistant' if all trypanosomes retained infectivity; but, if the prepatent period was longer in the test rodents than in the controls or if not all test rodents became infected, the stock should be designated 'subresistant'.

Unfortunately, few workers seem to have put these recommendations into practice and most continued to use the BIIT method described by Rickman and Robson (1970).

The BIIT was brought into further disrepute by the discovery that different cloned antigenic variants of the same stock gave widely divergent results in the BIIT (van Meirvenne *et al.*, 1976; Rickman, 1977). This change in serum resistance with antigenic variation would explain the change in BIIT response induced by rodent passage (Fairbairn, 1933a) and the finding of Geigy *et al.* (1975) that trypanosomes from the first peak of infection were more likely to give a negative BIIT result than trypanosomes from relapse populations.

* Scientific names of wild animals are given in Table 2.

Doubt on the relationship of BIIT results and demonstrable human infectivity also stemmed from the work of Fairbairn (1933b), who was able to infect himself with a *T. rhodesiense* stock that had been maintained in rats for nearly a year and was very susceptible to his own serum *in vitro*. Moreover, Geigy *et al.* (1975) failed to infect a volunteer with a plasma-resistant clone of a known man-infective hartebeest stock, while, similarly, Baker and McConnell (1973) also failed with a BIIT-positive bushbuck stock. In addition, many stocks of *T. lewisi*, *T. vivax* and *T. congolense* are highly resistant to human plasma, although these trypanosomes rarely infect man (Hawking, 1976b).

However, several successful infections of volunteers with BIIT-positive stocks were reported (Robson *et al.*, 1972; Geigy *et al.*, 1975) and most *Trypanozoon* stocks isolated from man are BIIT positive (Fairbairn, 1933a; Rickman and Robson, 1970; Hawking, 1976b). Thus, although individual BIIT results can be difficult to interpret, it is likely that most plasma-resistant *Trypanozoon* stocks are infective to man (Hawking, 1976b).

The way in which human blood exerts its trypanocidal effect is not known; the effect has variously been ascribed to immunoglobulin M (IgM), α_2 macroglobulins and high-density lipoprotein (Aaronovitch and Terry, 1972; Hawking *et al.*, 1973; Rifkin, 1978).

III. The Survey

A. Collection of data

1. *Enzyme electrophoresis*

Some 160 *Trypanozoon* stocks of diverse origins were surveyed using enzyme electrophoresis on thin-layer starch-gel as a means of intrinsic characterization; stock histories are contained in Tables 2 and 3. The following soluble enzymes from lysed bloodstream trypanosomes were examined: EC 2.6.1.2, alanine aminotransferase (ALAT); EC 2.6.1.1, aspartate aminotransferase (ASAT); EC 5.3.1.9, glucose phosphate isomerase (GPI); EC 2.7.5.1, phosphoglucomutase (PGM); EC 1.1.1.40, "malic" enzyme (ME); EC 1.1.1.37, malate dehydrogenase (MDH); EC 1.1.1.42, isocitrate dehydrogenase (ICD); EC 1.2.1.12, glyceraldehyde phosphate dehydrogenase (GAPDH); EC 1.1.1.103, threonine dehydrogenase (TDH); EC 3.2.2.1, nucleoside hydrolase (NH); EC 3.4.11, two peptidases: substrate L-leucylglycylglycine (PEP 1), L-leucyl-L-alanine (PEP 2).

Techniques for the preparation of trypanosome lysates and for electrophoresis were as described by Gibson *et al.* (1978), with the following modifications: (a) in addition to white TO mice, adult male and female Wistar and multimammate rats (*Rattus* (*Mastomys*) *natalensis*) were used for trypanosome passage; (b) it was not necessary to immunosuppress rodent hosts of fast-growing *Trypanozoon* stocks; (c) the electrophoretic method for PEP 2 was the same as that for PEP 1, except for the substitution of L-leucyl-L-alanine for L-leucylglycylglycine in the developing solution.

2. *Results*

The electrophoresis results for all stocks are given in Table 4; each stock was assigned to a particular zymodeme according to its combination of electrophoretic patterns for the 12 enzymes. All patterns encountered are shown diagrammatically in Fig. 1, except for those of GAPDH and NH, which are shown in Figs 2 and 15.

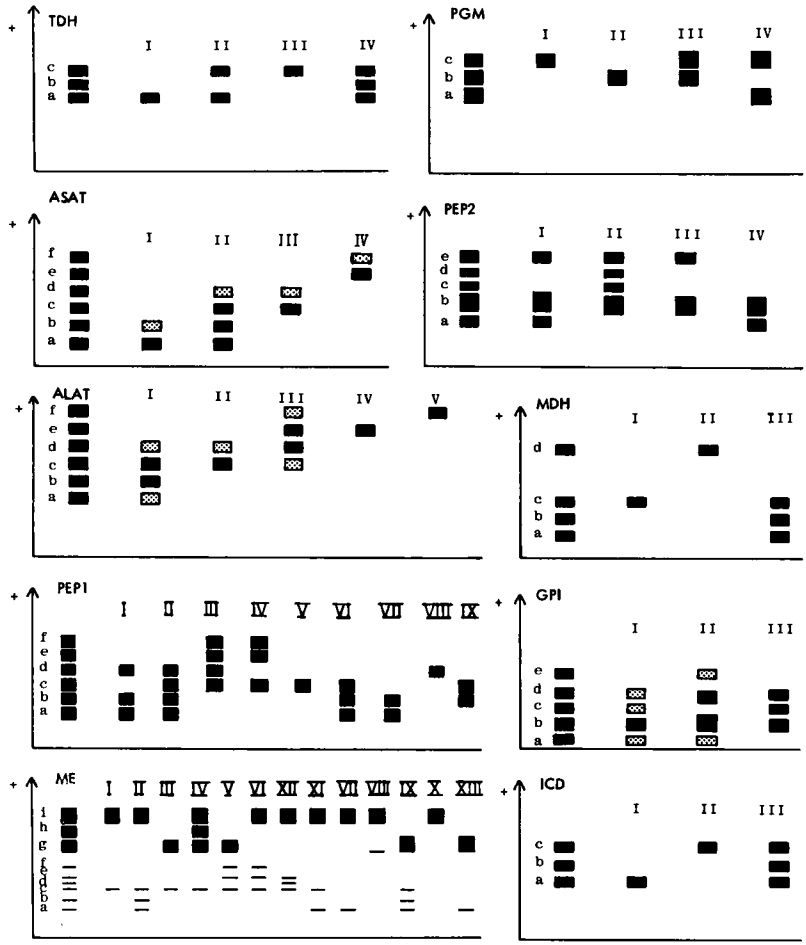


FIG. 1. Diagram of enzyme patterns of subgenus *Trypanozoon*; for GAPDH and NH see Figs 2 and 15. Each enzyme band is designated as a unit character by a lower case letter; each pattern, i.e. combination of bands, is designated by a roman numeral. Bands shown dotted were usually faint and the following bands may vary considerably in intensity: ALAT a and d, PEP 2 a and e, ICD a, all PEP 1 bands. Only the bands shown were considered in computer analysis, although other faint bands were seen sometimes. Enzyme names are abbreviated as in the text (section III A 1).

TABLE 2

Histories of stocks examined (other than those from Liberia)

Stock[a]		Primary isolation: Host	Locality	Year	P[b]	Other details[c]	Z[d]
ZAMBIA							
056	WEO line	Man—Esinery Sakala	Luwembe	1972	3		1
078	WEO line	Man—Margeret Phiri	Luwembe	1972	2		1
080	WEO line	Man—Yemi Daka	Luwembe	1972	2		1
082	WEO line	Man—Senti Ngandu	Luwembe	1972	2		1
TDRN 15	Clone	Man—Geoffrey Tembo	Luwembe	1978	–		1
072	WEO line	Man—Witness Mangoma	Isoka	1972	2		5
073	WEO line	Man—Milium Nakamba	Isoka	1972	2		2
074	WEO line	Man—Marina Nachinga	Isoka	1972	2		2
075	WEO line	Man—Mwaka Mwampashi	Isoka	1972	2		2
057	WEO line	Man—David Sinkala	Isoka	1974	2		3
058	WEO line	Man—Muwila Tayi	Kasempa	1974	2		2
059	WEO line	Man—Luka Kasonga	Kasempa	1974	1		4
076	WEO line	Man—Ndildo Abel	Chirundu	1972	2		3
TDRN 34		Man—Isabi Sebiyo	Mpongwe District W. of Ndola	1978	–		1
TDRN 21		Man—Nelia Mwale	Chipata	1971	–		4
TDRN 35		Man—Rachel Mwanza	Kakumbi	1978	–	BIIT equivocal (L. R. Rickman, personal communication)	3
HTD 2		Man—European	East or South Province	1977	2	From Italian "big game hunter" on safari	5
CRS 19		Hippopotamus *Hippopotamus amphibius*	Luangwa Valley	1971	1	BIIT −ve (Dillman and Awan, 1972)	2
CRS 21		*Hippopotamus amphibius*	Luangwa Valley	1971	1	BIIT −ve (Dillman and Awan, 1972)	6
CRS 22		*Hippopotamus amphibius*	Luangwa Valley	1971	1	BIIT −ve (Dillman and Awan, 1972)	9

CRS 24		*Hippopotamus amphibius*	Luangwa Valley	1971	1	BIIT −ve (Dillman and Awan, 1972)	10
J 10		Hyena *Crocuta crocuta*	Luangwa Valley	1973	1		11
J 11		Hyena *Crocuta crocuta*	Luangwa Valley	1973	1		12
H 3		Lion *Panthera leo*	Luangwa Valley	1974	1		10
H 15		Giraffe *Giraffa camelopardalis*	Luangwa Valley	1974	1		2
H 1		Waterbuck *Kobus ellipsiprymnus*	Luangwa Valley	1974	1		7
H 6		Waterbuck *Kobus ellipsiprymnus*	Luangwa Valley	1974	1		8
H 18	WEO line	Warthog *Phacochoerus aethiopicus*	Luangwa Valley	1974	2	BIIT +ve (A. J. Townsend, personal communication)	7
Liv/79		Man	Livingstone National Park	1979	1		5
BOTSWANA							
0186	TG line	Man—Monaxumo	Maun	1960	7		5
TANZANIA							
0256	WEO line	Man—Bunya	Kitanga River, Kasulu District	1960	–		1
01762	WEO line	Man—Shabani	Tabora/Kahama area	1959	2	Early case; 2 weeks' illness; no trypanosome in CSF (Apted *et al.*, 1963)	1
0259	WEO line	Hartebeest *Alcelaphus lichtensteini*	Tabora/Kahama area	1959	3		15
S-42 clone 1	TG line	Warthog *Phacochoerus aethiopicus*	Serengeti	1966	!	Not infective to 2 volunteers (Baker *et al.*, 1967). Serum-sensitive (S-42: Hawking, 1976b). BIIT +ve (S-42 parent stock, TG line: S. M. Lanham and C. M. Scott, personal communication).	16

TABLE 2 (*continued*)

Stock[a]	Primary isolation: Host	Locality	Year	P[b]	Other details[c]	Z[d]
TANZANIA (*continued*)						
S-102 TG line	Waterbuck *Kobus* sp.	Serengeti	1966	!	Not infective to 1 volunteer; BIIT −ve (LUMP 159: Targett and Wilson, 1973).	17
STIB 214	Hyena *Crocuta crocuta*	Serengeti	1971	1	BIIT −ve (L. Jenni, personal communication	19
STIB 215	Lion *Panthera leo*	Serengeti	1971	1	BIIT −ve (L. Jenni, personal communication).	20
STIB 221	Hartebeest *Alcelaphus buselaphus*	Serengeti	1971	1	BIIT equivocal (L. Jenni, personal communication).	21
STIB 250	Hartebeest *Alcelaphus buselaphus*	Serengeti	1971	1	BIIT +ve (L. Jenni, personal communication).	18
STIB 348 clone	Hartebeest *Alcelaphus buselaphus*	Serengeti	1971	4	BIIT −ve; not infective to 1 volunteer (Geigy *et al.*, 1975)	22
STIB 350 clone	Lion *Panthera leo*	Serengeti	1971	4	BIIT +ve; infected 1 volunteer (Geigy *et al.*, 1975)	18
KENYA						
LUMP 446 TG line	Reedbuck *Redunca redunca*	Lambwe Valley	1970	4	BIIT −ve (parent stock: Robson *et al.*, 1972; LUMP 446; Targett and Wilson, 1973; LUMP 446 TG line: S. M. Lanham and C. M. Scott, personal communication). Serum sensitive (Hawking, 1973). ALAT II, ASAT I (Godfrey and Kilgour, 1976).	24
LUMP 447 TG line	Man—Luka Watega	Wiga, Lambwe Valley	1970	–	BIIT +ve (LUMP 447: Targett and Wilson, 1973). ALAT II, ASAT I (Godfrey and Kilgour, 1976).	23

LUMP 448	TG line	Reedbuck *Redunca redunca*	Lambwe Valley	1970	5	BIIT +ve (parent stock: Robson *et al.*, 1972; LUMP 448: Targett and Wilson, 1973). ALAT II, ASAT I (Godfrey and Kilgour, 1976).	25
Otuoma	TG line	Man—Otuoma	W. Nyokal, Lambwe Valley	1970	–		23
LUMP 227	TG line	Cow	Alego, Central Nyanza	1965	!	Not infective to 1 volunteer (EATRO 839: Onyango *et al.*, 1966). BIIT −ve (LUMP 227: Targett and Wilson, 1973; LUMP 227, TG line: S. M. Lanham and C. M. Scott, personal communication). Serum sensitive (LUMP 227: Hawking, 1976b). ALAT II, ASAT I (Godfrey and Kilgour, 1976).	31
R 21 clone	TG line	Bushbuck *Tragelaphus scriptus*	Sakwa, Central Nyanza	1958	!	Infected 1 volunteer; tryparsamide resistant (Sakwa 21: Heisch *et al.*, 1958). BIIT +ve (EATRO 247: Rickman and Robson, 1970). ALAT II, ASAT I (Godfrey and Kilgour, 1976).	31
LUMP 258		Fly *Glossina pallidipes*	Kiboko	1969	1	Derived from MRC 257; fly K9	26
LUMP 266		Fly *Glossina pallidipes*	Kiboko	1969	1	Derived from MRC 241; fly K4	28
LUMP 277		Fly *Glossina pallidipes*	Kiboko	1969	1	Derived from MRC 244; fly K6	27
LUMP 444		Fly *Glossina pallidipes*	Sindo	1969	6	Derived from MRC 435; fly S5	29
LUMP 449		Fly *Glossina pallidipes*	Sindo	1969	5	Derived from MRC 431; fly S21. BIIT +ve (LUMP 449: C. M. Scott, personal communication).	29
LUMP 450		Fly *Glossina pallidipes*	Sindo	1969	7	Derived from MRC 442; fly S31	29
RWANDA							
LUMP 624		Man—Dr Vardendorpe	Kagera National Park	1971	2		7

TABLE 2 (*continued*)

Stock[a]		Primary Isolation: Host	Locality	Year	P[b]	Other details[c]	Z[d]
RWANDA (*continued*)							
LUMP 626		Man—Verspreeuwen	Kagera National Park	1971	2		30
UGANDA							
113 B	TG line	Man—Mugeni Muzungu	Busoga	1959	!	EATRO SS patient 354A (Robertson, 1960). ALAT I, ASAT I (Godfrey and Kilgour, 1976).	32
118 clone Y	TG line	Man—Mugeni Muzungu	Busoga	1959	!	Mel W relapse of above patient 354A. Patient died.	33
LUMP 1196		Man—Mugeni Muzungu	Busoga	1959	33	From EATRO 174. Mel W relapse as 118 clone Y.	31
LUMP 1197		Man—Mikayiri Obola	Busoga	1959	37	From EATRO 333. EATRO SS patient 314. Late-stage relapse after nitrofurazone (Robertson, 1960).	31
LUMP 1198		Man—Olase Bidonge	Busoga	1976	2	From EATRO 2274. EATRO SS patient 1234.	31
EATRO 427	BNI line	Cow	—	—	!	Plasma sensitive (Mehlitz, 1978).	34
Clone 099	NIMR line	Sheep	—	1960	!		36
B 207	TG line	Fly *Glossina pallidipes*	Lugala, Busoga	1955	!	Lugala 1; not infective to 2 volunteers. BIIT −ve (LUMP 143: Targett and Wilson, 1973).	35
EATRO 1066	BNI line	Fly *Glossina pallidipes*	Lugala, Busoga	1955	!	Plasma sensitive (D. Mehlitz, personal communication).	35
LUMP 63		Fly *Glossina pallidipes*	Lugala, Busoga	1960	27	ETat 1. LUMP 63 derived from TREU 276 (Lumsden and Herbert, 1975).	31

LUMP 139		Fly *Glossina pallidipes*	Lugala, Busoga	1960	56	ETat 10. LUMP 139 derived from TREU 511 which was derived directly from TREU 276 (Lumsden and Herbert, 1975). Infected laboratory worker (Robertson and Pickens, 1975).	31
ETHIOPIA							
Gambela 1	TG line	Man	Gambela, Illubabor Province	1967	16	Presumed late case — died after suramin. Tryparsamide resistant (Baker *et al.*, 1970). BIIT +ve (LUMP 21: Targett and Wilson, 1973). ALAT II, ASAT I Godfrey and Kilgour, 1976).	39
LUMP 997		Man—Ajuno Bom	Gok, Illubabor Province	1968	3	Gambela II. Late case relapse after suramin. BIIT +ve (LUMP 11: Targett and Wilson, 1973).	37
LUMP 1204		Man—Ochalla Onom	Dipa, Illubabor Province	1969	2	Gambela III. Blood +ve, no trypanosome in CSF, sick for 3 days. LUMP 10 BIIT +ve (Targett and Wilson, 1973) and serum resistant (Hawking, 1973). ALAT II, ASAT I (Godfrey and Kilgour, 1976).	37
LUMP 117		Fly *Glossina tachinoides*	Pinybago, Illubabor Province	1970	2	Gambela IV. Tryparsamide resistant; BIIT −ve (Baker and McConnell, 1973). Serum sensitive (EATRO 1714: Hawking, 1976b).	38
LUMP 116		Bushbuck *Tragelaphus scriptus*	Pinybago, Illubabor Province	1970	2	Gambela V. Tryparsamide resistant; BIIT +ve; not infective to 1 volunteer (Baker and McConnell, 1973). Serum subresistant (EATRO 1713: Hawking, 1973) and BIIT −ve (LUMP 116: Rickman and Robson, 1974).	5

TABLE 2 (*continued*)

Stock[a]		Primary Isolation: Host	Locality	Year	P[b]	Other Details[c]	Z[d]
NIGERIA							
B8/18 clone B	TG line	Pig	Nsukka	1962	!	Parent stock not infective to 2 volunteers (Godfrey and Killick-Kendrick, 1967). ALAT II, ASAT I (Godfrey and Kilgour, 1976). GPI I, ME I, G6PD (Bagster and Parr, 1973). BIIT −ve (S. M. Lanham and C. M. Scott, personal communication).	43
Tsuaa clone G	TG line	Man	Tsuaa	1968	!	ALAT I, ASAT I (Godfrey and Kilgour, 1976). Highly plasma resistant (D. Mehlitz, personal communication).	52
Bida 3 clone A	TG line	Man	Bida	1968	!	ALAT I, ASAT II (Godfrey and Kilgour 1976). Highly plasma resistant (Hawking, 1976b; D. Mehlitz, personal communication). Infected German laboratory worker 1977 (D. Mehlitz, personal communication).	53
Bida 2	TG line	Man	Bida	1968	!	Highly plasma resistant (Hawking, 1976b). ALAT II, ASAT II (Godfrey and Kilgour, 1976).	54
Kwang 9A	TG line	Man	Kwang	1967	!	ALAT II, ASAT I (Godfrey and Kilgour, 1976).	55
Gboko	TG line	Man	Gboko	1968	!	ALAT II, ASAT I (Godfrey and Kilgour, 1976).	56
IPIB-68		Horse	Ibadan	1968	–		44
R 10		Cow	Ibadan	1974	–		45

Ib 22		Muturu cow	Hausa village, Benin Province	1977	2	BIIT −ve (S. M. Lanham and C. M. Scott, personal communication).	46
Ib 23		Muturu cow	Hausa village, Benin Province	1977	3	BIIT −ve (S. M. Lanham and C. M. Scott, personal communication).	47
Ib 42		Muturu cow	Hausa village, Benin Province	1977	4	BIIT +ve (S. M. Lanham and C. M. Scott, personal communication); BIIT +ve material examined in this work.	48
Ib 60		Muturu cow	Obanigbe, Benin Province	1977	3	BIIT +ve (S. M. Lanham and C. M. Scott, personal communication); BIIT +ve and parent stocks examined in this work.	49
Ib 73		Muturu cow	Obanigbe, Benin Province	1977	3	BIIT −ve (S. M. Lanham and C. M. Scott, personal communication).	50
Ib 74		Muturu cow	Obanigbe, Benin Province	1977	4	BIIT +ve (S. M. Lanham and C. M. Scott, personal communication); BIIT +ve material examined in this work.	51
53/17	TG line	Camel *Camelus dromedarius*	Mongonu	1974	2		58
53/91	TG line	Camel *Camelus dromedarius*	Mongonu	1974	2		58
ZAIRE							
C-126	BNI line	Man—Bonré Gènérose	Kinshasa	1971	!	Highly plasma resistant (Mehlitz, 1978). Stock changed suddenly during passage from very slow-growing to very virulent; both types examined in this work and found to be identical.	57
THE GAMBIA							
Gb 1		Horse	Dankunku	1977	2		38
Gb 2		Ndama ox	Kantong Kunda	1977	2		42
Gb 3		Ndama/zebu cross	Kantong Kunda	1977	2		42
Gb 4		Ndama/zebu cross	Keneba	1977	2		42
Gb 5		Ndama/zebu cross	Juoli	1977	2		42
Gb 6		Ndama/zebu cross	Kantong Kunda	1977	2		42

TABLE 2 (*continued*)

Stock[a]	Primary isolation: Host	Locality	Year	P[b]	Other details[c]	Z[d]
COLOMBIA, SOUTH AMERICA						
E 2	Dog	Carimagua	1973	!		58
E 5	Capybara *Hydrochoerus hydrochaeris*	Carimagua	1973	!		58
E 8	Capybara *Hydrochoerus hydrochaeris*	Carimagua	1973	!		58
E 9	Horse	Carimagua	1973	!	Also examined as 4 clones 1,2,3,5, prepared by L. E. Ramirez.	58
E 10	Capybara *Hydrochoerus hydrochaeris*	Carimagua	1973	!		58
E 11	Horse	Carimagua	1973	!		58
UNKNOWN						
NIMR 1	Horse	—	1920s	!	Doubtful whether stock is *T. equiperdum* or *T. evansi*	59
Liverpool NIMR line strain/normal	Man	—	1920s	!		13
Liverpool NIMR line strain/stilb.	Man	—	1920s	!	Stilbamidine resistant stock of the above strain created in the 1950s	14

[a] The following abbreviations are used: LUMP, London University Medical Protozoology—stabilate from the World Health Organization reference collection maintained at the Department of Medical Protozoology, London School of Hygiene and Tropical Medicine (LSHTM), England. EATRO, East African Trypanosomiasis Research Organization. TG, Trypanosomiasis Group, LSHTM. WEO, Stabilate from the collection of Dr W. E. Ormerod, LSHTM. BNI, Bernhard-Nocht-Institut, Hamburg, Germany. NIMR, National Institute for Medical Research, London, England. A stock may have several stabilate numbers if it has been stabilated more than once and may be kept in different cryobanks; when a stock is kept in several different institutes, the separate stocks are referred to as "lines" (WHO, 1978). The recommended system (WHO, 1978) for the nomenclature of laboratory trypanosome stocks was not used here, since it makes no provision for the host from which the trypanosomes were isolated.

[b] P, number of passages already received by a stabilate before use in this study. !, old laboratory stock, numerous passages.

[c] Where no reference is quoted, details were taken from TG or LUMP records held by the Department of Medical Protozoology, LSHTM. BIIT, blood incubation infectivity test (see text). CSF, cerebrospinal fluid. SS, sleeping sickness.

[d] Z, number of the zymodeme to which a stock was allocated by its enzyme patterns; enzyme patterns for each group may be found in Table 4 and Fig. 1.

TABLE 3

Liberian stock histories

Stock[a]	Locality	P[b]	BIIT[c]	Z[d]	Stock[a]	Locality	P[b]	BIIT[c]	Z[d]
TSW 3/75[e]	Sehwee	3	+++	40	TSW 154/75	Wenten	2	−	39
TSW 4/75	Sehwee	6	+	41	TSW 155/75	Wenten	4	+++	39
TSW 5/75	Sehwee	6	−	40	TSW 162/75	Wenten	6	+	39
TSW 6/75	Sehwee	6	+	41	TSW 3/77	Compound	2	−	41
TSW 17/75	Sehwee	2	−	41	TSW 9/77	Compound	2	−	41
TSW 19/75	Sehwee	4	+	40	TSW 26/77	Compound	2	−	41
TSW 20/75	Sehwee	9	−	41	TSW 38/77[f]	Sehwee	2	+	40
TSW 23/75	Sehwee	4	−	41	TSW 47/77	Sehwee	2	−	40
TSW 24/75	Bindin	3	−	40	TSW 50/77	Sehwee	2	+	39
TSW 26/75	Bindin	4	−	40	TSW 56/77	Bindin	2	+	41
TSW 27/75	Bindin	7	−	40	TSW 57/77	Bindin	3	+	39
TSW 28/75	Bindin	6	−	41	TSW 58/77	Bindin	2	−	40
TSW 32/75	Bindin	5	+	39	TSW 73/77	Flompa	3	−	39
TSW 34/75	Bindin	4	−	40	TSW 79/77	Flompa	3	−	41
TD Bindin/75	Bindin	5	−	40	TSW 80/77	Flompa	3	−	41
TSW 90/75	Flompa	5	−	41	TSW 85/77	Gbahn	2	+++	41
TSW 92/75	Flompa	3	+	41	TSW 88/77	Gbahn	2	+++	39
TSW 98/75	Flompa	5	−	41	TSW 92/77	Gbahn	2	+	41
TSW 105/75	Flompa	3	−	41	TSW 95/77	Gbahn	2	+++	41
TSW 107/75	Flompa	5	+	41	TSW 106/77	Duoplay	2	+	41
TSW 113/75	Flompa	3	+	41	TSW 107/77	Duoplay	2	+	40
TSW 115/75	Flompa	7	+	41	TSW 124/77	Zianlay	2	+	40
TD 96/75	Flompa	5	+	41	TSW 154/77	Logarto	2	not done	40
TD 109/75	Flompa	3	−	41	TSW 223/77	Dongowa	3	+	41
TD 78/75	Taylorta	5	+++	41	TSW 293/77	Elizabeth Camp	2	−	41
TSW 125/75	Vanplay	3	−	41					

[a] TSW, stock isolated from pig; TD, stock isolated from dog. Stocks isolated in 1975 are indicated by 75 after the number, and similarly for those isolated in 1977.

[b] P, number of passages already received by a stabilate before use in this study.

[c] +++, highly resistant to human plasma. +, subresistant. −, sensitive. Results from Mehlitz (1978), Gibson *et al.* (1978) and D. Mehlitz and U. Zillman (personal communication).

[d] Z, number of zymodeme to which a stock was allocated by its enzyme patterns (see Table 4). 39, Group B (ALAT II); 40, Group A (ALAT I); 41, Group C (ALAT II).

[e] Also examined as two clones, A and B.

[f] Unique ALAT I pattern with bands *c* and *d* missing.

TABLE 4

Enzyme results[a] for all stocks

Zymodeme	Stock[b]	ALAT	ASAT	PGM	ICD	ME	TDH	MDH	GPI	PEP 1	PEP 2	NH
1	*Zh:* 056, 078, 080, 082, TDRN 34, TDRN 15 clone; *Th:* 01762, 0256	II	I	I	I	III	I	I	I	I	I	I
2	*Zh:* 073, 074, 075, 058; *Za:* CRS 19, H 15	II	I	I	I	IV	I	I	I	I	I	I
3	*Zh:* 057, 076, TDRN 35	II	I	I	I	IV	I	I	I	II	I	I
4	*Zh*: 059, TDRN 21	II	I	I	I	I	I	I	I	II	I	I
5	*Zh:* 072, HTD 2, Liv/79, *Bh:* 0186; *Ea:* LUMP 116	II	I	I	I	I	I	I	I	I	I	I
6	*Za:* CRS 21	II	I	I	II	I	III	II	I	III	II	I
7	*Za:* H 1, H 18; *Rh:* LUMP 624	II	I	I	I	II	I	I	I	II	I	I
8	*Za:* H 6	II	I	I	I	V	I	I	I	I	I	I
9	*Za:* CRS 22	II	I	I	II	I	I	II	I	IV	II	I
10	*Za:* CRS 24, H 3	II	I	I	II	I	II	II	I	V	III	I
11	*Za:* J 10	II	I	I	II	VI	II	II	I	V	III	I
12	*Za:* J 11	II	I	I	III	I	I	II	I	IV	II	I
13	*?h:* Liverpool strain/normal	II	I	I	I	IV	I	I	III	VIII	IV	I
14	*?h:* Liverpool strain/stilb.	II	I	I	I	IV	I	I	II	VIII	IV	I
15	*Ta:* 0259	II	I	I	I	VII	I	I	I	I	I	I
16	*Ta:* S-42 clone 1	II	I	III	I	II	I	I	I	I	I	I

17	*Ta:* S-102	II	I	III	III	II	I	I	I	II	I	I
18	*Ta:* STIB 250, 350	II	I	I	III	II	I	I	I	II	I	I
19	*Ta:* STIB 214	II	I	I	III	VIII	IV	I	I	VI	I	II
20	*Ta:* STIB 215	II	I	I	I	VI	IV	I	I	VI	I	II
21	*Ta:* STIB 221	II	I	I	III	VII	I	I	I	VI	I	I
22	*Ta:* STIB 348	II	I	I	III	I	I	I	I	II	I	I
23	*Kh:* LUMP 447, Otuomo	II	I	I	II	II	I	I	I	II	I	I
24	*Ka:* LUMP 446	II	I	I	III	I	I	I	I	VI	IV	I
25	*Ka:* LUMP 448	II	I	I	II	I	I	I	I	II	I	I
26	*Kf:* LUMP 258	II	I	I	I	III	II	I	I	II	I	II
27	*Kf:* LUMP 277	II	I	I	I	III	IV	I	I	II	I	II
28	*Kf:* LUMP 266	II	I	I	I	IX	III	III	I	II	I	II
29	*Kf:* LUMP 444, 449, 450	I	IV	I	II	VIII	I	I	I	VII	I	I
30	*Rh:* LUMP 626	II	I	I	I	II	I	I	I	I	I	I
31	*Uh:* LUMP 1196, 1197, 1198, 63, 139; *Ka:* R 21 clone, LUMP 227	II	I	III	III	I	I	I	I	II	I	I
32	*Uh:* 113 B	I	I	I	II	X	I	I	I	II	I	I
33	*Uh:* 118 clone Y	II	I	III	I	II	I	I	I	II	I	I
34	*Ua:* EATRO 427	II	I	IV	III	VII	I	I	I	II	I	I
35	*Uf:* B 207, EATRO 1066	II	I	IV	III	II	I	I	I	I	I	I
36	*Ua:* Clone 099	II	I	IV	III	VII	I	I	I	VI	I	I
37	*Eh:* LUMP 997, 1204	II	I	I	III	XI	I	I	I	II	I	I
38	*Ef:* LUMP 117; *Ga:* Gb 1	II	I	III	II	I	I	I	I	II	I	I
39	*Eh:* Gambela 1; *La:* group B	II	I	II	II	I	I	I	I	I	I	I

TABLE 4 (*continued*)

Zymodeme	Stock[b]	ALAT	ASAT	PGM	ICD	ME	TDH	MDH	GPI	PEP 1	PEP 2	NH
40	*La:* group A	I	I	II	II	I	I	I	I	I	I	I
41	*La:* group C	II	I	II	II	II	I	I	I	II	I	I
42	*Ga:* Gb2—6	II	I	II	II	II	I	I	I	VI	I	I
43	*Na:* B8/18 clone B	II	I	II	II	I	I	I	I	IX	III	I
44	*Na:* IPIB–68	V	I	II	II	I	I	I	I	I	IV	I
45	*Na:* R 10	IV	I	II	II	I	I	I	I	I	IV	I
46	*Na:* Ib 22	II	I	III	II	XII	I	I	I	II	I	I
47	*Na:* Ib 23	III	I	II	II	XII	I	I	I	II	I	I
48	*Na:* Ib 42	III	I	III	II	I	I	I	I	VI	I	I
49	*Na:* Ib 60	II	I	II	II	XII	I	I	I	VI	I	I
50	*Na:* Ib 73	II	I	II	II	XII	I	I	I	II	I	I
51	*Na:* Ib 74	III	I	III	II	I	I	I	I	II	I	I
52	*Nh:* Tsuaa clone G	I	I	II	II	II	I	I	I	I	IV	I
53	*Nh:* Bida 3 clone A	I	II	II	II	II	I	I	I	I	IV	I
54	*Nh:* Bida 2	II	II	II	II	II	I	I	I	I	IV	I
55	*Nh:* Kwang 9A	II	I	III	I	XIII	I	I	I	I	IV	I
56	*Nh:* Gboko	II	I	II	II	I	I	I	I	VI	IV	I
57	*Ch:* C-126	I	II	II	II	II	I	I	II	I	IV	I
58	*T. evansi*	II	I	II	II	II	I	I	I	VI	IV	I
59	*T. equiperdum*	III	I	II	II	II	I	I	I	VI	IV	I

[a] GAPDH pattern was the same for all stocks (see Fig. 2).

[b] Prefixes: *B*=Botswana; *C*=Zaire; *E*=Ethiopia; *G*=Gambia; *K*=Kenya; *L*=Liberia; *N*=Nigeria; *R*=Rwanda; *T*=Tanzania; *U*=Uganda; *Z*=Zambia; ?=unknown; *a*=animal (non-human mammal); *f*=tsetse fly (*Glossina*); *h*=human.

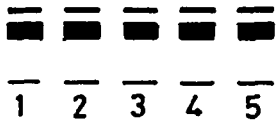


FIG. 2. Diagram of gel stained for glyceraldehyde phosphate dehydrogenase (GAPDH). No variation was found among *Trypanozoon* stocks. Left to right: (1) EATRO 1066; (2) TSW 105/75; (3) LUMP 626; (4) Otuoma; (5) Gambela 1.

3. *Computer analysis*

Zymodemes were coded as shown in Fig. 3, disregarding faint, inconsistent bands, and this information formed the input for the computer program GENSTAT (Rothamsted Experimental Station, 1977).

On the basis of the computed similarity indices (see Fig. 3), the zymodemes were grouped by furthest-neighbour hierarchical cluster analysis; in order to be a member of a cluster, a zymodeme had to be similar, to a stated minimum degree, to every other member of the cluster, i.e. complete linkage (Rothamsted Experimental Station, 1977). The results of cluster analysis were printed out as a dendrogram. Fig. 4A shows the dendrogram obtained using 45 unit characters (disregarding GPI, GAPDH and NH results) and 50 zymodemes (not including the following: C-126; STIB 214, 215, 221, 250, 348, 350; Clone 099; Liverpool *T. rhodesiense* strain); some changes in clustering

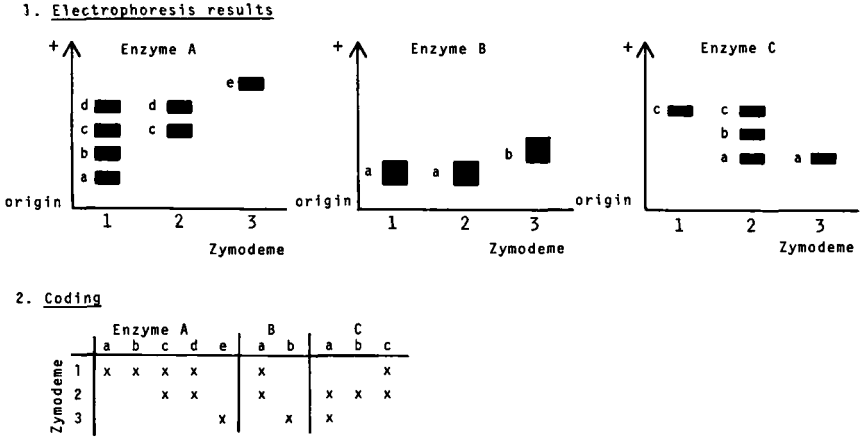
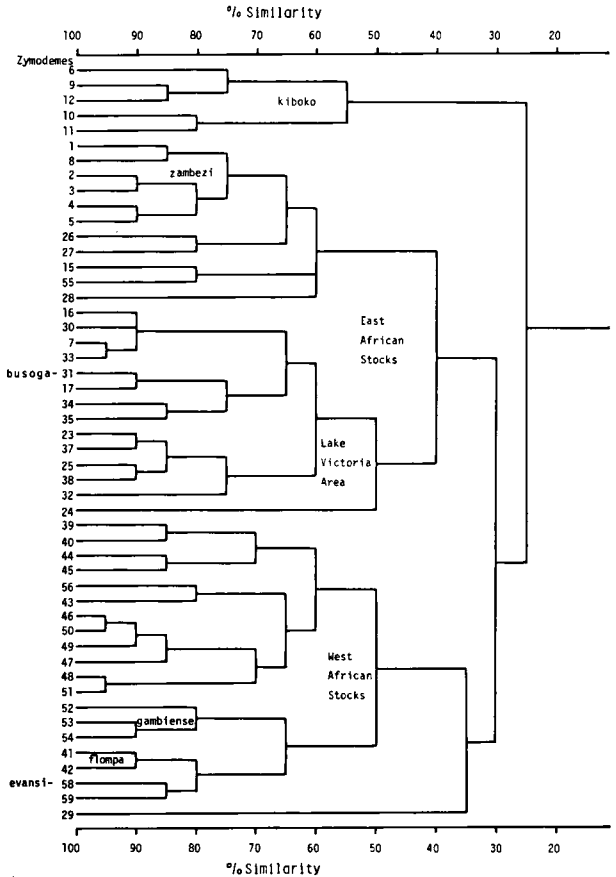


FIG. 3. Diagrammatic representation of the method of coding for computer analysis.

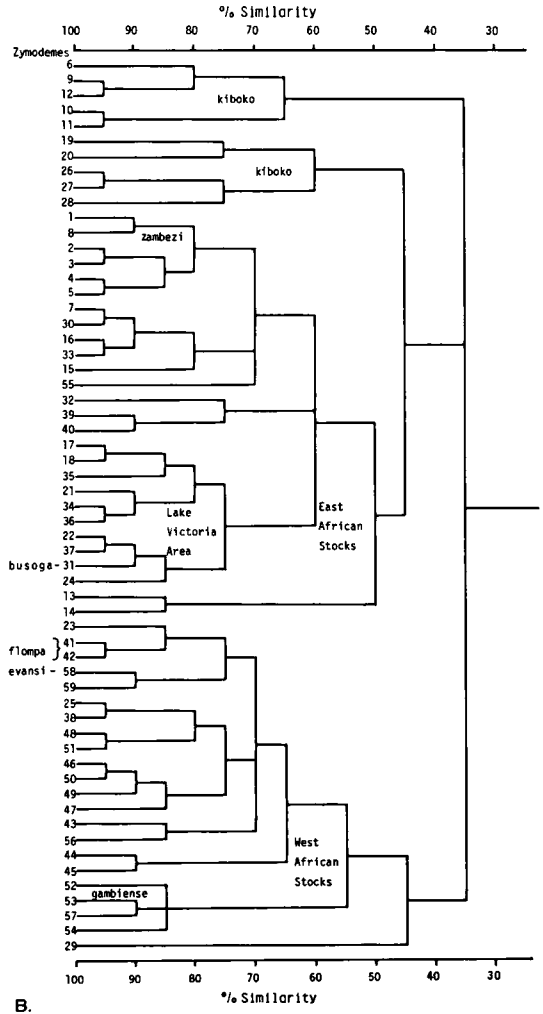
$$\text{Similarity index} = \frac{\text{No. of bands shared by both zymodemes}}{\text{Total no. of bands in either or both zymodemes}}$$

$$\text{i.e. } S = \frac{z}{x+y-z}$$

where S = similarity index, x = no. of bands in zymodeme X, y = no. of bands in zymodeme Y and z = no. of shared bands. Similarity index is expressed as a percentage. Thus in the examples illustrated, similarity index for zymodemes 1 and 2 = 4/8 i.e. 50%, similarity index for zymodemes 1 and 3 = 0/9 i.e. 0%, similarity index for zymodemes 2 and 3 = 1/8 i.e. 12%.



A.



B.

FIG. 4. Dendrograms showing the results of hierarchical cluster analysis: A, with 45 unit characters and 50 zymodemes; B, with 54 unit characters and 59 zymodemes. Notice how the clustering changes with the addition of more data.

patterns occurred when, at a later date, more data—54 unit characters (disregarding GAPDH, for which all stocks had the same pattern) and 59 zymodemes—were used (Fig. 4B).

IV. Interpretation of Results

A. Proposals for a working nomenclature

There can be no doubt, considering the electrophoresis results, that *Trypanozoon* stocks form a homogeneous group. Little (1–5% of all stocks) or no variation between stocks was found for six (ASAT, GPI, MDH, TDH, NH, GAPDH) of the 12 enzymes studied. A comparison of trypanosomes of three salivarian subgenera, *Trypanozoon*, *Nannomonas* and *Duttonella* (albeit, only a single representative of each of the two latter subgenera), showed distinctive electrophoretic patterns for each subgenus for 10 of the 12 enzymes examined. Thus the electrophoresis results agree with the morphological and behavioural separation of the three subgenera, and confirm the current view that *T. brucei*, *T. rhodesiense* and *T. gambiense* are too closely related to deserve separate species status (Ormerod, 1967; Hoare, 1972). In addition, the electrophoretic similarity of *T. evansi* and West African *T. brucei* stocks strongly suggests that *T. evansi* should also be included in this group. Unfortunately, since only one *T. equiperdum* stock (NIMR 1) of doubtful validity was examined, taxonomic revision cannot be extended to this species and it will not be considered further.

Other workers found different stocks of supposedly the same kinetoplastid species to vary considerably. Miles *et al.* (1977) discovered two distinct zymodemes that differed in all six enzymes studied (ASAT, ALAT, GPI, ME, PGM, G6PD) among *T. cruzi* stocks circulating in one district of Brazil, while Al-Taqi and Evans (1978) found five distinct zymodemes that differed in all seven enzymes used (ASAT, ALAT, GPI, ME, PGM, G6PD, MDH) among Kuwaiti stocks of *Leishmania tropica*. These contrasts to the electrophoretic similarity within subgenus *Trypanozoon* would seem to be a further reason for regarding the subgenus as containing only one species, *T. brucei*.

Despite the overall homogeneity of *Trypanozoon* stocks, several groupings differing from the customary ones emerged from the cluster anaysis. In particular, a striking dichotomy existed between East and West African stocks, whether from man or other animals (Fig. 4). This was especially evident in the ICD and PGM electrophoretic patterns (Figs 5 and 6; Table 4), but, also, variation in ALAT and ASAT was largely restricted to West African stocks and variation in MDH and TDH was completely restricted to East African stocks. However, several problems would remain if the one species, *T. brucei*, occurring in tropical Africa was simply divided into two subspecies, one West African and the other East African, both including man and other animals in their host range.

First, the intracontinental division was not perfect: 14 (19%) of the 73 East African stocks examined had a West African ICD pattern and 1 (1·6%) had a West African PGM pattern; 1 (1·4%) of the 74 West African stocks

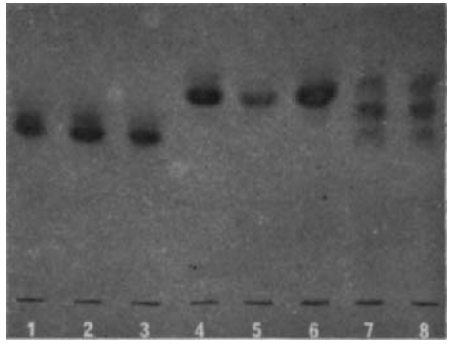


FIG. 5. Isocitrate dehydrogenase, ICD. Left to right: 1–3, East African pattern, ICD I, (1) 0186, (2) 01762, (3) 0256; 4–6, West African pattern, ICD II, (4) E 2, (5) E 5, (6) E 11; 7–8, Lake Victoria area pattern, ICD III, (7) LUMP 1196, (8) LUMP 1197.

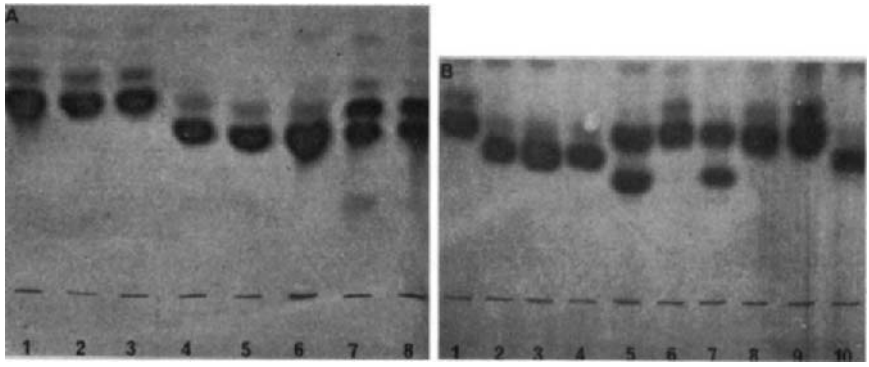


FIG. 6. Phosphoglucomutase, PGM. (A) Left to right: 1–3, East African pattern, PGM I; 4–6, West African pattern, PGM II; 7–8, Lake Victoria area pattern, PGM III; stocks as Fig. 5. (B) Left to right: (1) LUMP 624, PGM I; (2) TSW 28/75, PGM II; (3) TSW 90/75, PGM II; (4) TSW 155/75, PGM II; (5) EATRO 427, PGM IV; (6) LUMP 447, PGM I; (7) EATRO 1066, PGM IV; (8) LUMP 626, PGM I; (9) Otuoma, PGM I; (10) Gambela 1, PGM I.

examined had an East African ICD pattern, although this stock, Kwang 9A, was probably the subject of a laboratory error (see Section IV B 1). More importantly, a further grouping of equivalent rank and several subsidiary clusters are clearly shown in the dendrograms (Fig. 4), and it is likely that other new groupings would emerge from more extensive studies.

Finally, eliminating the taxonomic distinction between trypanosomes infective and non-infective to man in this way could only confuse those involved in the identification of reservoir hosts of the human trypanosomes. On the other hand, the present results here clearly show the present nomenclature to be inadequate, and it would be cowardly not to attempt reclassification.

It is generally thought that the present nomenclature should be retained for the benefit of clinicians and veterinarians (Ford, 1971; Hoare, 1972). However, this argument is invalid for the following reasons.

(a) Most clinicians working today in Africa do not differentiate between *T. b. gambiense* and *T. b. rhodesiense*; whatever the symptoms, the disease is simply diagnosed as rhodesian sleeping sickness if it occurs in East Africa and gambian sleeping sickness if it occurs in West Africa, and the same drugs, Suramin and Mel B, are used for treatment.

(b) There is no reason to doubt that both types of sleeping sickness are zoonoses, but at present there is no effective way to identify trypanosomes not infective to man. Thus the name *T. b. brucei* can never be used with certainty and is better not used at all.

(c) Since it is generally symptomless, *T. brucei* infection is not considered to be an important veterinary problem, except where horses, dogs or camels are concerned, and then the symptoms and treatment are identical with those for *T. evansi* infection.

It is therefore difficult to see what benefit clinicians and veterinarians derive from the present nomenclature and, since there is no other reason for the retention of these confusing names, *T. b. brucei*, *T. b. rhodesiense*, *T. b. gambiense* and *T. evansi* should be united under one name, *T. brucei*.

Obviously, this would be inconvenient for epidemiologists who would then need to use cumbersome descriptions such as "the chronic, man-infective *T. brucei* strain", instead of *T. b. gambiense*, or "the non-cyclically transmitted, non-infective to man *T. brucei* strain" instead of *T. evansi*. However, the names used for these concepts need not have taxonomic significance, as previously suggested by Ormerod (1967); there is already a widely used laboratory jargon in existence for the present *Trypanozoon* species and subspecies, which could easily be extended to newly recognized *Trypanozoon* groups with particular characteristics, e.g. "a gambiense" means a stock of *T. b. gambiense*, "an evansi" means a stock of *T. evansi*, and so on. Further proposals arising from the results of this study are outlined below; the list is not exhaustive, but it is hoped that these suggestions may serve as working definitions for the present.

1. Gambiense—*Definition:* man-infective *T. brucei* from West and Central Africa; ALAT I and/or ASAT pattern other than ASAT I; ICD II. Includes zymodemes 29, 40, 52, 53, 54, 57.

2. Zambezi—*Definition:* man-infective *T. brucei* from East Africa; ALAT II, ASAT I, PGM I, ICD I and ME I, II, III or IV. Includes zymodemes 1–5, 713, 14, 30.

3. Busoga—*Definition:* man-infective *T. brucei* from East Africa; ALAT II, ASAT I, PGM III and/or ICD III. Includes zymodemes 18, 31, 37.

4. Kiboko—*Definition: T. brucei* with TDH pattern other than TDH I and/or an MDH pattern other than MDH 1 and sometimes NH II; (probably not infective to man). Includes zymodemes 6, 9, 10, 11, 12, 19, 20, 26, 27, 28.

5. Flompa—*Definition: T. brucei* from West Africa with ALAT II, ASAT I, PGM II, ICD II, ME II and PEP 2 I; (probably not infective to man). Includes zymodemes 41, 42.

6. Evansi—*Definition: T. brucei* non-infective to man, naturally occurring outside tropical Africa and thus not tsetse transmitted; same electrophoretic characteristics as flompa, except for PEP I VI and PEP 2 IV. Zymodeme 58.

Again, in the following discussions, when the work of previous authors is considered, we shall quote the terms used by them for the various trypanosomes; otherwise, the proposed new terminology will be used. As the new terms do not refer to geographical areas, lower case initial letters should be used, except at the beginning of a sentence (Ormerod, 1967).

B. VALIDITY OF THE GROUPINGS

1. *Gambiense*

This grouping clearly corresponds to the currently accepted concept of *T. b. gambiense*. Opinions on the distinguishing characteristics of *T. b. gambiense* have changed considerably over the years. Originally, *T. gambiense* was named as a new species because, in contrast to *T. brucei* and *T. evansi*, it occurred in man. Later, a further man-infective species, *T. rhodesiense*, was described, which, unlike *T. gambiense*, had posteronuclear forms and was virulent to experimental animals (Stephens and Fantham, 1910; Yorke, 1910). However, posteronuclear forms of *T. gambiense* were soon demonstrated and strains of the parasite were found which caused rapid, heavy parasitaemia in experimental animals (Kleine, 1928; Lester, 1933).

With the first epidemics, the acute nature of rhodesian sleeping sickness compared to the usually chronic course of gambian sleeping sickness became apparent; but patients with acute disease were also observed in areas of gambian sleeping sickness (Kleine, 1928; Lester, 1933; van Hoof, 1947). In addition, mild or symptomless cases often occurred in areas of endemic rhodesian sleeping sickness (Ormerod, 1961; Rickman, 1974).

T. rhodesiense infections of both man and experimental animals were resistant to treatment with the arsenical drug tryparsamide, which readily cured gambian sleeping sickness. However, Lester (1933) and van Hoof (1947) found that tryparsamide resistance varied with different strains of *T. gambiense*, some being as resistant as *T. rhodesiense*.

At first, tsetse flies of the palpalis group were believed to be the only vectors of *T. gambiense*, while only tsetse flies of the morsitans group were believed to transmit *T. rhodesiense;* Willett (1956) went so far as to suggest that this difference in transmission was responsible for the difference in virulence of *T. gambiense* and *T. rhodesiense*, since morsitans-group flies injected a greater dose of infective trypanosomes per bite than did palpalis-group flies. However, palpalis-group flies were found to be transmitting *T. rhodesiense* in the 1964 Alego outbreak in Kenya (Willett, 1965) and probably also in the Gilo river outbreak in Ethiopia (Hutchinson, 1971), and it is now accepted that either or both fly groups may be involved in the transmission of each human trypanosome (Ford, 1971).

From circumstantial evidence (see Ashcroft, 1959) and later by direct proof (Heisch *et al.*, 1958), wild animals were recognized as reservoir hosts of

T. rhodesiense, and rhodesian sleeping sickness as a zoonosis. On the other hand, a vertebrate host of *T. gambiense* other than man was not needed to explain the epidemiology of gambian sleeping sickness, since outbreaks were supposedly initiated by human carriers of this chronic disease, and no such reservoir host was ever demonstrated (Ashcroft, 1963; Molyneux, 1973). This apparent difference between *T. rhodesiense* and *T. gambiense* has been interpreted as the response of one basic species of trypanosome to two different ecologies (Ashcroft, 1963; Ormerod, 1967), so that *T. rhodesiense* occurs in East Africa where wild animals are abundant and man scarce, and *T. gambiense* occurs in West Africa where man is abundant and wild animals scarce. Gibson *et al.* (1978) have now presented evidence that the pig probably harbours *T. b. gambiense* in Liberia.

Thus attempts to characterize *T. b. gambiense* by behaviour and morphology failed.

Intrinsic characterization by measurement of DNA buoyant density also proved ineffective (see section II B), but, using enzyme electrophoresis, Godfrey and Kilgour (1976) showed that *T. b. gambiense* could be identified by certain aminotransferase patterns. Of 16 stocks reputed to be *T. b. gambiense*, 13 had a slow ALAT pattern, ALAT I, and the remaining three stocks had ALAT II, a pattern found to be typical of *T. b. brucei* and *T. b. rhodesiense;* for a second aminotransferase, ASAT, two patterns (ASAT II and III) were found only in *T. b. gambiense* stocks. Two of the stocks without the *T. b. gambiense* ALAT marker, Gboko and Kwang 9A, were dismissed as misidentifications resulting from laboratory errors, since they were virulent for rodents, but the third, Bida 2, was considered to be a serious anomaly; in addition, one *T. b. rhodesiense* stock (113B) had ALAT I. All stocks found to have ALAT I (Fig. 7) in this survey were either suspected or known to cause gambian sleeping sickness. A substantial number of stocks from Liberian pigs and dogs had ALAT I, and, although it might be argued that ALAT I is therefore not exclusive to gambiense and, if enough

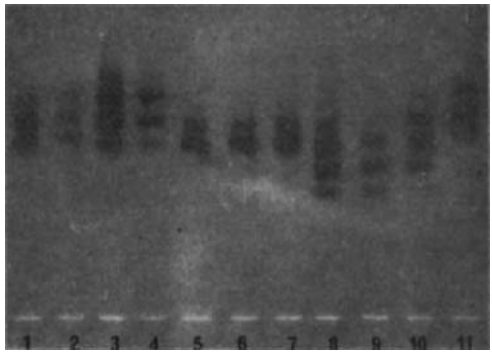


FIG. 7. Alanine aminotransferase, ALAT. Left to right: (1) TSW 162/75, ALAT II; (2) TSW 155/75, ALAT II; (3) and (4) NIMR 1, ALAT III, (5) TSW 50/77, ALAT II; (6) TSW 57/77, ALAT II; (7) TSW 79/77, ALAT II; (8) TSW 26/75, ALAT I; (9) TSW 34/75, ALAT I; (10) TSW 24/75, ALAT I; (11) TSW 109/75, ALAT II.

stocks are examined, may also be found in trypanosomes non-infective to man, the additional evidence of (a) human plasma resistance in the BIIT, and (b) low virulence to rodents (stocks TSW 3/75, TSW 26/75, TSW 47/77), strongly suggests that these Liberian stocks are also gambiense. Similarly, there is every reason to believe, from the same behavioural criteria, that the three Kenyan fly stocks from Sindo with ALAT I are again gambiense.

The finding of the ALAT marker in two *T. b. gambiense* stocks (Tsuaa clone G and Bida 3 clone A), previously examined by Godfrey and Kilgour (1976), was confirmed; one of these stocks recently accidentally infected a German laboratory worker (D. Mehlitz, personal communication), thus demonstrating that the trypanosomes were still infective to man after cloning and about 9 years of rodent passage. In addition, the ALAT marker was found in a stock from man in Zaire (C-126); C-126 also had the ASAT marker and an odd GPI pattern (Fig. 8).

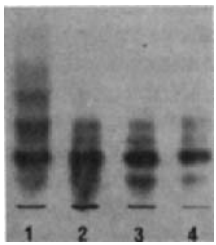


FIG. 8. Glucose phosphate isomerase, GPI. Left to right: (1) C-126, GPI III; (2) TSW 20/75, GPI I; (3) TSW 34/75, GPI I; (4) Bida 3 clone A, GPI I.

The four anomalous stocks (113B, Kwang 9A, Gboko, Bida 2) detected in the previous survey (Godfrey and Kilgour, 1976) were re-examined. Stock 113B, which had ALAT I, but also was reputedly a *T. b. rhodesiense* stock from Uganda, was electrophoretically different (Fig. 9) from the other stocks (118 clone Y, LUMP 1196) isolated from the same patient. Since the patient concerned relapsed after drug treatment, it is possible that the relapse trypanosome population, which was represented by 118 clone Y and LUMP 1196, was different from the original population, represented by 113B. Stock 113B was similar to West African gambiense stocks in ALAT, ICD and ME electrophoretic patterns, and gambian sleeping sickness was still endemic in southeast Uganda up to 1967 (Apted, 1970).

However, it seems unlikely that two different trypanosome zymodemes were present in the relapse population, and the inescapable conclusion is that one or more laboratory errors occurred. LUMP 1196, having been derived directly from a stock maintained by EATRO since isolation, was probably the least likely of the three stocks to have been mixed up with another stock. Moreover, LUMP 1196 was similar to, whereas 113B and 118 clone Y were dissimilar from, other stocks from south-east Uganda. Therefore, only LUMP 1196 can with any certainty have been derived from the original trypanosome population in EATRO sleeping sickness patient

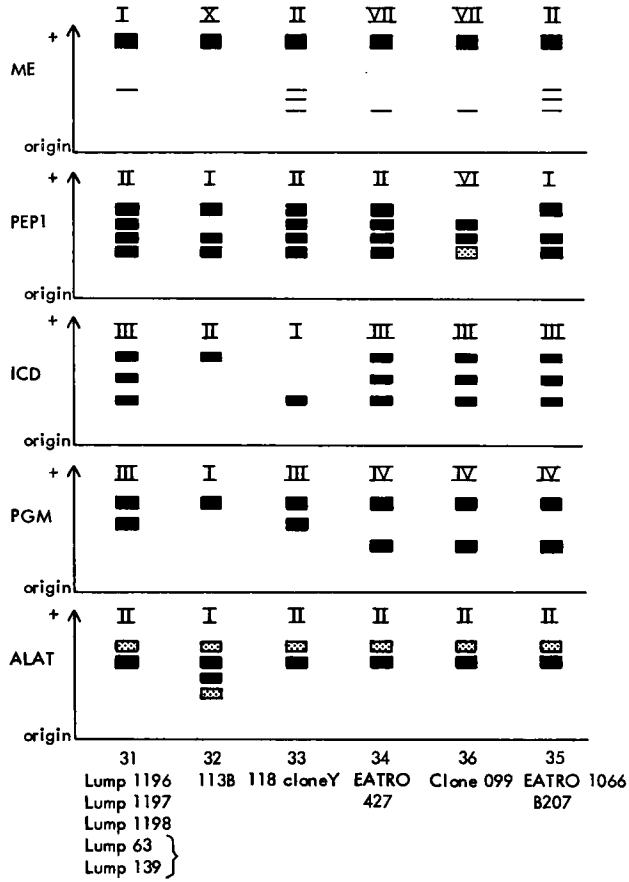


FIG. 9. Diagram of Ugandan *Trypanozoon* zymodemes; stocks otherwise electrophoretically identical.

354; stocks 113B and 118 clone Y must for the present be regarded as laboratory stocks of unknown origin, and thus 113B does not constitute a serious anomaly in the hypothesis that ALAT I is a marker for gambiense.

Similarly, it is extremely likely, as suggested by Godfrey and Kilgour (1976), that mistakes have occurred during the laboratory passage of Gboko and Kwang 9A, supposedly two Nigerian *T. b. gambiense* stocks, without the ALAT marker. Gboko was electrophoretically more similar to stocks isolated from Nigerian animals than man, and Kwang 9A had greater affinity with stocks from East Africa than from West Africa (Figs 4 and 10).

In contrast, Bida 2, although it had ALAT II instead of I, fitted in well with other Nigerian stocks from man (Fig. 10), and shared their idiosyncratic ME pattern, with weak minor bands, and PEP 1 pattern, with a slightly faster migrating *d* band than usual. Therefore, as Godfrey and Kilgour (1976)

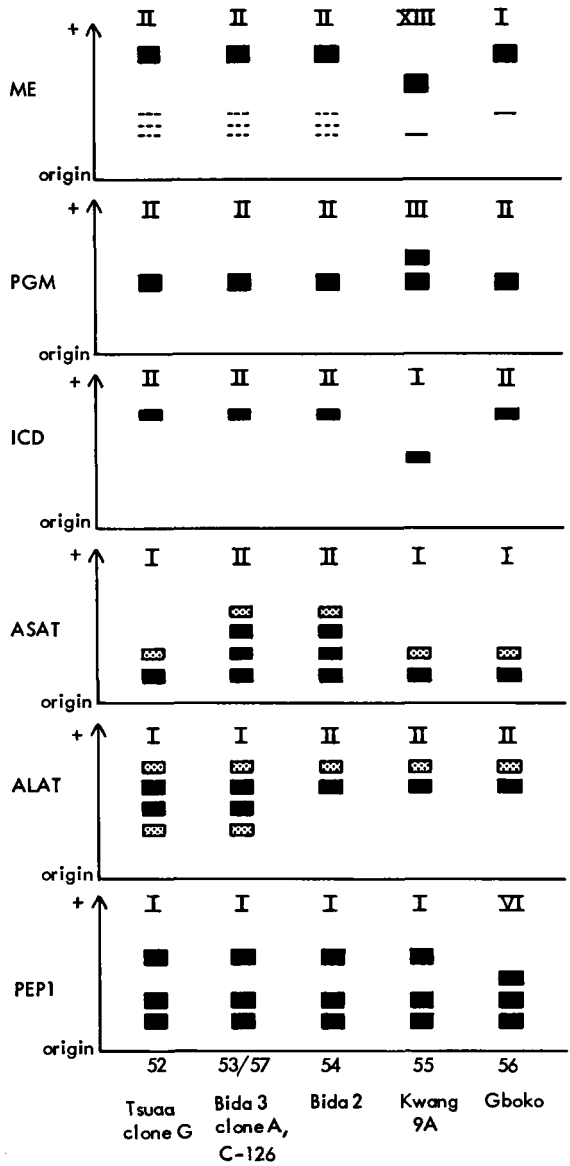


FIG. 10. Diagram of West African *Trypanozoon* zymodemes from man. GPI variation not shown; stocks otherwise electrophoretically identical. Dashed ME bands were very weak.

concluded, there is no reason to doubt its identity, especially since it had another marker for *T. b. gambiense*, ASAT II.

Rather than a particular pattern, any variation in ASAT from the most frequently occurring pattern, ASAT I, appears to characterize gambiense,

although ASAT I may sometimes occur. Godfrey and Kilgour (1976) found two unusual ASAT patterns (ASAT II and III) only among the *T. b. gambiense* stocks they examined; and here, similarly, two unusual ASAT patterns (ASAT II and IV, Fig. 11) were found only among stocks suspected or known to have caused gambian sleeping sickness (Bida 3 clone A; Bida 2; C-126; Sindo fly stocks LUMP 444, 449, 450).

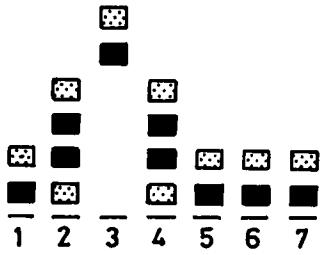


FIG. 11. Diagram of aspartate aminotransferase, (ASAT) gel. Left to right: (1) B8/18 clone B, ASAT I; (2) and (4) C-126, ASAT II; (3) LUMP 450, ASAT IV; (5) Gb 1, ASAT I; (6) Gb 2, ASAT I; (7) Gb 3, ASAT I.

Thus the data so far obtained indicate that gambiense may be identified by the presence of ALAT I and/or an ASAT pattern other than ASAT I. However, it should be pointed out that stocks with either or both of these markers did not segregate as a distinct grouping in cluster analysis (Fig. 4).

2. *Zambezi and* 3, *busoga*

Broadly, zambezi and busoga correspond to the classical concept of *T. b. rhodesiense*. No distinctive marker like those of gambiense was discovered, although it seems likely that all trypanosomes of certain zymodemes are potentially pathogenic to man.

In particular, most of the man-infective trypanosomes from the Busoga district of south-east Uganda belonged to one zymodeme (31, Fig. 9), together with two stocks from Central Nyanza, Kenya; one of the latter stocks, R21 clone, which was originally isolated from a bushbuck, had infected a volunteer, but there was no evidence of man-infectivity for the other stock, LUMP 227 (Table 2). Nonetheless, all trypanosomes of zymodeme 31 should be regarded as potentially infective to man, especially considering that others in this zymodeme, the ETat series, represented here by LUMP 63 and 139, came to be designated *T. b. rhodesiense* only after the accidental infection of a laboratory worker (Robertson and Pickens, 1975; Lumsden and Herbert, 1975).

Zymodeme 31, and two other busoga zymodemes from northern East Africa (18 and 37, from northern Tanzania and Ethiopia respectively) were

characterized by multibanded PGM and/or ICD electrophoretic patterns; in contrast, zambezi zymodemes from southern East Africa (1–5, from Botswana, Zambia and mid-west Tanzania) and also Rwanda (7 and 30) had single-banded PGM and ICD patterns of the so-called East African type (Figs 5 and 6); this north/south division clearly emerges from the dendrograms (Fig. 4).

These findings agree with Ormerod's earlier division of the rhodesian nosodeme into northern and southern "strains" (Ormerod, 1961, 1963, 1967). He observed that the "southern disease", found in Botswana, Zambia, Rhodesia and southern Tanzania, was characterized by few and mild symptoms, the presence of human carriers and a trypanosome strain with large cytoplasmic granules; in contrast, strains of *T. b. rhodesiense* found in Uganda, Kenya and northern Tanzania were more virulent, giving rise to acute disease, and had small cytoplasmic granules.

However, in the light of the electrophoretic evidence, which shows a substantial dissimilarity between busoga and zambezi, it seems unlikely that the acute rhodesian trypanosomiasis of Uganda resulted from the northward spread of an increasingly virulent strain during this century (Ormerod, 1961) and an alternative origin must be sought.

4. *Kiboko*

The kiboko zymodeme has not so far been found in man and thus, together with flompa (and possibly others), probably corresponds to the accepted concept of *T. b. brucei.* It is the only zymodeme exhibiting TDH/MDH polymorphism (Figs 12 and 13), which distinguishes kiboko in rather the same way that ALAT/ASAT markers distinguish gambiense.

Eight kiboko stocks came from wild animals in two well-separated endemic areas of rhodesian sleeping sickness (STIB 214 and 215 from Serengeti National Park, Tanzania; CRS 21, 22 and 24, H 3, J 10 and J 11 from the

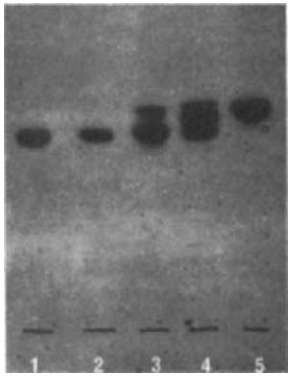


FIG. 12. Threonine dehydrogenase, TDH. Left to right: (1) LUMP 63, TDH I; (2) LUMP 139, TDH I; (3) LUMP 258, TDH II; (4) LUMP 277, TDH IV; (5) LUMP 266, TDH III.

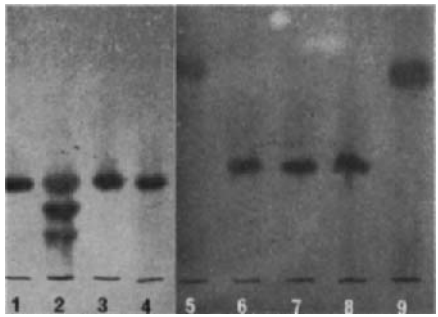


FIG. 13. Malate dehydrogenase, MDH. Left to right: (1) LUMP 139, MDH I; (2) LUMP 266, MDH III; (3) LUMP 450, MDH I; (4) Gb 1, MDH I; (5) J 11, MDH II; (6) H 1, MDH I; (7) TSW 223/77, MDH I; (8) TSW 80/77, MDH I; (9) J 10, MDH II.

Luangwa Valley Reserve, Zambia); these stocks were enzymically distinct from the local man-infective trypanosomes and existed alongside the human zymodemes in the same species of wild animal. This suggests that no barrier exists to transmission between wild animals and man, and hence that the kiboko zymodeme is truly not infective to man. TDH and/or MDH variation may then denote an inability to infect man. Three other kiboko stocks from *G. pallidipes* originated from the Kiboko area of Kenya where human trypanosomiasis has never been recorded. Interestingly, most of the kiboko stocks multiplied remarkably quickly in rodents.

Hierarchical cluster analysis segregated the above 11 stocks from all others into two very distinct small groups; the six Zambian stocks formed one because, in addition to TDH/MDH variation, they had unusual peptidase patterns (Fig. 14), while the three Kenyan and two Tanzanian stocks shared an unusual NH pattern (Fig. 15).

Using the same kind of reasoning, it might be argued that PGM IV (Fig. 6), found in only four Ugandan stocks isolated from *G. pallidipes* and domestic animals, is a marker for another type of non-man-infective *T. brucei*; the locally prevalent human zymodeme was 31, with PGM III. However, more data are required before it can be assumed that these possibly aberrant, old laboratory stocks belong to another major subdivision of *T. brucei*.

5. *Flompa*

As stated above, the flompa zymodeme is probably not involved in human disease and constitutes part of the old *T. b. brucei*.

The majority of *T. brucei* stocks isolated in The Gambia and Liberia were of two closely similar zymodemes, 41 and 42, neither of which possessed the ALAT or ASAT markers characteristic of gambiense. Therefore, it seems likely, since there was either little or no human trypanosomiasis in The Gambia and Liberia between 1975 and 1977 when the stocks were isolated, that they are not man-infective.

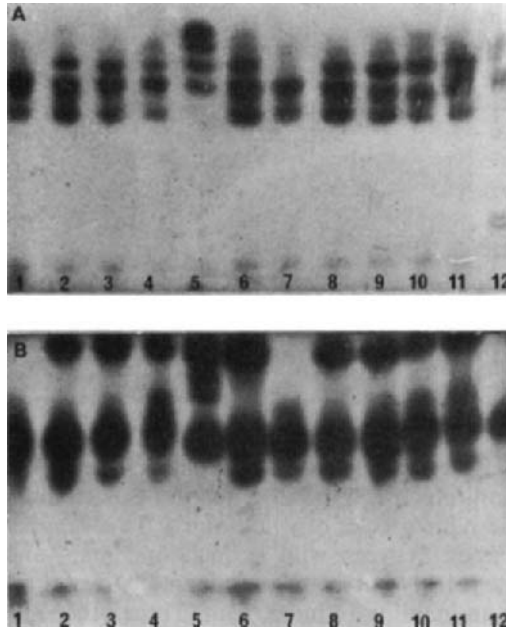


FIG. 14. Peptidase. A, PEP 1, substrate L-leucylglycylglycine. B, PEP 2, substrate L-leucyl; L-alanine. Left to right: (1) NIMR 1, PEP 1 VI, PEP 2 IV; (2) S-102, PEP 1 II, PEP 2 I- (3) TSW 95/77, PEP 1 II, PEP 2 I; (4) LUMP 227, PEP 1 II, PEP 2 I; (5) CRS 21, PEP 1 III, PEP 2 II; (6) TSW 85/77, PEP 1 II, PEP 2 I; (7) E 53/17, PEP 1 VI, PEP 2 IV; (8) LUMP 117, PEP 1 II, PEP 2 I; (9) LUMP 116, PEP 1 I, PEP 2 I; (10) TSW 88/77, PEP 1 I, PEP 2 I; (11) TSW 3/77, PEP 1 II, PEP 2 I; (12) B 8/18 clone B, PEP 1 IX, PEP 2 III.

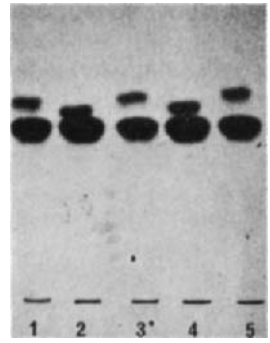


FIG. 15. Nucleoside hydrolase, NH. Left to right, 1,3,5, B 8/18 clone B, NH I; 2,4, LUMP 266, NH II.

6. *Evansi*

Evansi corresponds to the current concept of *T. evansi* which may be described as a minimally pleomorphic member of the subgenus *Trypanozoon* that is transmitted non-cyclically by bloodsucking flies other than tsetse,

and occurs outside the tsetse belts of Africa. However, for practical purposes, the differentiation of *T. evansi* from other *Trypanozoon* trypanosomes rests on geographical origin alone, because it would be almost impossible to distinguish *T. evansi* from *T. b. brucei* satisfactorily if both were found in animals in tropical Africa.

In this survey, the evansi zymodeme was found to be very similar to West African tsetse-transmitted stock (Fig. 4), and furthermore, evansi stocks from West Africa and South America were identical, which can be no mere coincidence, considering that *Trypanozoon* stocks from the two sides of Africa were clearly distinguished by enzyme markers. These results lend considerable support to the suggested introduction of *T. evansi* into South America from origins in West Africa.

According to Hoare (1965, 1972), *T. evansi* probably arose from *T. brucei*, on the northern edge of the tsetse belt in Africa, where trypanosomes underwent less cyclical transmission by tsetse, but more non-cyclical (or mechanical) transmission by other blood-sucking flies (e.g. Tabanidae); camels travelling in and out of the tsetse belt would have been ideal hosts for such a change and for the spread of trypanosomes across the Sahara desert. Once trypanosomiasis was established among domestic animals in North Africa, its further spread was ensured by caravans to the Middle East and Asia, and by the export of Arab horses; such horses would have been used during the Spanish conquest of South America in the 16th century and it is likely that *T. evansi* was first introduced into South America in this way (Hoare, 1965).

Thus the relationship between evansi and other kinds of *T. brucei* would appear to be very close, although there remains the fundamental difference that evansi does not, and probably cannot, undergo part of its life cycle in the tsetse fly.

C. CLUSTER ANALYSIS

Cluster analysis is widely used in the classification of data, but its appropriateness in all situations has been questioned (Cormack, 1971; Everitt, 1979); even a random set of data can be shown to contain clusters. However, it is hardly necessary to justify the use of a hierarchical classification for biological organisms, since it is standard practice.

The difficulties encountered in the interpretation of electrophoretic data have already been discussed, but further problems arose when the data were subjected to computer analysis. The approach differed fundamentally in that, by eye, whole electrophoretic patterns were compared, whereas the computer program compared individual bands. For example, the slow ALAT pattern, ALAT I, although considered to be of great importance as a marker for gambiense, was 50% similar to the usual *T. brucei* pattern, ALAT II, when compared band for band. Similarly, by computer analysis, the rare MDH and TDH polymorphisms had as much significance as a variation in one ME minor band. This obvious shortcoming could be rectified by some form of *a posteriori* weighting as suggested by Avise (1975), although the objectivity of the analysis would then be in doubt. Also, as noted by Cormack (1971),

since infinitely more weight is attached to observed than unobserved variables, the whole discussion of weighting becomes irrelevant.

In principle, the addition of further data should not fundamentally alter existing internal relationships in a sound classification; however, the clusters generated by the present analysis are disconcertingly unstable (compare Figs 4A and 4B).

This may be due to insufficient data, but seems more likely to be a fault inherent in the clustering method; initial cluster formation greatly restricts the way in which later zymodemes group, so that the inclusion or exclusion of a particular zymodeme can considerably alter the shape of the final dendrogram. The use of a different clustering technique might resolve this problem; for example, instead of the whole dendrogram being formed at once, in the single-cluster formation technique each cluster initiated is completed before another is begun (with the disadvantage that overlapping clusters result) (Cormack, 1971).

Overall, though, since the dendrograms produced agree with an intuitive analysis of the electrophoretic results and since the clusters can be defined by variables other than those used in deriving them (e.g. host species, geographical origin), we concluded that the cluster analysis technique was both suitable and useful for our data.

D. EPIDEMIOLOGY

In this section, an attempt is made to correlate some of the electrophoretic results with available epidemiological data. For general accounts of the epidemiology and spread of human trypanosomiasis in Africa, the reader is referred to Ormerod (1961), Willett (1965), Duggan (1970), Apted (1970), Ford (1971), Hoare (1972) and de Raadt (1976).

1. *Zambia and Botswana*

(a) *Introduction.* Sleeping sickness has not until recently been considered to be a major health problem in Zambia; this may in part have been due to the lack of medical personnel in rural areas, the difficulty of diagnosis and the fear and mistrust of the people (Buyst, 1976).

Foulkes (1970) admitted 300 trypanosomiasis patients to the Kasempa hospital between 1960 and 1968; the Kafue river area suffered epidemics in the past (Ormerod, 1961) and is in a large *G. morsitans* belt (Fig. 16). Buyst (1974) reported a major outbreak at the head of the Luangwa valley; between 1971 and 1974, 410 patients were diagnosed at Isoka. Almost simultaneously a small but severe outbreak occurred lower down the valley in the Petauke district (Rickman, 1974). A noticeable feature of these two outbreaks was the large number of women and children affected, suggesting close contact with *Glossina* in the villages (Rickman, 1974; Buyst, 1977b). Bush grew close to the huts and tsetse were observed biting inside villages in the Luwembe (Petauke) outbreak (Rickman, 1974). In Buyst's opinion, the position of Isoka on the edge of the Luangwa valley flybelt (Fig. 16), the scarcity of wild animal hosts

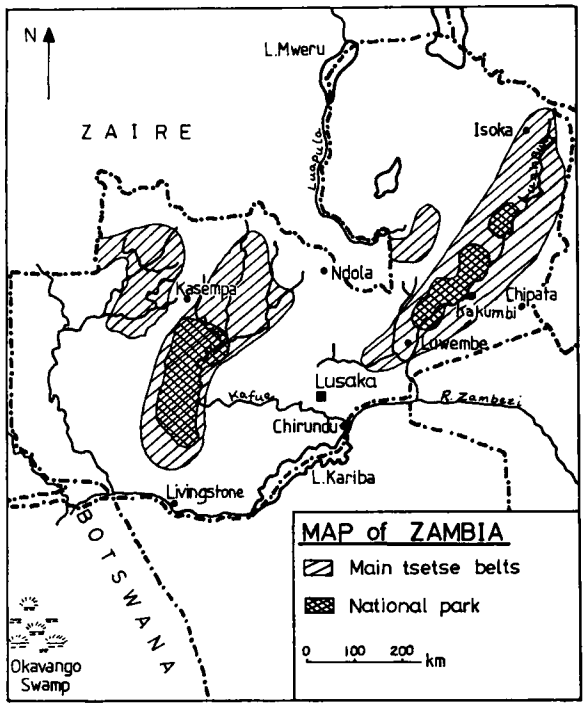


FIG. 16. Map of Zambia showing main tsetse belts and National Parks.

and the occurrence of small, scattered villages all contributed to close man–fly contact (Buyst, 1976, 1977a).

All three observers noted the generally mild nature of the disease. Foulkes (1970) contrasted the severe course in patients without previous tsetse contact, all being febrile and incapacitated within 2 weeks of infection, with the almost symptomless course, until the CNS became involved, in local patients. Buyst (1974) recorded three instances of 'spontaneous cure' and surveys in the Petauke district revealed the presence of 'symptomless carriers', who paradoxically later died (Rickman, 1974).

Both the Kafue and the Luangwa valley contain large wild animal reserves (Fig. 16). Wild animals spread from the flooded lower Luangwa valley into the Isoka district during the rains and concentrate again by the dwindling river in the dry season; this seasonal migration has been correlated with the increasing incidence of trypanosomiasis in women and children in the dry season as the tsetse congregate near villages because their wild hosts have disappeared (Buyst, 1976, 1977a).

In Botswana, the major human trypanosomiasis focus is the Okavango swamp area (Fig. 16) near Maun in the small but spreading *G. morsitans* belt (Ormerod, 1961). The incidence of the disease rose from about 40 cases per year in 1957–1970 to 272 in 1971 (Ormerod, 1961; de Raadt, 1976).

(b) *Electrophoresis.* In this survey, 17 *Trypanozoon* stocks isolated from man in Zambia between 1972 and 1979 were used. Nine stocks were from the Isoka and Luwembe epidemic foci, 1972–4; one further stock, cloned by Mr L. R. Rickman, was obtained from the Luwembe focus in 1978. Other stock histories are in Table 2, and Fig. 16 shows the places of origin. A further 11 *Trypanozoon* stocks examined (see Table 2) were from wild animals in the Luangwa valley reserve, which lies between the two epidemic sleeping sickness foci. Only one stock from Botswana, 0186, was studied (see Table 2). Among the human stocks, variation was found in only two enzymes, the highly variable ME and PEP 1; electrophoretic patterns for the other 10 enzymes were identical. ICD, PGM and ALAT patterns were those typical of East African *Trypanozoon* stocks (Figs 5, 6 and 7). Three ME patterns (Fig. 17) and two PEP 1 patterns (Fig. 18) were found, which divided the stocks into

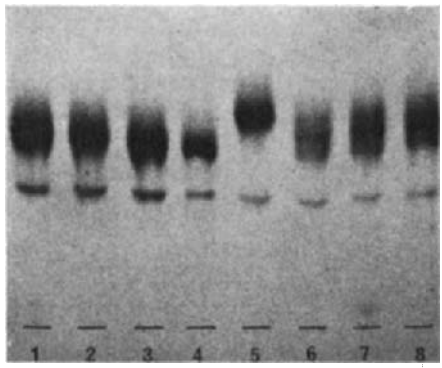


FIG. 17. "Malic" enzyme, ME. Variation among Zambian stocks from man. Left to right: 1–4, ME III, (1) 056, (2) 078, (3) 080, (4) 082; (5) 072, ME I; 6–8, ME IV, (6) 072, (7) 074, (8) 075.

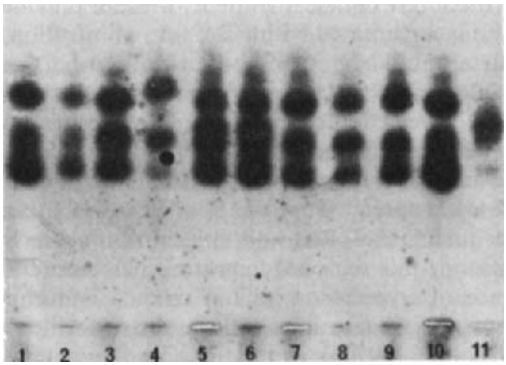


FIG. 18. Peptidase, PEP 1. Left to right: 1–4, PEP 1 I, (1) TSW 3/75 clone B; (2) TSW 3/75 clone A; (3) TSW 3/75; (4) Bida 3 clone A; (5) and (6). PEP 1 II, (5) TSW 293/77; (6) TSW 80/77; 7–10, PEP 1 I, (7) TSW 73/77; (8) and (9) TSW 154/75; (10) TSW 124/77; (11) B 8/18 clone B, PEP 1 IX.

five groups (Fig. 19). Zymodeme 1 contained all the five Luwembe focus stocks, while 2 and 3 contained four of the five Isoka focus stocks; thus trypanosomes from the two foci of epidemic sleeping sickness were distinguishable by the different mobilities of their ME.

Stocks from Kasempa (058), Kakumbi (TDRN 35) and Chirundu (076) also had the Isoka ME pattern, ME IV, and TDRN 34 had the Luwembe ME pattern, ME III. A clone of 058 had ME IV (C. A. Letch, personal communication) so this pattern does not result from a mixed infection of ME I and III stocks. The Botswana stock, 0186, was identical to Zambian stocks of zymodeme 5; this agrees with Ormerod's conclusion that Botswana and Zambian stocks were of the same type (Ormerod, 1967).

More variation was found among the wild animal stocks than among those from man: differences were found in the electrophoretic patterns of six enzymes (ME, ICD, PEP 1, PEP 2, TDH, MDH) compared with two (ME, PEP 1) in the human samples. Two of the 11 stocks, giraffe H 15 and hippopotamus CRS 19, were identical to the Isoka human stocks, zymodeme 2 (Fig. 19). Two other stocks, warthog H 18 and waterbuck H 1, differed only

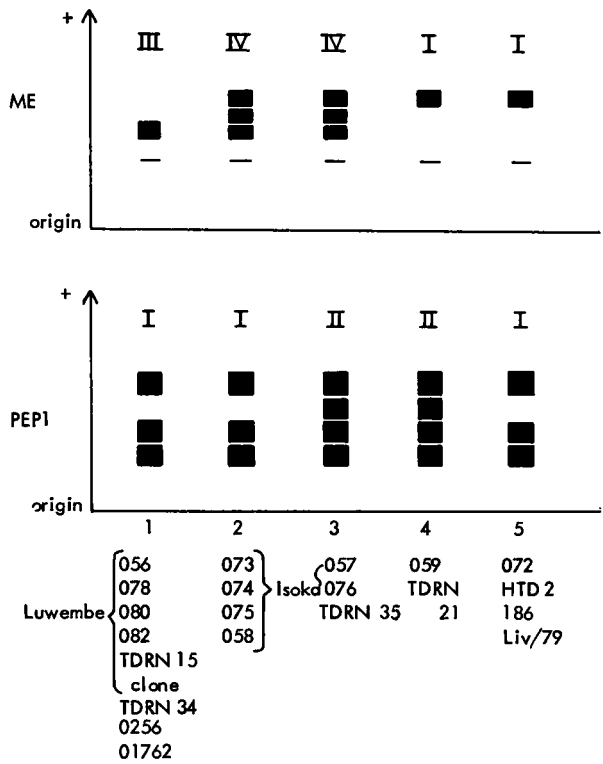


FIG. 19. Diagram of combinations of ME and PEP 1 electrophoretic patterns found among trypanosomes from man in Zambia, Botswana and Tanzania. All stocks otherwise electrophoretically identical. Arabic numbers 1–5 refer to zymodemes.

in ME pattern from zymodeme 4, with three minor ME bands instead of only one (ME II, Fig. 20); these two stocks were identical to a human stock from Rwanda, LUMP 624. Similarly, waterbuck H 6 differed from zymodeme 1 only in minor ME bands (ME V, Fig. 20). Thus five of the eleven stocks were either identical to, or differed only in the highly polymorphic ME from, stocks isolated from man in the same area.

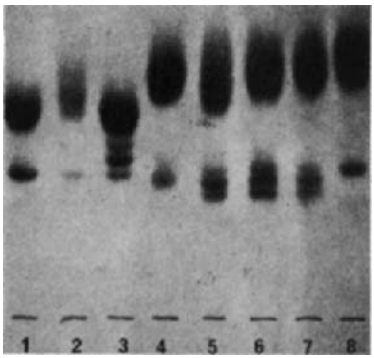


FIG. 20. Malic enzyme, ME. Left to right: (1) 078, ME III; (2) CRS 19, ME IV; (3) H 6, ME V; (4) TSW 26/75, ME I; (5) TSW 26/77, ME II; (6) TSW 56/77, ME II; (7) TSW 92/77, ME II; (8) TSW 38/77, ME I.

However, as discussed earlier (section IV B 4), the remaining six stocks were remarkably different from any other *Trypanozoon* stocks examined from Zambia or elsewhere. All six had a fast-moving MDH (Fig. 13) and four had a fast TDH band either alone or in combination with the usual slow TDH band (Fig. 12). In addition, two unusual PEP 1 and PEP 2 patterns were found among the stocks (Figs 14 and 21), always in the same combination. Five stocks had ME I, but hyena J 10 had a unique pattern, ME VI. For ICD, instead of the expected East African pattern, five stocks had the fast West African band and hyena J 11 had a triple-banded pattern (Fig. 5). Only two of the six stocks were identical—lion H 3 and hippopotamus CRS 24. Fig. 21 shows the combinations of patterns for six enzymes among the six stocks in contrast with a human stock of zymodeme 5.

(c) *Discussion.* Trypanosomes of different zymodemes were responsible for two simultaneous epidemics of sleeping sickness in the same valley in Zambia. One of these zymodemes persisted in the Luwembe area for at least 6 years. Rickman (1974) deduced that "Hargreaves", an early focus of infection in the Luangwa valley *circa* 1910, and Luwembe were the same place*. Possibly, then, the same zymodeme was responsible for human trypanosomiasis there 70 years ago. Two *T. b. rhodesiense* stocks, 01762 and 0256, which originated from the 1957–60 Tanzanian outbreaks of sleeping sickness around Tabora and Kasulu (Apted *et al.*, 1963), also belonged to zymodeme 1, and this lends support to the suggested movement of a *T. b. rhodesiense* strain northwards through Tanzania from an origin in the Zambezi basin (Ormerod, 1961).

* See note on p. 246.

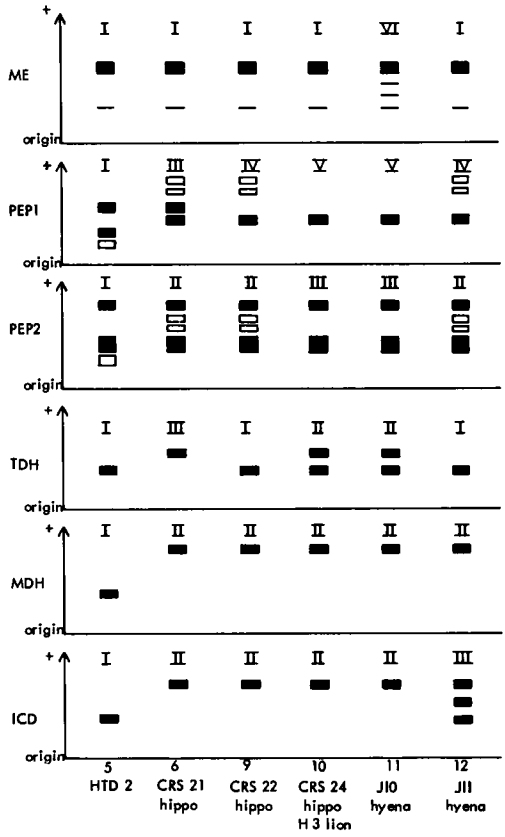


FIG. 21. Diagram of combinations of enzyme patterns found among Zambian wild animal *Trypanozoon* stocks compared with a human stock of zymodeme 5. All stocks otherwise electrophoretically identical. Arabic numbers refer to zymodemes. Note that unshaded bands appear with both peptide substrates.

Alternatively, at some time in the past, zymodeme 1 was introduced into the Luwembe area; Hargreaves was a crossing place of the Luangwa river on the road from Nyasaland (=Malawi) via the Zambian copper belt to the Congo (=Zaire) (Rickman, 1974).

The ME pattern (ME IV) associated with the other epidemic area, Isoka, may also have persisted in the Luangwa valley for very many years. Both stocks of the Liverpool *T. b. rhodesiense* "strain", which, in all likelihood, had been isolated in the Luangwa valley during the early 1920s, had ME IV, and the ICD, PGM and ALAT patterns of the "strain" were also consistent with those of zymodemes 2 and 3 (see Table 4). This "strain" was probably that used by Yorke and co-workers for many years at the Liverpool School of Tropical Medicine as their standard *T. rhodesiense* 'strain' (see for example, Yorke *et al.*, 1929). The year of isolation of the 'strain' was 1923 (Yorke

et al., 1929) and it presumably originated from the Luangwa valley, Zambia, where Yorke and others from the Liverpool School had investigated an outbreak of rhodesian sleeping sickness *circa* 1912.

Trypanosomes belonging to one of the zambezi zymodemes (Isoka) were found in wild animals, a giraffe and a hippopotamus. The giraffe stock gave an equivocal result in the BIIT, but the hippopotamus stock was sensitive to human plasma (Table 2). As discussed above, BIIT results are not always reliable, so for the present it seems safer to assume that both stocks are potential human pathogens.

Three other wild animal stocks from warthog and waterbuck were similar to the stocks isolated from man and two of the three showed resistance to human plasma (Table 2). Thus about half of the wild animal stocks may have been infective to man. If so, wild animals formed a substantial reservoir for zambezi in the Luangwa valley, for although *T. brucei* infection rates were low (Keymer, 1969; Dillman and Awan, 1972), very large numbers of wild animals were present—over 10 000 hippopotamus alone were reputed to live in the valley (Dillman and Awan, 1972). Interestingly, two Rwandan stocks from man (LUMP 624 and 626) closely resembled these Zambian wild animal stocks, differing only in the highly variable PEP 1; these Rwandan infections—both in Europeans, with presumably little resistance to trypanosome challenge—were probably derived from wild animal hosts, since they were acquired on a visit to a national park.

The tsetse species of the Luangwa valley are *G. morsitans* and *G. pallidipes* (Dillman and Awan, 1972). Bloodmeal analysis of East African tsetse showed that warthog and giraffe were the favoured hosts of *G. morsitans*, whereas *G. pallidipes* favoured bushbuck (Ford, 1970); warthog, however, were not considered as effective reservoir hosts of *T. b. rhodesiense* because of the scanty and short-lived parasitaemia produced (if indeed this can be regarded as an adequate measure of transmission capability), and neither were giraffe, in spite of their high infection rate (37%; Ford, 1970), because their habits brought them into little contact with man (Apted, 1970). Waterbuck supplied few meals for tsetse, but were nevertheless frequently found infected (52%; Ford, 1970); presumably the waterbuck was a 'dead end' in the cycle of tsetse transmission for the trypanosomes found in its blood, and clearly could not be an effective reservoir host of *T. b. rhodesiense*.

According to Glasgow (1970), hippopotamus submerged to defend themselves against tabanid flies and incidentally tsetse; this behaviour may account for the low *Trypanozoon* infection rate (5·3%) found by Dillman and Awan (1972), although their sampling procedure engendered a substantial delay in isolating any trypanosomes. Alternatively, hippopotamus, like warthogs, may have some resistance to trypanosome infection.

Three other hippopotamus stocks grouped with stocks from two hyenas and one lion in kiboko, a zymodeme quite distinct from the above human and other wild animal zymodemes, which belonged to zambezi. The two groups of stocks may have been transmitted independently in different cycles. Perhaps the carnivores' infections were acquired by eating infected prey, although this leaves the origin of the hippopotamus infections a mystery,

and it would be absurd to suggest that lions and hyenas in the Luangwa valley existed on a diet of hippopotamus.

Recent surveys of wild animals in the Serengeti, Tanzania, revealed that over 50% of lions and 35% of hyenas were infected with trypanosomes (Geigy and Kauffmann, 1973). Some of these infections might have been acquired by eating infected prey (Geigy and Kauffmann, 1973); Moloo *et al.* (1973) transmitted *T. brucei* to cats (19%) and dogs (46%) by feeding them on infected goats, although the goats had only scanty parasitaemia.

The growth characteristics of these hippopotamus, lion and hyena stocks were similar to those of evansi stocks in rodents, rapidly producing very heavy parasitaemias, although the Zambian stocks were far more pleomorphic than evansi stocks. Perhaps the virulence of the Zambian stocks was due to non-cyclical transmission by biting flies other than tsetse, such as Tabanidae. Two transmission cycles could have existed: man–tsetse–wild animal (e.g. giraffe, warthog, waterbuck, hippopotamus) and tabanid–wild animal (e.g. lion, hyena, hippopotamus). When hippopotamus congregate in small herds in a river, it is easy to imagine how the flies' feeding could be interrupted several times as each animal bitten submerged in defence.

However, three Kenyan stocks from tsetse (*G. pallidipes*) were similar to the Zambian wild animal stocks both electrophoretically and in growth characteristics, so these features cannot necessarily be entirely associated with non-cyclical transmission. But this leads to the possibility of two different cycles involving *G. pallidipes* and *G. morsitans*. *G. pallidipes* always inhabits thicket or the forest edge, whereas *G. morsitans* is a savannah tsetse (Ford, 1971); thus the host species available to the two species of fly are different, because *G. pallidipes* is restricted to fellow-inhabitants of thicket, like bushbuck, whereas *G. morsitans* is free to range after savannah dwellers like giraffe. Interestingly, hyena sometimes build their lairs in dense vegetation (Walker, E. P., 1964) but there the speculation must end. Whatever the nature of the transmission cycles may be, they are not completely segregated since trypanosomes of both sorts were isolated from hippopotamus.

2. *The Lake Victoria area*

(a) *Introduction.* The first recorded appearance of sleeping sickness was in a major epidemic around the shores of Lake Victoria at the turn of the century; the causative organism was identified as *T. gambiense*, the only man-infective trypanosome known at the time, and the vector as *G. palpalis* (=*G. fuscipes*) (Bruce *et al.*, 1903). Gambian sleeping sickness remained endemic in the lakeshore regions of Uganda and Kenya up to at least 1968 (Apted, 1970; Watson, 1972).

Apparently resulting from the northward spread of *T. rhodesiense*, a further epidemic of more acute sleeping sickness broke out in the 1940s in lakeshore Uganda (Mackichan, 1944) and crossed the Uganda/Kenya border, to affect first Central Nyanza province (1955) and later (1960) the Lambwe valley, South Nyanza (Willett, 1965). The vector of the new disease was thought to be *G. pallidipes* alone until *G. fuscipes* was for the first time

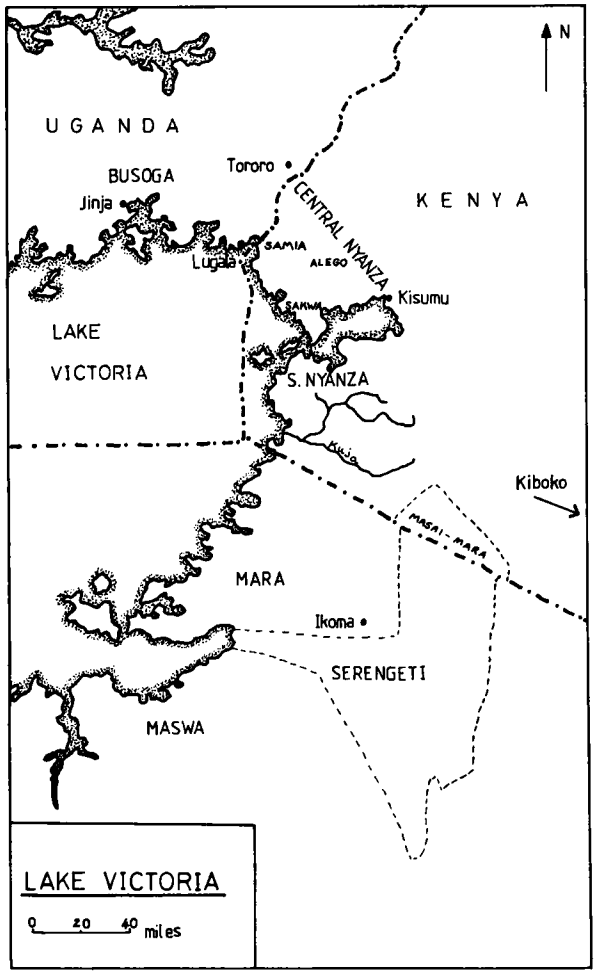


FIG. 22. Map of eastern shores of Lake Victoria.

also implicated in the transmission of *T. rhodesiense* in the Alego outbreak of 1964 (Willett, 1965; Apted, 1970). By 1958, *T. rhodesiense* had almost completely replaced *T. gambiense* in south-east Uganda (Robertson and Baker, 1958; Apted, 1970), and similarly, between 1964 and 1970, gambian sleeping sickness was apparently eradicated from Central and South Nyanza provinces, Kenya (Apted, 1970; Allsopp and Baldry, 1972).

(b) *Electrophoresis. T. brucei* stocks from four main regions of the Lake Victoria area were examined: Busoga district, Uganda; Central Nyanza, Kenya; South Nyanza, Kenya; Serengeti National Park, Tanzania (Figs 22, 25 and Table 2).

As recorded above, five of the man-infective stocks from south-east

Uganda—LUMP 1196, 1197, 1198, 63, 139—were identical to the Central Nyanzan stocks, R 21 clone and LUMP 227.

Although originally isolated from the same patient, stocks 113B and 118 clone Y were different both from each other and from LUMP 1196 (see section IV B 1 and Fig. 9).

A unique PGM pattern (Fig. 6) was found for the remaining Ugandan stocks, EATRO 427 and 1066, Clone 099 and B 207; all four stocks also had an ICD triplet (Fig. 5). Stocks EATRO 1066 and B 207 were identical electrophoretically, a not too surprising result considering that they were different lines taken from the same primary isolation (Fig. 9).

Among the Kenyan stocks from South Nyanza, variation was found in five enzymes—ME, ICD, PEP 1, ALAT and ASAT—dividing the stocks into four zymodemes (Fig. 23).

The two human stocks from the Lambwe valley, LUMP 447 and Otuoma, were identical, but different from the two reedbuck stocks, LUMP 446 and 448. These in turn differed from each other.

The three identical Sindo stocks were markedly different from those of the Lambwe valley. The ALAT pattern was ALAT I (Fig. 7), previously

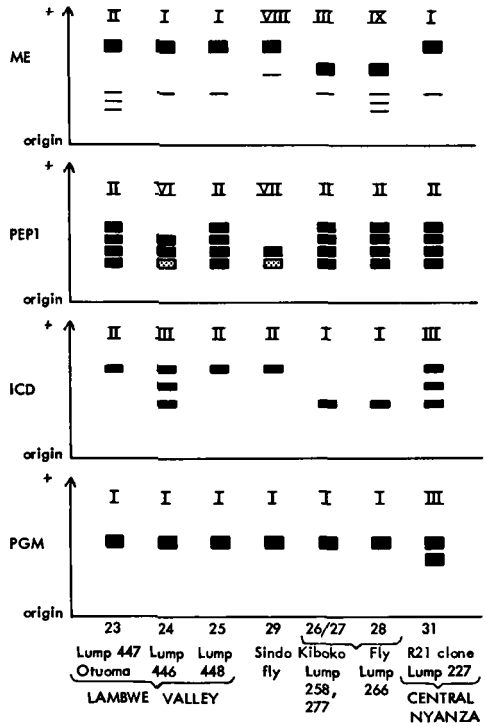


FIG. 23. Combinations of enzyme patterns found among Kenyan stocks; variation in ALAT, ASAT, MDH, TDH and NH not shown. Arabic numbers refer to zymodemes.

recorded for *T. b. gambiense* (Godfrey and Kilgour, 1976). Patterns for ASAT, ME and PEP 1 were unique (Figs 11 and 23).

The electrophoretic results for the eight Tanzanian stocks are shown in Fig. 24; only two stocks (STIB 250 and 350) were identical. STIB 214 and 215 had unusual TDH and NH patterns, like the Kenyan *G. pallidipes* stocks from Kiboko.

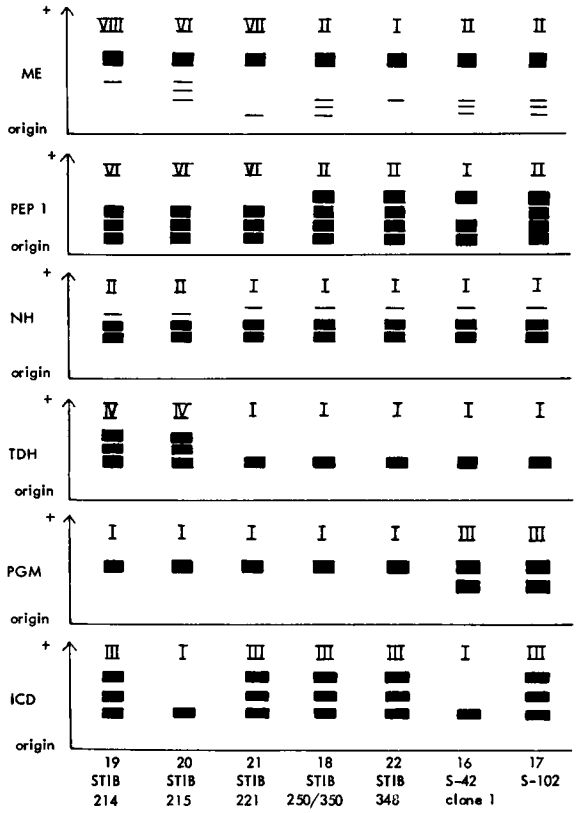


FIG. 24. Diagram of Tanzanian *Trypanozoon* zymodemes from wild animals; all stocks otherwise electrophoretically identical. STIB 215 had an extra, possibly artefactual, slow TDH band, not shown here.

(c) *Discussion. Trypanozoon* stocks from the Lake Victoria area typically had PGM and/or ICD multibanded patterns; the possible significance of this characteristic will be discussed below (section IV E 1).

Rhodesian trypanosomiasis appeared in the Lambwe valley in 1960 (Willett, 1965). Baldry (1972) gave two possible origins: either *T. rhodesiense* spread southwards across the Kavirondo Gulf from its northern foci in Samia and Sakwa into South Nyanza, or the trypanosome was carried northwards from its foci in Maswa and Mara in Tanzania (Fig. 22); settlers and their domestic stock moved into South Nyanza from both Central

Nyanza and northern Tanzania, and further, there was frequent traffic of fishermen along the coast of Lake Victoria.

The enzyme results lend little support to the idea of a southward spread from Central Nyanza, although this seems to be the commonly held belief (Willett, 1965; Ford, 1971). The Central Nyanzan stocks, R 21 clone and LUMP 227, were not similar to the South Nyanzan stocks. R 21 clone and LUMP 227 were identical electrophoretically, although R 21 was isolated 7 years before LUMP 227; the two stocks were also identical to five stocks from south-east Uganda collected between 1959 and 1976 and all of known infectivity to man. The spread of *T. b. rhodesiense* from south-east Uganda across the border into Kenya has not been disputed (Mackichan, 1944; Morris, 1960; Willett, 1965; Baldry, 1972). It seems unlikely that a zymodeme that had remained constant for at least 17 years and spread widely, would change in several ways during a 10 year stay in a new area.

A northward spread from Tanzania seems more likely considering the electrophoresis results: STIB 250 and 350, both of which were probably man-infective (see Table 2), differed only in ICD from the Lambwe valley human stocks, Otuoma and LUMP 447. These two stocks were electrophoretically identical, a not unexpected finding during an epidemic. However, according to Watson (1972), people from West Nyokal, the patient Otuoma's home, frequently crossed the valley to a market at Wiga, where LUMP 447 was isolated, and probably acquired their infections there (Fig. 25). It is not without significance that two (including the donor of LUMP 447) of the five new patients with trypanosomiasis found in this general area at the southern end of the Lambwe valley in 1970, were immigrant Maragoli tribesmen recently arrived with their cattle from the Musoma district of Tanzania (Baldry, 1972; Watson, 1972). So maybe the idea of a northward spread of pathogenic trypanosomes with immigrants from northern Tanzania is correct.

Alternatively, perhaps man-infective trypanosomes had been present in the Lambwe valley for some time, since their existence in an area will not be noticed unless man is present. The Lambwe valley was densely populated at the beginning of this century, but by 1935 a series of cattle epizootics and epidemics, including gambian sleeping sickness, had led to extensive depopulation; abandoned farmland rapidly returned to bush which was invaded by *G. pallidipes*. The trypanosomiasis hazard to man and livestock discouraged settlers and finally the government had to introduce a resettlement scheme in 1959, just before rhodesian sleeping sickness appeared in the valley (Ford, 1971; Watson, 1972). Thus either the settlers brought the disease into the area or it was already present in the wild animal population and was transmitted to the new human population; Baldry (1972) described an outbreak in 1971 among new settlers from Central Nyanza in the Lambwe valley that might have had a zoonotic origin.

Rhodesian sleeping sickness existed at low endemic levels at the south-east corner of Lake Victoria since the Maswa and Ikoma epidemics of the 1920s which arose independently of the main epidemic in Western Tanzania (Fairbairn, 1948); wild animals roamed freely between the Serengeti and Lambwe

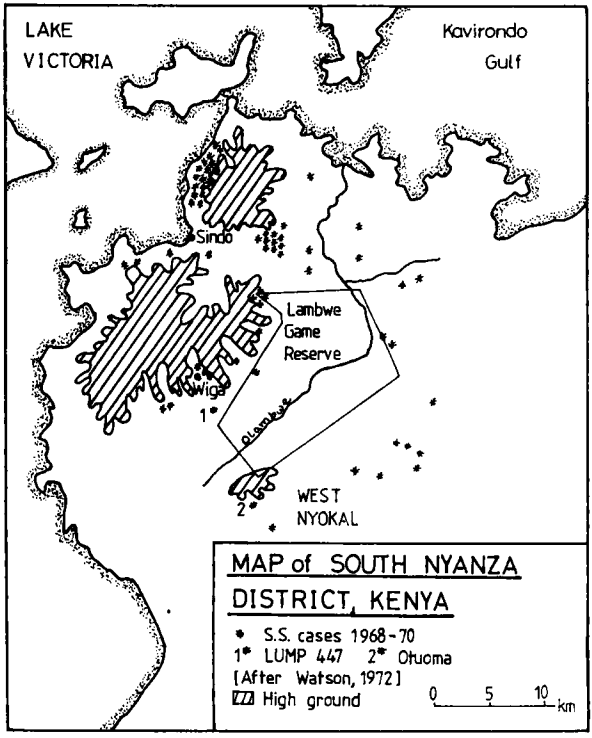


FIG. 25. Map of South Nyanza district, Kenya; SS=sleeping sickness. (After Watson, 1972.)

valley until recently, when the intervening area became too densely settled (Baldry, 1972). Over half of the trypanosome infections found in wild animals in the Lambwe valley during the WHO project were of subgenus *Trypanozoon* (Allsopp, 1972). Thus man-infective trypanosomes may have been introduced long ago into the Lambwe valley by wild animals from the areas of northern Tanzania endemic for sleeping sickness, and remained in wild animal hosts until recently.

Interestingly, human trypanosomiasis remained endemic in the Musoma district until 1954, when it apparently disappeared following the closure of the goldmines and subsequent depopulation of Ikoma, only to reappear in 1964 when the tourist industry associated with the Serengeti National Park attracted people to the district again (Baker *et al.*, 1967; Geigy *et al.*, 1973). But there is no need to search for a single origin of rhodesian trypanosomiasis in the Lambwe valley; as Baldry (1972) himself stressed, several introductions from several different established foci may have occurred. Stocks of the Central Nyanzan zymodeme were possibly circulating at the northern end of the Lambwe valley.

It was surprising that the two South Nyanzan groups were so different, considering that the Lambwe valley and Sindo are no more than 20 km

apart (Fig. 25), that the trypanosomes were isolated at about the same time and that the *G. pallidipes* belt sampled at Sindo was continuous with that of the Roo and Lambwe valleys (Allsopp and Baldry, 1972; Goedbloed *et al.*, 1971; D. M. Minter, personal communication).

The Sindo stocks were probably gambiense: LUMP 444 and 449 grew slowly in rodents; all three stocks had ALAT I, a pattern associated with *T. b. gambiense* by Godfrey and Kilgour (1976); variation was found for ASAT, which occurred only in gambiense stocks isolated from man in this study and in that of Godfrey and Kilgour (1976); LUMP 449 showed resistance to human plasma (Table 2).

Gambian human trypanosomiasis was apparently absent from South Nyanza during the WHO 1968–71 Lambwe valley project, although some cases were reported in early 1968 along the Lake Victoria shore; *G. fuscipes* had been eradicated from the lakeshore between 1954 and 1957, but had to be cleared again by insecticide spraying in 1967–68 and 1970 (Allsopp and Baldry, 1972; Watson, 1972).

Of the trypanosomiasis patients diagnosed in South Nyanza between 1968 and 1971, about a third were from the shore near Sindo (Baldry, 1972); it is by no means certain that all the trypanosomiasis was of the acute, rhodesian type (L. R. Rickman, unpublished observation). It seems likely that some of these patients were infected with trypanosomes of the Sindo tsetse zymodeme, since the people's greatest contact with fly probably occurred while they grazed their cattle in the Roo valley near Sindo (D. M. Minter, personal communication); around Sindo, hosts of *G. pallidipes* other than man and cattle were scarce and the majority of *Trypanozoon* isolates from this vector formed a homogeneous group: from 7500 Sindo *G. pallidipes*, 33 *Trypanozoon* isolates were of a chronic type and only six of an acute type (Goedbloed *et al.*, 1971); the three of these chronic type stocks examined had identical enzyme electrophoretic patterns.

The obvious conclusion is that *G. pallidipes* was transmitting gambiense in the Sindo area in 1969; if so, this would be the first example of gambiense transmission by a morsitans-group fly in East Africa. Possibly the eradication of the usual vector of gambiense, *G. fuscipes*, led to this change in transmission, although it is difficult to see why, unless *G. pallidipes* invaded the cleared lakeside territory. Moreover, this was not the first isolation of chronic *Trypanozoon* stocks from *G. pallidipes* in Kenya: two stocks were isolated from *G. pallidipes* in Sakwa location in 1958 (Baker, 1958). It seems more likely that both *G. pallidipes* and *G. fuscipes* were involved in the transmission of *T. b. gambiense*.

Why then was no chronic human trypanosomiasis found after the eradication of *G. fuscipes*? Maybe the idea that *T. b. gambiense* increases in virulence after transmission by *G. pallidipes* because of the greater number of infective trypanosomes injected per bite (Willett, 1956) is correct after all; all 58 salivary gland infections found among Sindo flies were heavily positive (Goedbloed *et al.*, 1971). Alternatively, perhaps the patients with gambian trypanosomiasis were simply thought to have a less acute type of rhodesian trypanosomiasis, since gambian trypanosomiasis should have been eradicated

with its vector, *G. fuscipes*. Until more stocks, especially old ones, from the Sindo area are examined these questions must remain unanswered.

The *T. brucei* stocks of unknown status derived from wild and domestic mammals or tsetse in the Lake Victoria area fell into three groups after enzyme electrophoresis (excluding the three Sindo *G. pallidipes* stocks). The members of the first group were identical to the local man-infective stocks: LUMP 227 and R 21 belonged to the busoga zymodeme; STIB 250 was identical to STIB 350 which had infected a volunteer (Table 2).

Members of the second group resembled, but were not identical to, man-infective stocks, and grouped with them in cluster analysis (Fig. 4); these included LUMP 448, S-42 clone 1, S-102 and STIB 348. In particular, LUMP 448, from a Lambwe valley reedbuck, differed only in minor bands of the highly variable ME from the man-derived Lambwe valley stocks, Otuoma and LUMP 447, and was resistant to human plasma in several different tests (Table 2). There is also a possibility that the parent stock of S-42 clone 1 was man-infective, since it was recently shown to be resistant to human plasma (S. M. Lanham and C. M. Scott, personal communication). However, stock S-42 when originally isolated was non-infective to two volunteers (Baker *et al.*, 1967), and has since been used as a 'safe' *T. b. brucei* stock.

The third group comprised trypanosomes that were electrophoretically distinct from man-infective stocks, and in all probability were not involved in man–fly transmission cycles, since there was little evidence of man-infectivity or human plasma resistance among them (Table 2).

As noted earlier, four Ugandan stocks from *G. pallidipes* or domestic animals (EATRO 427; 1066; clone 099; B207) had an unusual PGM pattern which distinguished them from the locally occurring busoga zymodeme (section IV B 4). Similarly, LUMP 446, a Lambwe valley reedbuck stock, and STIB 221, from a Tanzanian hartebeest, had PEP 1 VI (Fig. 14)—a pattern seen in evansi and West African *T. brucei* stocks; STIB 221 also had an unusual ME pattern, ME VII.

STIB 214 and 215, from a lion and a hyena respectively, were quite unlike other Tanzanian stocks electrophoretically (Fig. 24), having more affinity with three Kenyan *G. pallidipes* stocks from Kiboko (with which they were grouped in cluster analysis; Fig. 4) and six Zambian wild animal stocks. The possibility that these stocks were isolated from others by their transmission cycle has already been discussed.

3. *Ethiopia*

(a) *Introduction.* Sleeping sickness was long thought to exist in Abyssinia to the south and west of Addis Ababa near to the known foci of the disease in Sudan and Uganda; *G. palpalis* (=*G. fuscipes*) and *G. morsitans* were abundant along the Acobo and Chibese rivers and *G. pallidipes* had also been found (Sheppard, 1946). But it was not until 1967 that the first microscopically confirmed diagnosis of human trypanosomiasis was made in south-western Ethiopia and further patients were soon discovered (Baker *et al.*, 1970); by 1970, a total of 232 cases had been found (McConnell *et al.*, 1970).

Baker *et al.* (1970) thought that the disease was of the rhodesian type because of its acute nature in man and because the trypanosomes were virulent to laboratory rats and resistant to tryparsamide, and posteronuclear forms were prevalent. Further, the epidemiology of the disease around Gambela (Fig. 26) was typical of rhodesian sleeping sickness; the vector was probably *G. morsitans* and the sporadic incidence among men with occupations that took them frequently into the bush, suggested the existence of a wild animal reservoir of the disease (Baker *et al.*, 1970).

To the south in the Gilo river area (Fig. 26) where many people were infected, *G. tachinoides* and perhaps *G. pallidipes* were the main vectors, giving rise to domestic foci of trypanosomiasis, although *G. morsitans* may have been responsible for some sporadic infections (McConnell *et al.*, 1970). By 1970, this area had produced 210 patients compared with four patients from the Gambela area (Hutchinson, 1971).

FIG. 26. Map of southwest Ethiopia.

Baker *et al.* (1970) favoured a recent Sudanese origin for the disease. However, there is no evidence that rhodesian trypanosomiasis has ever existed in the Sudan. Archibald (1922) and Archibald and Riding (1926) described two patients with virulent trypanosomiasis, who failed to respond to the arsenical Atoxyl, from an area of endemic gambian sleeping sickness in the Sudan; the trypanosome stocks isolated from these patients were virulent to rodents and had a high percentage of posteronuclear forms. However, Lester (1933) found stocks of *T. gambiense* in Nigeria with similar characteristics and noted that arsenical (tryparsamide)-resistant infections of gambian sleeping sickness occurred from time to time in both endemic and epidemic areas. Also rhodesian sleeping sickness was not very widespread in the early 1920s and it seems surprising that the disease did not break out in epidemic form if it had reached the Sudan. Therefore these two isolated reports of virulent trypanosomiasis in the Sudan were probably not of rhodesian sleeping sickness at all, but atypical cases of gambian sleeping sickness.

The nearest known foci of rhodesian sleeping sickness to Ethiopia in the 1960s were those in south-east Uganda and Nyanza province, Kenya; no report of rhodesian trypanosomiasis in the north of Uganda or Kenya was found.

Baker *et al.* (1970) also suggested, but thought it unlikely, either that the disease had existed for some time at a low endemic level undetected by the medical authorities and unnoticed by the people, or that it had existed only in wild animals and tsetse until some ecological change brought man into the cycle; no evidence for such an ecological change was found.

However, Hutchinson (1971) remarked that medical services in the area were slight and noted that 50–100 deaths occurred before the people realized that a new disease was present and began seeking medical aid. Further, abnormally high numbers of tsetse were present in 1969, probably because of the recent favourable weather conditions; the relatively dry rainy season in 1968 would have reduced losses of tsetse pupae through flooding, and the following wet dry season 1968–69 would have prolonged the life of adult flies.

Nevertheless, Hutchinson (1971) also thought that human trypanosomiasis was most probably a recent introduction and was certain that epidemic trypanosomiasis was a new phenomenon in Ethiopia. Both Sudanese and Ethiopians had been attracted from afar to a gold mine at Dambala and in the early 1960s many Sudanese refugees had settled in the Gilo river area; moreover, it seemed improbable that the first few trypanosomiasis patients should have been diagnosed independently in the same year if the disease were endemic (Hutchinson, 1971).

(b) *Electrophoresis.* Five stocks were examined. Of three stocks from man, Gambela 1 was from the Gambela area and LUMP 997 and 1204 were from Gok and Dipa in the Gilo river area (Figs 26 and 27). Gambela 1 was isolated from a man who had been ill for 2 years; Baker *et al.* (1970) doubted that this illness was wholly due to trypanosomiasis because of the rapidity of the

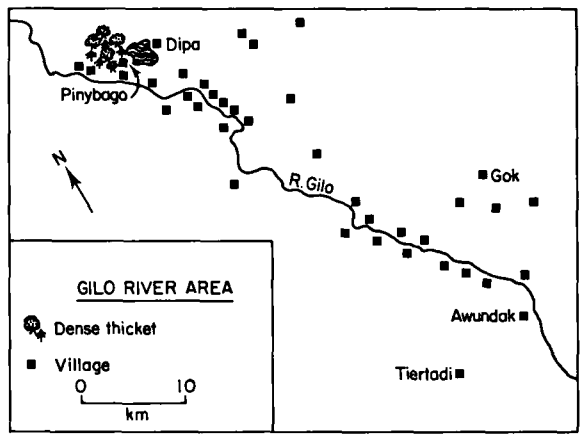


FIG. 27. Map of Gilo river area, Ethiopia (after Hutchinson, 1971).

disease in other patients; however, this patient apparently had CNS involvement, since he did not respond to Suramin and died.

Two tryparsamide-resistant stocks were isolated from *G. tachinoides* (LUMP 117) and a bushbuck, *Tragelaphus scriptus* (LUMP 116). The fly-derived stock was sensitive to human plasma on two occasions (Table 2); however, the bushbuck stock was resistant to human plasma but failed to infect a volunteer (Baker and McConnell, 1973). All further BIIT results on this stock showed it to be sensitive to human plasma (Table 2).

Variation was found in four enzymes: PGM, ME, ICD and PEP 1; Fig. 28 shows the combinations of patterns found. All stocks had ALAT II and the usual *T. brucei* patterns for the other enzymes. Stocks from the northern and southern Illubabor province rhodesian trypanosomiasis foci were different. Gambela 1 had West African ICD and PGM patterns and was identical to group B (=zymodeme 39) Liberian stocks from pigs. LUMP 997 and 1204 from the southern focus were identical and had an East African PGM pattern, an ICD triplet with a very weak lower band, and a unique ME pattern; in cluster analysis these two stocks grouped with a Tanzanian hartebeest stock (zymodeme 22) and the busoga zymodeme, 31 (Fig. 4). The bushbuck stock, LUMP 116, had East African PGM and ICD patterns and was identical to zymodeme 5, Zambian and Botswana stocks from man (Fig. 19).

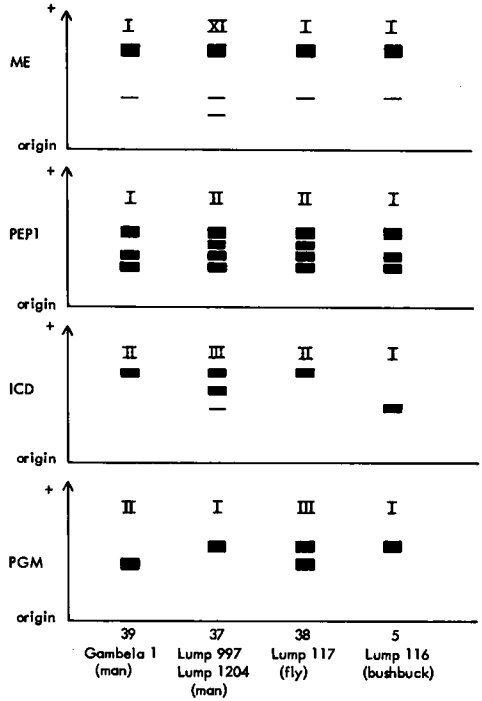


FIG. 28. Diagram of Ethiopian zymodemes; stocks otherwise electrophoretically identical.

The *G. tachinoides* stock, LUMP 117, was identical to a horse stock from The Gambia, Gb 1, and grouped with West African stocks in cluster analysis (Fig. 4).

(c) *Discussion.* Gambela 1 was very different from other Ethiopian stocks electrophoretically and clinically: the patient from whom this stock was isolated had been ill for 2 years, an unusually long time compared to the majority of Ethiopian patients in whom the disease had an acute onset (Baker *et al.*, 1970); also he was the first patient found in the Gambela area and was diagnosed during a malaria survey (Baker *et al.*, 1970; Hutchinson, 1971). Gambela is about 75 km from the Gilo river, the main epidemic focus where the other Ethiopian stocks were isolated (Fig. 26); thus, not only was the epidemiology of trypanosomiasis in the two areas different, but also the trypanosome zymodemes, although this conclusion is based on evidence from only one stock. In all probability, the epidemic foci on the Gilo river did not develop from trypanosomes introduced from the Gambela area and it was simply a coincidence that human trypanosomiasis was discovered first in Gambela and then along the Gilo river. Later patients from Gambela might well have been infected from the main Gilo epidemic focus, as Hutchinson (1971) thought later patients from the Akobo (=Acobo) river further to the south had been.

Thus, if human trypanosomiasis was introduced into Ethiopia, it happened at least twice.

The affinity of Gambela 1 with West African stocks strongly suggests that it was introduced from the west, i.e. the Sudan. There is no reason to believe that this introduction was recent; considering the scanty medical services, general isolation and poverty of south-west Ethiopia and the fact that 50–100 deaths had to occur before the people became aware of a new disease (Hutchinson, 1971), human trypanosomiasis may have existed at a low endemic level for many years without discovery.

The two stocks from the main epidemic area along the Gilo river were different from all other stocks from East and West Africa, but were most similar to human stocks from the Lambwe valley, Kenya. There can be little doubt that human trypanosomiasis was introduced into the Gilo river area; conditions for transmission were so favourable, with people living in fly-infested thickets (Hutchinson, 1971), that only the trypanosome was necessary for an epidemic to result; since no epidemic seems to have occurred before 1967 (Hutchinson, 1971), the trypanosome must have been introduced at about that time. The virulence of the disease also suggests that a new, non-immune population was affected (Hutchinson, 1971).

How the introduction was made remains a mystery, but the goldmines of Dambala, which had been visited by both of the first two trypanosomiasis patients found in Ethiopia (Hutchinson, 1971), may be of importance. It is noteworthy that sleeping sickness disappeared from Ikoma, Tanzania, with the closure, *circa* 1954, of the goldmines which had attracted large numbers of foreign settlers (Geigy *et al.*, 1973). Perhaps these goldminers moved north to Ethiopia.

Pinybago, from where LUMP 116 and 117 originated, was in an area of

dense thicket infested with *G. tachinoides* (Hutchinson, 1971). It seems likely that the people of Pinybago, of whom one in five had had trypanosomiasis by 1970 (Hutchinson, 1971), were infected with trypanosomes of the same zymodeme as the people of Dipa across the lake (Fig. 27); there was considerable movement of people between Dipa and Pinybago, and the very few Dipa people who contracted trypanosomiasis probably did so in Pinybago rather than Dipa, where tsetse contact was less intense (Hutchinson, 1971). Therefore we may assume that the human trypanosome zymodeme in Pinybago was that of LUMP 1204, which originated from Dipa. In that case, three zymodemes were circulating around Pinybago: LUMP 1204 in man, LUMP 116 in bushbuck and LUMP 117 in *G. tachinoides*. According to Hutchinson (1971), these three components were probably linked as shown in Fig. 29; it is not surprising that three different trypanosome zymodemes existed in the same ecosystem and probably other zymodemes were also present.

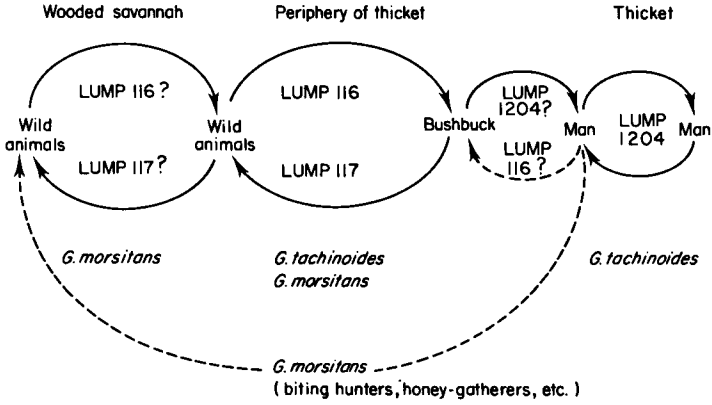


FIG. 29. Possible transmission cycles coexisting in the vicinity of Pinybago, S.W. Ethiopia (after Hutchinson, 1971).

The bushbuck zymodeme, LUMP 116, may have been part of a man–fly–bushbuck cycle. This zymodeme was that circulating in man in Zambia and Botswana, and the early positive BIIT results on the bushbuck isolate suggested that the trypanosomes were infective to man. The later negative BIIT results may reflect changes due to abnormal laboratory passage (Targett and Wilson, 1973), antigenic variation (van Meirvenne *et al.*, 1976; Rickman, 1977) or an increase in the proportion of plasma-sensitive trypanosomes in a mixed population (Rickman and Robson, 1974); the failure of the bushbuck isolate to infect a single volunteer was inconclusive, since in the past volunteers have often failed to become infected with known man-infective stocks.

Yet it seems unlikely that more than one trypanosome zymodeme was circulating among men in the Gilo epidemic, especially in Pinybago. Hutchinson (1971) traced the spread of human trypanosomiasis westwards from Tiertadi and Awundak to Gok and thence to Pinybago, where an explosive outbreak followed the finding of two patients who had both

recently visited Gok. This is borne out by the finding that LUMP 997 from Gok was identical to LUMP 1204 from Dipa, which in turn was probably identical to human stocks from Pinybago as explained earlier. Thus man-infective trypanosomes would have been established secondarily in the wild animals of the Gilo area and would have been of the same zymodeme as the human stocks.

4. *Animal reservoirs of gambian sleeping sickness*

(a) *Introduction.* Concerning the existence of an animal reservoir of human trypanosomiasis in West Africa, Mehlitz (1977) reported the finding of human plasma-resistant *Trypanozoon* stocks from pigs in Liberia, and Kageruka *et al.* (1977) reported a similar finding from pigs in Bas-Zaire.

Most of the available evidence for the existence of animal reservoir hosts of gambiense arises from the successful experimental infection of various wild and domestic animals (including pigs, dogs, cattle, antelope, monkeys and Gambian pouched rats, *Cricetomys gambianus*) with trypanosomes isolated from man, and the demonstration that such infections are of long duration and can be transmitted by tsetse from animal to animal and sometimes back to man (Molyneux, 1973). Such experiments indicate the potential of certain animals as reservoir hosts of gambiense; only the finding of animals naturally infected with gambiense would have epidemiological significance.

Yorke and Blacklock (1915) claimed to have identified the reservoir of the human trypanosome in Sierra Leone, but had merely found a *Trypanozoon* stock of low virulence to rats in the blood of an ox. Low virulence to rodents, although often observed with newly isolated *T. b. gambiense* (Gray, 1972), is not definitive; Godfrey (1977) suggested that *T. b. brucei* of low virulence to rodents may have been overlooked in the past since rodent inoculation became the standard method for isolating *T. b. brucei* in the field, giving rise to the mistaken idea that *T. b. brucei* is always highly virulent to rodents. Dräger and Mehlitz (1978) recorded several *Trypanozoon* stocks from buffalo in Botswana that failed to infect rodents. Thus the low virulence of a *Trypanozoon* stock is not proof of its being gambiense, although it may add support to other evidence.

The obvious way to test the infectivity to man of a trypanosome stock is to inoculate a volunteer, but human volunteer experiments with gambiense would be too dangerous, since early infection might be difficult to detect and invasion of the CNS could occur insidiously. Nevertheless, Denecke (1941) successfully infected himself with *T. brucei* isolated from a dog in Fernando Poo, an area of endemic gambian sleeping sickness, although the possibility that the infection resulted from a tsetse bite cannot be ruled out.

To summarize, the existence of an animal reservoir of gambian sleeping sickness still awaits clarification.

(b) *Electrophoresis.* Two groups of West African *T. brucei* stocks from domestic animals were suspected to include man-infective stocks on the basis of positive BIIT results; these were 51 stocks from Liberian pigs and dogs (Table 3) and six stocks from Nigerian cattle (Table 2).

However, the gambiense marker, ALAT I, was found in only 14 of the Liberian stocks, and its occurrence could not be correlated with the BIIT results (Gibson *et al.*, 1978; D. Mehlitz and U. Zillman, personal communication). Three of the Nigerian stocks had another unusual ALAT pattern, ALAT III (Fig. 7), but this was also shared by NIMR 1, which was probably evansi. The combinations of enzyme patterns found among these stocks are shown in Figs 30 and 31.

(c) *Discussion.* Five *T. brucei* stocks isolated from Liberian pigs were both resistant to human plasma and had the ALAT isoenzyme pattern characteristic of the causative organism of gambian trypanosomiasis (Godfrey and Kilgour, 1976). It seems reasonable to conclude that these stocks are or contain gambiense and that the domestic pig is a reservoir of human trypanosomiasis in West Africa, as previously suggested by van Hoof (1947) and Watson (1960).

The problem of the remaining stocks, for which the combined BIIT and isoenzyme results were equivocal, has been discussed by Gibson *et al.* (1978). The question is whether these trypanosomes present a threat to man. They are certainly abundant; 27% of the pig and dog stocks from Central Province, Liberia had the ALAT marker and 47% had some degree of plasma resistance.

However, as far as could be assessed, sleeping sickness was occurring only in the Suakoko area in 1977, where seven patients had been diagnosed earlier

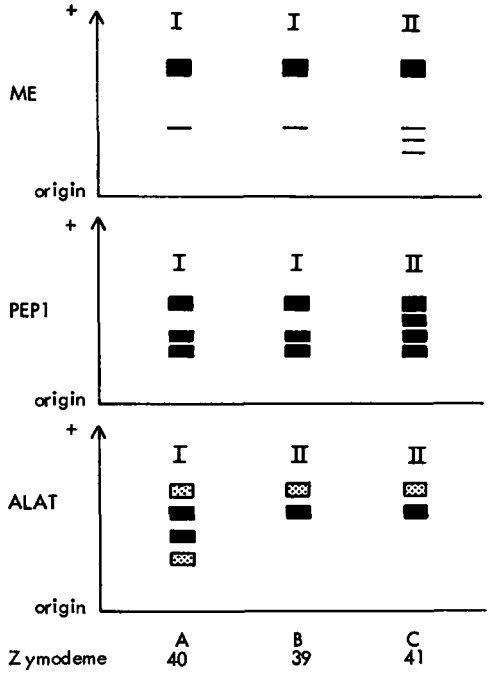


FIG. 30. Diagram of Liberian *Trypanozoon* zymodemes from pigs and dogs; stocks otherwise electrophoretically identical.

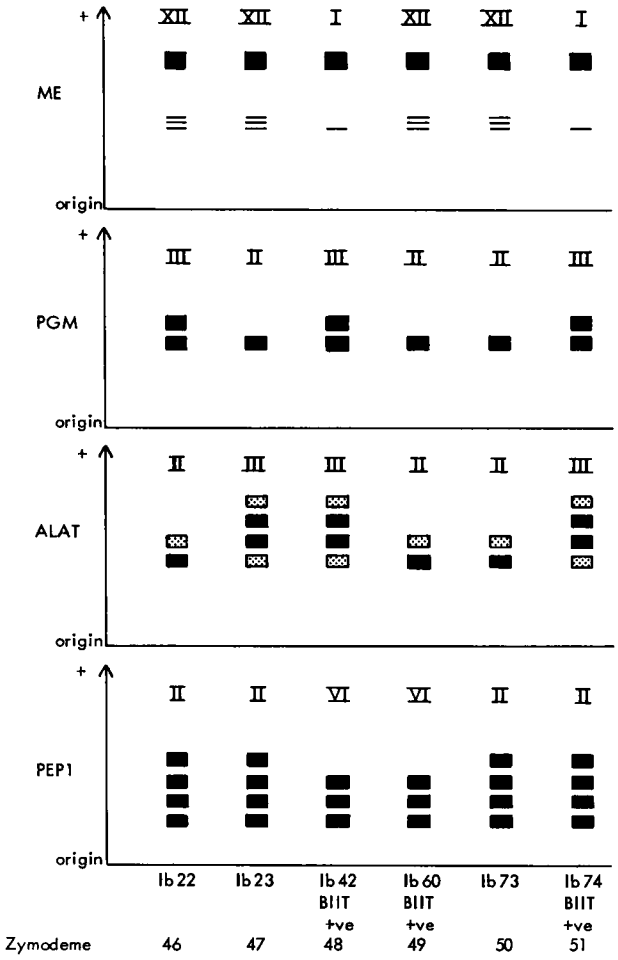


FIG. 31. Diagram of southwest Nigerian *Trypanozoon* zymodemes from cattle; stocks otherwise electrophoretically identical.

in the year at Phebe hospital; six of these were late cases, of which at least four died following treatment. The hospital had found only 18 cases of sleeping sickness in all since opening in 1964. Ganta hospital had seen no sleeping sickness case since 1973. On the other hand, the overall impression was of a general unawareness of the possibility of sleeping sickness, except at Suakoko, and there was no longer a hospital or doctor in the Kissi area, where an epidemic of sleeping sickness had occurred in the 1940s.

Judging from our own field observations and the large numbers found to be infected, pigs must have been an important food source for the tsetse. Baldry (1964) showed a close association between *G. tachinoides* and domestic pigs in Eastern Nigeria where pig meals formed almost 95% of fly feeds in the dry

season; an 86% trypanosome infection rate was found in the pigs (Killick-Kendrick and Godfrey, 1963). Baldry (1964) suggested that, although sleeping sickness was unknown in the area, it might appear if the pigs disappeared; *G. tachinoides* is a known vector of sleeping sickness in Northern Nigeria where Jordan *et al.* (1962) found man to be its major food source and no pig meal was recorded. With regard to this, Hutchinson (1954) recorded that first the cattle and then the pigs were much reduced in numbers by epidemics before the Kissi epidemic of the 1940s in Sierra Leone. The cattle epidemic, 1930–31, was pleuropneumonia and numbers did not recover for 10 years; the pig epidemic, 1937, occurring at the same time as the incidence of sleeping sickness started to rise, was associated with the human disease by the people who then killed their remaining pigs. Perhaps a similar situation has occurred in the Suakoko area where pigs were few in 1977, although plentiful in 1975.

However, this can only be speculation. Detailed epidemiological studies must be carried out before the importance of pigs and dogs as reservoir hosts of gambiense can be assessed.

E. FURTHER CONSIDERATIONS

1. *Possibility of hybridization*

Most of the stocks with a mixture of East and West African electrophoretic characteristics originated from the Lake Victoria area, but others were found in The Gambia, Nigeria, Zambia and Ethiopia.

Two double-banded PGM patterns, PGM III and IV (Fig. 6) occurred; PGM III appeared to be simply a combination of the East African band, PGM I, and the West African band, PGM II. Similarly, the triple-banded ICD pattern, ICD III, which also occurred among these stocks, seemed to contain the East African band, ICD I, and the West African band, ICD II (Fig. 5).

A study of Table 4 reveals that ICD III occurred only in areas where both East and West African ICD patterns were found in other stocks; most stocks with PGM III and all stocks with PGM IV originated in the Lake Victoria area, where it is known that the distributions of the East and West African forms of human trypanosomiasis overlapped during this century.

These multi-banded patterns did not arise from a mixed infection of East and West African stocks, because eight stocks with PGM III or PGM IV and/or ICD III were clones. Moreover, such a mixture of stocks would have resulted in both PGM and ICD multi-banded patterns always being found together, which was not so, and, although a mixture of trypanosomes with PGM I and PGM II would have given rise to PGM III, a mixture of trypanosomes with ICD I and ICD II would not have produced ICD III.

Although it may be simply a coincidence that trypanosomes with a combination of East and West African PGM and ICD patterns occurred in the Lake Victoria area, it is possible that hybridization has taken place, since the observed enzyme patterns agree very well with those expected from two

different homozygotes and the heterozygote cross (Fig. 32). PGM IV could result from a cross between a PGM I individual and one containing a rare allele for a slowly migrating PGM, or from a point mutation of the slower band of the PGM III pattern. The sometimes disproportionately weak slow band of the ICD III pattern may be explained by assuming that enzymes with *b* subunits are favoured, perhaps because they react more efficiently or are more stable than the enzymes with *a* subunits.

Thus the simple and obvious conclusion is that the multibanded patterns are those of heterozygotes, the hypothesis depending on the assumptions that PGM is monomeric and ICD dimeric in *T. brucei*, and that two loci exist for both enzymes in the *T. brucei* genome.

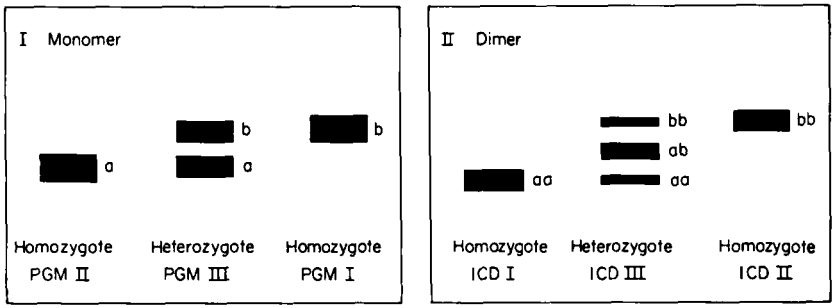


FIG. 32. The electrophoretic patterns expected from two homozygotes and the heterozygote cross for (i) a monomeric enzyme and (ii) a dimeric enzyme, assuming that no factor influences recombination of subunits (Harris and Hopkinson, 1976). Note the similarity with the patterns found for PGM and ICD among *Trypanozoon* stocks. PGM is known only as a monomer and ICD as a dimer in other genera (Harris and Hopkinson, 1976).

Genetic exchange may not be a regular feature of the life-cycle of *T. brucei*, but at some time in the past, perhaps in exceptional circumstances, it probably occurred. Hybrid vigour may account for the predominance of the multi-banded ICD and PGM zymodemes in certain areas such as around Lake Victoria; Ormerod (1961, 1967) noted the virulence and high degree of drug resistance shown by *T. rhodesiense* in south-east Uganda.

Speculating further on the possibility of genetic exchange in trypanosomes, it can be seen that among zambezi stocks, ME IV corresponds to the expected result from a cross between stocks with ME I and ME III (Fig. 17), given that ME is dimeric (or composed of two dimeric units = tetrameric) in trypanosomes; in man ME is tetrameric (Harris and Hopkinson, 1976). Similarly, TDH IV could arise from a cross between stocks with TDH I and III (Fig. 12), given that TDH is dimeric in trypanosomes, and the multi-banded ASAT and ALAT patterns might also be the products of a heterozygote. Some of the *T. brucei* zymodemes found among cattle in two Southwest Nigerian villages (Fig. 31) may also have been the products of hybridization. Unfortunately, genetic exchange has never been demonstrated among trypanosomes, only asexual reproduction by binary or multiple fission (Hoare, 1972). However,

Walker, P. J. (1964) decided that the subject of sex in trypanosomes had been studied by ineffective techniques and saw no reason why such a highly specialized group should not have a unique or unexpected system of genetic exchange still awaiting discovery.

Clearly, if genetic exchange does occur between *T. brucei* trypanosomes, the whole discussion of speciation and subspeciation takes on a new dimension. However, until direct proof is obtained, sex in trypanosomes must remain only a possibility.

2. *Highly polymorphic enzymes*

With most enzymes studied, only a few different electrophoretic patterns occurred, whereas ME and PEP 1 were highly variable.

The *T. brucei* ME pattern typically possessed distinct major and minor components (Fig. 1); this kind of isoenzyme pattern formation is indicative of multiple loci for ME (Harris and Hopkinson, 1976).

The high variability of ME was conferred mainly by the minor component, i.e. the fine slowly migrating bands; these varied both in number and position. V. Kilgour (personal communication), applying samples to the gel at equal levels of activity, found these bands to be very weak in laboratory gambiense and other old stocks, and we found them to be apparently absent from one such stock, 113B.

It is known that when *T. brucei* trypanosomes are long maintained in the laboratory, they lose the ability to transform into short stumpy forms in which the mitochondrion is activated (Hoare, 1972). Therefore, it might be suggested that the minor bands represent a mitochondrial ME component controlled by kinetoplast DNA. However, many of the old laboratory stocks and all evansi stocks examined had strongly active minor ME components. Further, among certain stocks, for example those from Liberia, ME patterns were consistent and particular ones could be correlated with particular patterns for other enzymes, i.e. ALAT and PEP 1. Thus it would seem that ME minor bands are simply very variable for some unknown reason.

A further point awaiting clarification is the observation that stocks derived from man and animals in the same area often differed only in minor ME bands (e.g. Liberian group A and Nigerian man-derived gambiense, zymodemes 40 and 52–54, Kenyan LUMP 448 and 447, zymodemes 23 and 25, Zambian 059/TDRN 21 and warthog H18/waterbuck H1, zymodemes 4 and 7; see Table 4).

PEP 1 and PEP 2 variability is understandable considering that at least two different peptidases were being examined simultaneously in each case, because of the non-specificity of the staining method (see Harris and Hopkinson, 1976). However, as with ME, the possibility that long laboratory maintenance may affect results is disconcerting. Several old laboratory stocks (e.g. B8/18 clone B; Nigerian gambiense stocks; Liverpool *T. rhodesiense* "strain"; S-42 clone 1; B207; LUMP 63, 139; 113B) had weakly staining peptidase bands, usually also reduced in number. Nor can the possibility be ignored that short stumpy transformation might lead to an alteration in the

peptidases produced. Evansi stocks had fewer peptidase bands than pleomorphic stocks (e.g. from Liberia), although the remaining bands were strongly staining. Clearly, further work is essential before peptidase patterns can be interpreted with any confidence, especially since PEP 1 markers may be useful for the identification of gambiense.

3. *Historical spread of sleeping sickness trypanosomes*

Since man-infective stocks from East and West Africa were more closely related to their respective local animal stocks than to each other, the isoenzyme results support the view that the human disease trypanosomes arose

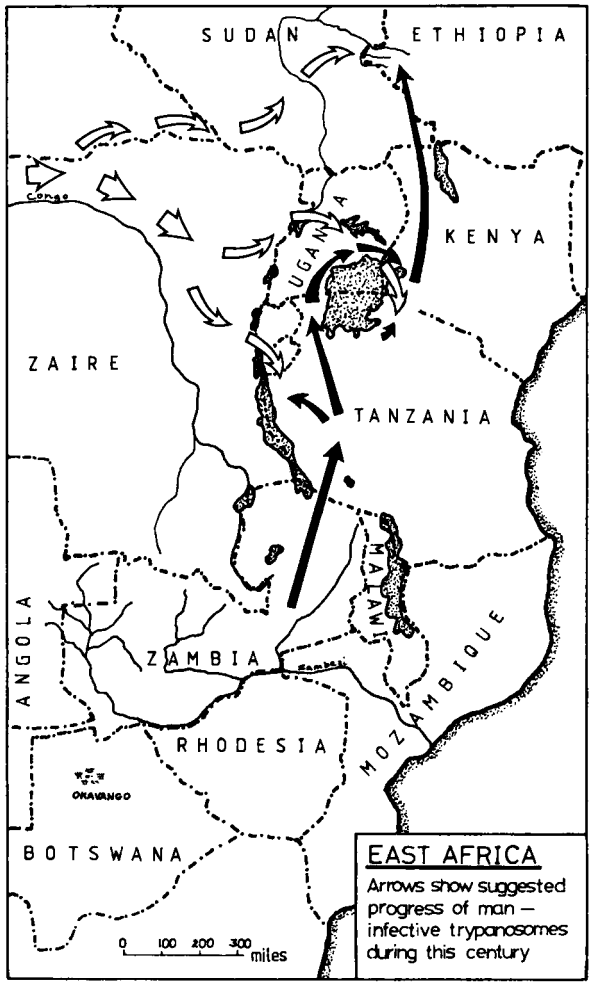


FIG. 33. Map of East Africa, showing possible routes of spread of human trypanosomiasis

independently from original *T. brucei* populations in East and West Africa rather than one from the other (Ormerod, 1967). Whether the original zambezi appeared in the Zambezi valley and spread northwards or appeared elsewhere and spread southwards is unclear from the isoenzyme results, but similar zambezi zymodemes have been present in Rwanda, Tanzania, Zambia and Botswana for the past 20 and possibly 50 years. These zymodemes were distinct from that causing acute human trypanosomiasis in south-east Uganda and north-west Kenya, which confirms Ormerod's earlier division of the rhodesian nosodeme into the northern and southern 'strains' (Section IV B 2; Ormerod 1961, 1963, 1967). It seems likely therefore that zambezi and busoga had separate origins. Alternatively, given that hybridization is possible in trypanosomes, perhaps busoga represents the virulent hybrid produced when the northward spreading zambezi met gambiense, already present in Uganda, *circa* 1940; the hybrid subsequently spread into north-west Kenya (Fig. 33). Similarly, in Ethiopia, perhaps man-infective trypanosomes of a West African zymodeme met the still northward moving zambezi and the hybrid gave rise to the Gilo epidemic *circa* 1970.

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References

Aaronovitch, S. and Terry, R. J. (1972). The trypanolytic factor in normal human serum. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **66**, 344.

Allsopp, R. (1972). The role of game animals in the maintenance of endemic and enzootic trypanosomiases in the Lambwe Valley, South Nyanza District, Kenya. *Bulletin of the World Health Organization*, **47**, 735–746.

Allsopp, R. and Baldry, D. A. T. (1972). A general description of the Lambwe Valley area of South Nyanza District Kenya. *Bulletin of the World Health Organization*, **47**, 691–698.

Al-Taqi, M. and Evans, D. A. (1978). Characterization of *Leishmania* spp. from Kuwait by isoenzyme electrophoresis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **72**, 56–65.

Apted, F. I. C. (1970). The epidemiology of Rhodesian sleeping sickness. *In* "The African Trypanosomiases" (Mulligan, H. W., ed.), pp. 645–660, George Allen and Unwin, London.

Apted, F. I. C., Ormerod, W. E., Smyly, D. P. and Stronach, B. W. (1963). A comparative study of the epidemiology of endemic Rhodesian sleeping sickness in different parts of Africa. *Journal of Tropical Medicine and Hygiene*, **66**, 1–16.

Archibald, R. G. (1922). *Trypanosoma rhodesiense* in a case of sleeping sickness from the Sudan. *Annals of Tropical Medicine and Parasitology*, **16**, 339–340.

Archibald, R. G. and Riding, D. (1926). A second case of sleeping sickness in the Sudan caused by *Trypanosoma rhodesiense*. *Annals of Tropical Medicine and Parasitology*, **20**, 161–166.

Ashcroft, M. T. (1959). A critical review of the epidemiology of human trypanosomiasis in Africa. *Tropical Diseases Bulletin*, **56**, 1073–1093.

Ashcroft, M. T. (1963). Some biological aspects of the epidemiology of sleeping sickness. *Journal of Tropical Medicine and Hygiene*, **66**, 133–136.

Avise, J. C. (1975). Systematic value of electrophoretic data. *Systematic Zoology*, **23**, 465–481.

Bagster, I. A. and Parr, C. W. (1973). Trypanosome identification by electrophoresis of soluble enzymes. *Nature (London)*, **244**, 364–366.

Baker, J. R. (1958). Atypical strains of the *Trypanosoma brucei* group. *East African Trypanosomiasis Research Organization Annual Report, January–December*, 1958, pp. 20–22.

Baker, J. R. and McConnell, E. (1973). Strains of *Trypanosoma (Trypanozoon) brucei* Spp. isolated in Ethiopia from *Glossina tachinoides* and *Tragelaphus scriptus*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **67**, 153–154.

Baker, J. R., Sachs, R. and Laufer, I. (1967). Trypanosomes of wild mammals in an area northwest of the Serengeti National Park, Tanzania. *Zeitschrift für Tropenmedizin und Parasitologie*, **18**, 280–284.

Baker, J. R., McConnell, E., Kent, D. C. and Hady, J. (1970). Human trypanosomiasis in Ethiopia. Ecology of Illubabor Province and epidemiology in the Baro River area. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **64**, 523–530.

Baker, J. R., Miles, M. A., Godfrey D. G. and Barrett, T. V. (1978). Biochemical characterization of some species of *Trypanosoma (Schizotrypanum)* from bats (Microchiroptera). *American Journal of Tropical Medicine and Hygiene*, **27**, 483–491.

Baldry, D. A. T. (1964). Observations on a close association between *Glossina tachinoides* and domestic pigs near Nsukka, Eastern Nigeria. II. Ecology and trypanosome infection rates in *G. tachinoides*. *Annals of Tropical Medicine and Parasitology*, **58**, 32–44.

Baldry, D. A. T. (1972). A history of Rhodesian sleeping sickness in the Lambwe Valley. *Bulletin of the World Health Organization*, **47**, 699–718.

Borden, D., Whitt, G. S. and Nanney, D. L. (1973). Isozymic heterogeneity in *Tetrahymena* strains. *Science*, **181**, 279–280.

Bruce, D., Nabarro, D. and Greig, E. D. W. (1903). Further report on sleeping sickness in Uganda. *Reports of the Sleeping Sickness Commission of the Royal Society*, **4**, 3–87.

Buyst, H. (1974). The epidemiology, clinical features, treatment and history of sleeping sickness on the northern edge of the Luangwa flybelt. *Medical Journal of Zambia*, **8**, 2–12.

Buyst, H. (1976). Sleeping sickness research in Zambia. *East African Medical Journal*, **53**, 452–458.

Buyst, H. (1977a). The epidemiology of sleeping sickness in the historical Luangwa Valley. *Annales de la Société belge de Médecine tropicale*, **57**, 349–359.

Buyst, H. (1977b). Sleeping sickness in children. *Annales de la Société belge de Médecine tropicale*, **57**, 201–211.

Carter, R. (1970). Enzyme variation in *Plasmodium berghei*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **64**, 401–406.

Carter, R. and Voller, A. (1975). The distribution of enzyme variation in populations of *Plasmodium falciparum* in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **69**, 371–376.

Chance, M. (1979). The identification of *Leishmania*. *Symposia of the British Society for Parasitology*, **17**, 55–74.

Cormack, R. M. (1971). A review of classification. *Journal of the Royal Statistical Society, Series A*, **134**, 321–367.

Denecke, K. (1941). Menschenpathogene Trypanosomen des Hundes auf Fernando Poo. Ein Betrag zur Epidemiologie der Schlafkrankheit. *Archiv für Hygiene und Bakteriologie*, **126**, 38–42.

De Raadt, P. (1976). African sleeping sickness today. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **70**, 114–116.

Dillmann, J. S. S. and Awan, M. A. Q. (1972). The isolation of *Trypanosoma brucei* from *Hippopotamus amphibius* in the Luangwa Valley, Zambia. *Tropical Animal Health and Production*, **4**, 135–137.

Dräger, N. and Mehlitz, D. (1978). Investigations on the prevalence of trypanosome carriers and the antibody response in wildlife in Northern Botswana. *Tropenmedizin und Parasitologie*, **29**, 223–233.

Duggan, A. J. (1970). An historical perpective. *In* "The African Trypanosomiases" (H. W. Mulligan, ed.), pp. xli–lxxxviii. George Allen and Unwin, London.

Everitt, B. S. (1979). Unresolved problems in cluster analysis. *Biometrics*, **35**, 169–181.

Fairbairn, H. (1933a). The action of human serum *in vitro* on sixty-four recently isolated strains of *T. rhodesiense*. *Annals of Tropical Medicine and Parasitology*, **27**, 185–205.

Fairbairn, H. (1933b). Experimental infection of man with a strain of *Trypanosoma rhodesiense* apparently susceptible to normal human serum *in vitro*. *Annals of Tropical Medicine and Parasitology*, **27**, 251–264.

Fairbairn, H. (1948). Sleeping sickness in Tanganyika Territory, 1922–1946. *Tropical Diseases Bulletin*, **45**, 1–17.

Fairbairn, H. and Burtt, E. (1946). The infectivity to man of a strain of *Trypanosoma rhodesiense* transmitted by *Glossina morsitans* through sheep and antelope: evidence that man requires a minimum infective dose of metacyclic trypanosomes. *Annals of Tropical Medicine and Parasitology*, **40**, 270–313.

Ford, J. (1970). The geographical distribution of *Glossina*. *In* "The African Trypanosomiases" (H. W. Mulligan, ed.), pp. 274–297. George Allen and Unwin, London.

Ford, J. (1971). "The role of the trypanosomiases in African ecology. A study of the tsetse fly problem." Clarendon Press, Oxford.

Foulkes, J. (1970). Human trypanosomiasis in Zambia. *Medical Journal of Zambia*, **4**, 167–177.

Geigy, R. and Kauffmann, M. (1973). Sleeping sickness survey in the Serengeti area (Tanzania) 1971. I. Examination of large mammals for trypanosomes. *Acta Tropica*, **30**, 12–23.

Geigy, R., Mwambu, P. M. and Kauffmann, M. (1971). Sleeping sickness survey in Musoma District, Tanzania. IV. Examination of wild mammals as a potential reservoir for *T. rhodesiense*. *Acta Tropica*, **28**, 211–220.

Geigy, R., Kauffmann, M., Mayende, J. S. P., Mwambu, P. M. and Onyango, R. J. (1973). Isolation of *Trypanosoma* (*Trypanozoon*) *rhodesiense* from game and domestic animals in Musoma district, Tanzania. *Acta Tropica*, **30**, 49–56.

Geigy, R., Jenni, L., Kauffmann, M., Onyango, R. J. and Weiss, N. (1975). Identification of *T. brucei*-subgroup strains isolated from game. *Acta Tropica*, **32**, 190–205.

Gibson, W., Mehlitz, D., Lanham, S. M. and Godfrey, D. G. (1978). The identification of *Trypanosoma brucei gambiense* in Liberian pigs and dogs by isoenzymes and by resistance to human plasma. *Tropenmedizin und Parasitologie*, **29**, 335–345.

Glasgow, J. P. (1970). The *Glossina* community. *In* "The African Trypanosomiases" (H. W. Mulligan, ed.), pp. 348–381. George Allen and Unwin, London.

Godfrey, D. G. (1977). Problems in distinguishing between the morphologically similar trypanosomes of mammals. *Protozoology*, **3**, 33–49.

Godfrey, D. G. (1979). The zymodemes of trypanosomes. *Symposia of the British Society for Parasitology*, **17**, 31–53.

Godfrey, D. G. and Kilgour, V. (1976). Enzyme electrophoresis in characterizing the causative organism of Gambian trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **70**, 219–224.

Godfrey, D. G., and Killick-Kendrick, R. (1967). Cyclically transmitted infections of *Trypanosoma brucei*, *T. rhodesiense* and *T. gambiense* in chimpanzees. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **61**, 781–791.

Goedbloed, E., Ligthart, G. A. and Minter, D. M. (1971). A comparison of *Trypanosoma brucei* subgroup isolates from *Glossina pallidipes* in two areas of Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **65**, 261–263.

Goedbloed, E., Ligthart, G. A., Minter, D. M., Wilson, A. J., Dar, F. K. and Paris, J. (1973). Serological studies of trypanosomiasis in East Africa. II. Comparisons of antigenic types of *Trypanosoma brucei* subgroup organisms isolated from wild tsetse flies. *Annals of Tropical Medicine and Parasitology*, **67**, 31–43.

Gray, A. R. (1972). Variable agglutinogenic antigens of *Trypanosoma gambiense* and their distribution among isolates of the trypanosome in different places in Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **66**, 263–284.

Harris, H. and Hopkinson, D. A. (1976). "Handbook of enzyme electrophoresis in human genetics." North-Holland Publishing Company, Amsterdam.

Hawking, F. (1973). The differentiation of *Trypanosoma rhodesiense* from *T. brucei* by means of human serum. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **67**, 517–527.

Hawking, F. (1976a). The resistance to human plasma of *Trypanosoma brucei*, *T. rhodesiense* and *T. gambiense*. I. Analysis of the composition of trypanosome strains. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **70**, 504–512.

Hawking, F. (1976b). The resistance to human plasma of *Trypanosoma brucei*, *T. rhodesiense* and *T. gambiense*. II. Survey of strains from East Africa and Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **70**, 513–520.

Hawking, F., Ramsden, D. B. and Whytock, S. (1973). The trypanocidal action of human serum and of baboon plasma. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **67**, 501–516.

Heisch, R. B., McMahon, J. P. and Manson-Bahr, P. E. C. (1958). The isolation of *Trypanosoma rhodesiense* from a bushbuck. *British Medical Journal*, **2**, 1203–1204.

Hoare, C. A. (1965). Vampire bats as vectors and hosts of equine and bovine trypanosomes. *Acta Tropica*, **22**, 204–216.

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

Hutchinson, M. P. (1954). The epidemiology of human trypanosomiasis in British West Africa. III. Sierra Leone. *Annals of Tropical Medicine and Parasitology*, **48**, 75–94.

Hutchinson, M. P. (1971). Human trypanosomiasis in south west Ethiopia (March 1967–March 1970). *Ethiopian Medical Journal*, **9**, 3–69.

Jordan, A. M., Lee-Jones, F. and Weitz, B. (1962). The natural hosts of tsetse flies in Northern Nigeria. *Annals of Tropical Medicine and Parasitology*, **56**, 430–442.

Kageruka, P., Colaert, J. and Nkuku-Pela, N. (1977). Strain of *Trypanosoma (Trypanozoon) brucei* isolated from pigs in Bas-Zaire. *Annales de la Société belge de Médecine tropicale*, **57**, 85–88.

Keymer, I. F. (1969). A survey of trypanosome infections in wild ungulates in the Luangwa Valley, Zambia. *Annals of Tropical Medicine and Parasitology*, **63**, 195–200.

Kilgour, V. and Godfrey, D. G. (1973). Species-characteristic isoenzymes of two aminotransferases in trypanosomes. *Nature (London), New Biology*, **244**, 69–70.

Kilgour, V. and Godfrey, D. G. (1977). Persistence in the field of two characteristic isoenzyme patterns in Nigerian *Trypanosoma vivax*. *Annals of Tropical Medicine and Parasitology*, **71**, 387–9.

Kilgour, V., Godfrey, D. G. and Na'isa, B. (1975). Isoenzymes of two aminotransferases among *Trypanosoma vivax* in Nigerian cattle. *Annals of Tropical Medicine and Parasitology*, **69**, 329–335.

Killick-Kendrick, R. and Godfrey, D. G. (1963). Observations on a close association between *Glossina tachinoides* and domestic pigs near Nsukka, Nigeria. *Trypanosoma congolese* and *T. brucei* infections in the pigs. *Annals of Tropical Medicine and Parasitology*, **57**, 225–231.

Kleine, F. K. (1928). Report on the new sleeping sickness focus at Ikoma. *Final Report of the League of Nations International Commission on Human Trypanosomiasis, Geneva*, pp. 7–20.

Lester, H. M. O. (1933). The characteristics of some Nigerian strains of the polymorphic trypanosomes. *Annals of Tropical Medicine and Parasitology*, **27**, 361–395.

Letch, C. A. (1979). Host restriction, morphology and isoenzymes among trypanosomes of some British freshwater fishes. *Parasitology*, **79**, 107–117.

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

Lumsden, W. H. R. and Herbert, W. J. (1975). Pedigrees of the Edinburgh *Trypanosoma (Trypanozoon)* antigenic types (ETat). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **69**, 205–208.

Mackichan, I. W. (1944). Rhodesian sleeping sickness in eastern Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **38**, 49–60.

McConnell, E., Hutchinson, M. P. and Baker, J. R. (1970). Human trypanosomiasis in Ethiopia: the Gilo river area. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **64**, 683–691.

Mehlitz, D. (1977). The behaviour in the blood incubation infectivity test of four *Trypanozoon* strains isolated from pigs in Liberia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **71**, 86.

Mehlitz, D. (1978). Untersuchungen zur Empfänglichkeit von *Mastomys natalensis* für *Trypanosoma (Trypanozoon) brucei gambiense*. *Tropenmedizin und Parasitologie*, **29**, 101–107.

Miles, M. A., Toyé, P. J., Oswald, S. C. and Godfrey, D. G. (1977). The identification by isoenzyme patterns of two distinct strain groups of *Trypanosoma cruzi* circulating independently in a rural area of Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **71**, 217–225.

Miles, M. A., Souza, A., Povoa, M., Shaw, J. J., Lainson, R. and Toyé, P. J. (1978). Isozymic heterogeneity of *Trypanosoma cruzi* in the first autochthonous patients with Chagas' disease in Amazonian Brazil. *Nature (London)*, **272**, 819–821.

Moloo, S. K., Losos, G. J. and Kutuza, S. B. (1973). Transmission of *Trypanosoma brucei* to cats and dogs by feeding on infected goats. *Annals of Tropical Medicine and Parasitology*, **67**, 331–334.

Molyneux, D. H. (1973). Animal reservoirs and Gambian trypanosomiasis. *Annales de la Société belge de Médecine tropicale*, **53**, 605–618.

Morris, K. R. S. (1960). Studies on the epidemiology of sleeping sickness in East Africa. II. Sleeping sickness in Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **54**, 71–86.

Mwambu, P. M. and Mayende, J. S. P. (1971). Sleeping sickness survey in Musoma District, Tanzania. III. Survey of cattle for the evidence of *T. rhodesiense* infections. *Acta Tropica*, **28**, 206–210.

Newton, B. A. and Burnett, J. K. (1972). DNA of Kinetoplastidae: a comparative study. *In* "Comparative Biochemistry of Parasites" (H. Van den Bossche, ed.), pp. 185–198. Academic Press, London.

Onyango, R. J., Van Hoeve, K. and de Raadt, P. (1966). The epidemiology of *Trypanosoma rhodesiense* sleeping sickness in Alego Location, Central Nyanza, Kenya, I. Evidence that cattle may act as reservoir hosts of trypanosomes infective to man. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **60**, 175–182.

Ormerod, W. E. (1961). The epidemic spread of Rhodesian sleeping sickness 1908–1960. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **55**, 525–538.

Ormerod, W. E. (1963). A comparative study of growth and morphology of strains of *Trypanosoma rhodesiense*. *Experimental Parasitology*, **13**, 374–385.

Ormerod, W. E. (1967). Taxonomy of the sleeping sickness trypanosomes. *Journal of Parasitology*, **53**, 824–830.

Paris, J., Wilson, A. J. and Gray, A. R. (1976). A study of the antigenic relationships of isolates of *Trypanosoma brucei* from three areas in East Africa. *Annals of Tropical Medicine and Parasitology*, **70**, 45–51.

Peters, W., Chance, M. L., Mutinga, M. J., Ngoka, J. M. and Schnur, L. F. (1977). The identification of human and animal isolates of *Leishmania* from Kenya. *Annals of Tropical Medicine and Parasitology*, **71**, 501–502.

Reeves, R. E. and Bischoff, J. M. (1968). Classification of *Entamoeba* species by means of electrophoretic properties of amebal enzymes. *Journal of Parasitology*, **54**, 594–600.

Rickman, L. R. (1974). Investigations into an outbreak of human trypanosomiasis in the lower Luangwa Valley, Eastern province, Zambia. *East African Medical Journal*, **51**, 467–487.

Rickman, L. R. (1977). Variation in the test response of clone-derived *Trypanosoma (Trypanozoon) brucei* and *T. (T.) b. rhodesiense* relapse antigenic variants, examined by a modified blood incubation infectivity test and its possible significance in rhodesian sleeping sickness transmission. *Medical Journal of Zambia*, **11**, 31–41.

Rickman, L. R. and Robson, J. (1970). The testing of proven *Trypanosoma brucei* and *T. rhodesiense* strains by the blood incubation infectivity test. *Bulletin of the World Health Organization*, **42**, 911–916.

Rickman, L. R. and Robson, J. (1974). Some observations on the identification of *Trypanosoma* (*Trypanozoon*) *brucei* species [sic] strains isolated from non-human hosts. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **68**, 166–167.

Rifkin, M. R. (1978). Identification of the trypanocidal factor in normal human serum: high density lipoprotein. *Proceedings of the National Academy of Sciences of the U.S.A.*, **75**, 3450–3454.

Robertson, D. H. H. (1960). The trypanosome collection: trypanosomes from man. *East African Trypanosomiasis Research Organization Annual Report January–December*, 1959, pp. 4–6.

Robertson, D. H. H. and Baker, J. R. (1958). Human trypanosomiasis in south-east Uganda. 1. A study of the epidemiology and present virulence of the disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **52**, 337–348.

Robertson, D. H. H. and Pickens, S. (1975). Accidental laboratory infection with *Trypanosoma brucei rhodesiense*—a case report. *Communicable diseases Scotland weekly report*, 9/8/75, pp. iii–vi.

Robson, J., Rickman, L. R., Allsopp, R. and Scott, D. (1972). The composition of the *Trypanosoma brucei* subgroup in nonhuman reservoirs in the Lambwe Valley, Kenya, with particular reference to the distribution of *T. rhodesiense*. *Bulletin of the World Health Organization*, **46**, 765–770.

Rothamstead Experimental Station (1977). "GENSTAT, a General Statistical Program" (user's manual).

Sargeaunt, P. G., Williams, J. E. and Grene, J. D. (1978). The differentiation of invasive and non-invasive *Entamoeba histolytica* by isoenzyme electrophoresis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **72**, 519–521.

Sheppard, R. L. (1946). Geographical distribution of sleeping sickness in Africa. *In* "A survey of recent work on trypanosomiasis and tsetse flies, 1932–1944" (Wilcocks, C., Corson, J. F. and Sheppard, R. L.), pp. 1–21. Bureau of Hygiene and Tropical Diseases, London, Review Monograph 1.

Shirley, M. W. and Rollinson, D. (1979). Coccidia: the recognition and characterization of populations of *Eimeria*. *Symposia of the British Society for Parasitology*, **17**, 7–30.

Stephens, J. W. W. and Fantham, H. B. (1910). On the peculiar morphology of a trypanosome from a case of sleeping sickness and the possibility of its being a new species (*T. rhodesiense*). *Annals of Tropical Medicine and Parasitology*, **4**, 343–350.

Tait, A. (1969). Syngen differences in electrophoretic mobility of certain enzymes in *Paramecium aurelia*. *Journal of Protozoology*, **16**, supplement, 28.

Targett, G. A. T. and Wilson, V. C. L. C. (1973). The blood incubation infectivity test as a means of distinguishing between *Trypanosoma brucei brucei* and *T. brucei rhodesiense*. *International Journal for Parasitology*, **3**, 5–11.

Van Hoof, L. M. J. J. (1947). Observations on trypanosomiasis in the Belgian Congo. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **40**, 728–761.

Van Meirvenne, N., Magnus, E. and Janssens, P. G. (1976). The effect of normal human serum on trypanosomes of distinct antigenic type (ETat 1 to 12) isolated from a strain of *Trypanosoma brucei rhodesiense*. *Annales de la Société belge de Médecine tropicale*, **56**, 55–63.

Van Meirvenne, N., Magnus, E. and Vervoort, T. (1977). Comparisons of variable antigenic types produced by trypanosome strains of the subgenus *Trypanozoon*. *Annales de la Société belge de Médecine tropicale*, **57**, 409–423.

Walker, E. P. and others (1964). "Mammals of the World". The Johns Hopkins Press, Baltimore.

Walker, P. J. (1964). Reproduction and heredity in trypanosomes. A critical review dealing mainly with the African species in the mammalian host. *International Review of Cytology*, **17**, 51–98.

Watson, H. J. C. (1960). Animal reservoirs of *T. gambiense*. *West African Institute for Trypanosomiasis Research Annual Report*, 1960, pp. 12–13.

Watson, H. J. C. (1972). The epidemiology of human sleeping sickness in the Lambwe Valley, South Nyanza, Kenya. *Bulletin of the World Health Organization*, **47**, 719–726.

WHO (1978). Proposals for the nomenclature of salivarian trypanosomes and for the maintenance of reference collections. *Bulletin of the World Health Organization*, **56**, 467–480.

Willett, K. C. (1956). The problem of *Trypanosoma rhodesiense*, its history and distribution, and its relationships to *T. gambiense* and *T. brucei*. *East African Medical Journal*, **33**, 473–479.

Willett, K. C. (1965). Some observations on the recent epidemiology of sleeping sickness in Nyanza region, Kenya, and its relation to the general epidemiology of Gambian and Rhodesian sleeping sickness in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **59**, 374–394.

Willett, K. C. and Fairbairn, H. (1955). The Tinde experiment: a study of *Trypanosoma rhodesiense* during 18 years of cyclical transmission. *Annals of Tropical Medicine and Parasitology*, **49**, 278–292.

Yorke, W. (1910). On the pathogenicity of a trypanosome (*T. rhodesiense*, Stephens and Fantham) from a case of sleeping sickness contracted in Rhodesia. *Annals of Tropical Medicine and Parasitology*, **4**, 351–368.

Yorke, W. and Blacklock, B. (1915). The reservoir of the human trypanosome in Sierra Leone. *Annals of Tropical Medicine and Parasitology*, **9**, 383–390.

Yorke, W., Adams, A. R. D. and Murgatroyd, F. (1929). Studies in chemotherapy. I. A method for maintaining pathogenic trypanosomes alive *in vitro* at 37°C for 24 hours. *Annals of Tropical Medicine and Parasitology*, **23**, 501–518.

Note added in proof (see p. 216). However, H. S. Stannus (*Nyasaland Sleeping Sickness Diary*, 1910, part 12, p. 15) identified "Hargreaves" with Chutika, near Kakumbi.

Immunity to *Trypanosoma cruzi**

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I. The Parasite and Chagas' Disease

Chagas' disease is a predominantly rural disease transmitted by blood-sucking vectors, which affects millions of people in Latin American countries. Migration of patients from rural areas, transmission by blood transfusion and congenital cases are transforming Chagas' disease into an urban problem in affected areas. Cardiac and/or digestive manifestations (megacolon and megaoesophagus) are found in a variable percentage of infected people and sudden death is a frequent event in the course of the disease. No cures by treatment have been proved parasitologically and immunological methods to prevent infection have not been developed.

Trypanosoma cruzi, the causative agent of Chagas' disease, is a digenetic trypanosomatid which circulates in the bloodstream of the vertebrate host as trypomastigotes and has also an obligatory intracellular phase in which the parasite multiplies as amastigotes which differentiate into trypomastigotes. From an immunological point of view, therefore, the parasite presents stages that are directly exposed to the effector elements of the immune response, such as antibodies and macrophages, as well as stages that are sequestrated within host cells. *T. cruzi* infection is characterized by an acute phase with large numbers of parasites and by a sub-patent chronic phase in which circulating and tissue stages are difficult to detect. The mechanisms involved in the resistance to the parasite and in the control of parasitism during the chronic phase are not known. Chagas' disease is life-long and spontaneous

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cures do not occur. The mechanisms of evasion which permit the parasites to overcome the host's immune response also are not clear. Finally, there is increasing evidence that auto-immune processes participate in the pathogenesis of the cardiac and digestive forms of the disease. These many obscure aspects and challenging problems explain the increasing use of *T. cruzi* as a model for the study of humoral and cellular immunity in both basic and applied immunology.

II. Antigenic Constitution

The antigenic heterogeneity of *T. cruzi* populations was established by Nussenzweig *et al.* (1962, 1963a) who typed strains isolated from man, vector and sylvatic animals by using, with culture form antigens, agglutination and precipitation tests in agar gel. After specific absorption of immune sera with culture forms, the strains could be separated into two types, *A* and *B*. The type-specific antigens (probably polysaccharides from the organisms' membrane) cross-reacted, suggesting a structural similarity between them. One strain derived from a naturally-infected carnivore was antigenically different from both *A* and *B* (type *C*). The serological types were not confined to particular animal species and were not related to the geographical distribution of the parasite populations studied (Nussenzweig and Goble, 1966). Types *A* and *B* could not be separated by protection tests (Nussenzweig *et al.*, 1963b), confirming previous observations on the existence of cross-resistance among different *T. cruzi* strains in the vertebrate host. Bergendi *et al.* (1970) studied the immunological characteristics of fractions from a soluble extract of *T. cruzi* culture forms using Ouchterlony double-diffusion and immunoelectrophoresis tests. Reactions performed with a human immune serum demonstrated the presence of five precipitin bands. Five antigenic fractions with different physicochemical properties could be identified but only three were antigenically reactive, a finding which the authors consider as very similar to that described by Nussenzweig and Goble (1966).

A detailed immunoelectrophoretic analysis of a soluble extract from *T. cruzi* culture forms detected at least 30 antigenic components (Afchain, 1976). One of the bands (component 5) showed remarkable intensity, was present in three different strains and remained fairly stable over a period of 3 years observation. Absorption of anti-*T. cruzi* immune sera with extracts from different trypanosomatids (*T. dionisii*, *T. rangeli*, *T. brucei* and *L. donovani*) demonstrated that this fraction was highly specific since no cross-reactions were observed. Antibodies against antigen 5 were detected in sera of most chronic chagasic patients. The preparation of a monospecific rabbit antiserum against antigen 5, by immunofluorescence and immunoperoxidase tests, allowed the localization of antigen 5 on the surface of culture epimastigotes and bloodstream forms, suggesting a glycoprotein nature for the antigen (Afchain *et al.*, 1978).

Protein typing by disc electrophoresis was performed with extracts from culture forms from *T. cruzi* and *T. cruzi*-like stocks of sylvatic origin. Although some basic common bands could be identified in all stocks, the

protein patterns were different and could be used as fingerprints for the populations examined (Ebert *et al.*, 1978). No significant differences could be observed between *T. cruzi* stocks isolated from humans and from sylvatic animals.

Membrane components have been little investigated in *T. cruzi*. A lipopeptidophosphoglycan was extracted from a macromolecular complex obtained from culture forms (Lederkremer *et al.*, 1976, 1977). This fraction was purified from other glycoproteins and was composed of mannose, galactose and glucose in the proportions 35 : 22 : 1. This compound inhibited concanavalin A-induced agglutination of epimastigotes (Alves and Colli, 1974) and was specifically concentrated in a membrane-enriched subcellular fraction. Adenylyl cyclase activity and protein-bound ^{131}I were significantly increased in this enriched fraction, strong evidence for the membrane location of the lipopeptidophosphoglycan. Nevertheless, the authors consider premature any analogy between this *T. cruzi* membrane component and the glycoproteins from the cell coat of *T. brucei*. Gottlieb (1977) isolated a polysaccharide-containing fraction from cultured epimastigote forms of *T. cruzi* which on acid hydrolysis also yielded galactose and mannose. By using antiserum prepared in rabbits against this fraction, Gottlieb detected a trypanosome-derived circulating polysaccharide fraction in the plasma of experimentally infected animals. The existence of common neutral sugars, such as mannose and galactose, between this circulating antigen ('exo-antigen') and the lipopeptidophosphoglycan fraction suggested a possible relationship between the two. The latter, however, failed to induce antibody synthesis in animals (Alves *et al.*, 1979).

The work of Gottlieb (1977) added evidence for the presence of circulating exo-antigens in *T. cruzi* infections. Dzbenski (1974) described the detection by double diffusion, immunoelectrophoresis and immunochromatography of an exo-antigen in the blood of infected animals. This circulating antigen had a molecular weight of 70,000, contained carbohydrates and bore some resemblance to the exo-antigen of *T. brucei*. Circulating antigens have been also described by Araujo (1976) in the plasma of acutely infected mice; those antigens reacted against serum of mice chronically infected with *T. cruzi* by complement fixation test and by counterimmunoelectrophoresis. Sera from humans with chronic Chagas' disease also reacted against the circulating antigens.

The origin of the circulating antigen has been discussed by Gottlieb (1977), who mentioned three possibilities: (a) secretion by the parasite; (b) release of soluble surface components; (c) leakage from the cell during separation of the plasma or by parasite degradation. Considering that the circulating antigen is precipitated by concanavalin A, that the parasites present concanavalin A receptors and, finally, that carbohydrates are identified in the membrane surface of *T. cruzi* by cytochemical methods, Gottlieb suggested a surface origin for the circulating antigen. If this is the case, an analogy with the surface variant antigen of *T. brucei* could be made. Dzbenski (1974) suggested this similarity, based on the irregular presence of the exo-antigen in the many investigated blood samples from infected animals. Actually,

there is no sound evidence to support this analogy: the peculiar surface coat characteristic of *T. brucei* bloodstream forms is not apparently present in *T. cruzi* (Maria *et al.*, 1972) and antigenic variation has not yet been shown in *T. cruzi* infections. The implications of the presence of circulating antigens in both animals and humans are not clear and the possible role of immune complexes in the course of infection in Chagas' disease has not yet been fully investigated. Castro and Ribeiro dos Santos (1977) reported that kidneys of rats chronically infected with *T. cruzi* presented alterations in the mesangial area and basement membrane as well as deposits of immunoglobulins, C3b and fibrinogen, suggesting an immunocomplex nephropathy. Specific antigens however, could not be detected in the kidneys.

Ketteridge (1978) extracted a lipopolysaccharide from culture forms, but provided no indication of its location and no evidence that it was a surface component. A detailed study of subcellular fractions of trypomastigote and amastigote forms derived from *T. cruzi*-infected tissue cultures was published by Teixeira and Santos-Buch (1974). The homogenates obtained after disruption in a high-speed rotation tissue homogenizer, and differential centrifugation, yielded six fractions. The highest haemagglutination titres in rabbits were induced by the total homogenate and by the cytosol fraction, whereas the most intense cell-mediated immune response (evaluated by inhibition of mononuclear cell migration) was observed with a membrane-rich fraction. A number of subcellular fractions obtained by differential centrifugation and characterized by enzyme and electron-microscopy analysis have been described by Segura *et al.* (1974, 1977).

III. Natural Immunity to *T. Cruzi*

A wide range of hosts, including wild and domestic animals, are parasitized by *T. cruzi* (see review by Brener, 1973). Infection with *T. cruzi*-like parasites has been reported in more than 100 mammalian species from many different orders. Amphibians and birds, however, are refractory to *T. cruzi* (Dias, 1933, 1944). The mechanism of the avian resistance to trypanosomal infections has been recently described by Kierszenbaum *et al.* (1976). The resistance is apparently related to the complement-dependent capacity of chicken sera to lyse bloodstream forms *in vitro*. Parasites injected into decomplemented chickens could be detected for at least 24 h in the bloodstream but disappeared within 1 minute in normal animals. Hormonal bursectomy or corticosteroid administration did not render chickens susceptible to *T. cruzi*. Antibodies did not play any role in this complement-dependent lysis since agammaglobulinaemic chickens also were able to destroy the trypomastigotes. Nevertheless, with at least one of the methods used by the authors, bursectomy, the existence of gammaglobulin cannot be completely excluded (Leslie and Martin, 1973). The lytic effect of chicken serum was detected in the absence of calcium ions but required magnesium, evidence that the alternative pathway of complement was involved in the process of parasite destruction. Since the mechanism by which parasites skin-inoculated into chickens are destroyed is not apparently, phagocytosis, complement-mediated

lysis seems to be the most important process operating in this natural resistance (Nery-Guimãraes, 1972). Some biological characteristics (age, sex) and environmental conditions (temperature) which increase the vertebrate host resistance have been reviewed by Brener (1973).

IV. The Immune Response

A. HUMORAL IMMUNITY

1. *Immunoglobulins*

Patients in both acute and chronic Chagas' disease do not display the impressive high levels of IgM present in African trypanosomiasis. Data concerning the immunoglobulin levels in *T. cruzi* infections are controversial and conflicting statements are found in the literature. Lelchuk *et al.* (1970) detected normal serum concentrations of IgG, IgM and IgA in patients in the acute phase, although anti-trypanosome IgM antibodies were regularly observed. Vattuone *et al.* (1973), however, reported that IgM and IgG concentrations were significantly higher in the acute phase than in chronic cases or in uninfected controls. Schmunis *et al.* (1978a) found that the concentration of IgM in patients in the acute phase was higher than the average concentration determined in healthy controls. Some patients presented increases of IgG and/or IgA concentrations. Hanson *et al.*, (1974) investigated immunoglobulin levels in a laboratory-acquired acute case of Chagas' disease. IgM and IgG were within normal limits on day 19 of the infection, increased between 40–100 days and were normal again on day 159. Camargo and Amago Neto (1974) reported that specific IgM-antibodies were detected by immunofluorescence in 19 acute cases of post-transfusional or vector-derived origin; such antibodies could not be detected in 40 chronic cases of Chagas' disease. A detailed electrophoretic study of serum immunoglobulins in CFl mice experimentally infected with *T. cruzi* was published by Hanson (1977).

At the chronic stage also results are controversial. Lelchuk *et al.* (1970) found normal levels of IgM and IgA, and a significant but small increase in IgG. According to Vattuone *et al.* (1973), IgG levels were significantly increased in chronic patients compared with the control group. Marsden *et al.* (1970), however, found that in patients with chronic Chagas' disease, IgG, IgM and IgA levels were rather similar to those detected in uninfected people living in the same area.

The reasons for the discrepancies reported are not clear. Some differences, however, could be explained by methodological variations related to the samples used in the experiments. In the case of Lelchuk *et al.* (1970), for instance, the average concentration of IgM in the control group was higher than that reported by Vattuone *et al.* (1973), a fact which probably reduced the significance of data from the test and control groups. Marsden *et al.* (1970), on the other hand, selected their control group from people with negative serology but living in the same village as the infected group, a procedure which is unlikely to have been followed in the other related studies.

2. *Role of antibodies in host resistance*

Circulating antibodies in Chagas' disease have been known since early investigators detected complement-fixing antibodies in human patients (Guerreiro and Machado, 1913). Further investigations demonstrated the existence of specific agglutinins and precipitins in both acute and chronic stages of the disease (Muniz and Freitas, 1944a, 1944b). A number of serological tests (complement fixation, immunofluorescence, haemagglutination, ELISA) are now available for the diagnosis of Chagas' disease. Nevertheless, the role played by antibodies in the resistance against *T. cruzi* remained controversial for many years. Culberston and Kolodny (1938) and Kolodny (1940) were among the first authors to demonstrate the protective effect of immune sera by passive-transfer experiments. Injection of sera from animals that survived the acute phase of *T. cruzi* infection into normal rats induced a significant decrease in parasitaemia and mortality following a challenge with virulent parasites. Similar results have been reported by Kagan and Norman (1961). According to Hauschka *et al.* (1950), however, repeated daily injections of immune sera from mice in the chronic phase did not protect normal mice against a lethal homologous challenge. In mice with already established patent infection, the administration of anti serum against culture forms of *T. cruzi* affected neither the course of parasitaemia nor mortality rates (Voller and Shaw, 1965).

More recent investigations have clearly demonstrated the participation of antibodies in the host resistance against *T. cruzi* and have shed light on the discrepancies in results reported in earlier papers. The effects of immune sera, from mice with chronic infections, on bloodstream forms from two *T. cruzi* strains (*Y* and *CL*), as well as the protective action of those sera *in vivo*, have been investigated by Krettli and Brener (1976) and Krettli (1978). Trypomastigotes of the *Y* strain were strongly agglutinated *in vitro* by homologous and by anti-*CL* immune sera, forming clumps of variable numbers of parasites (Fig. 1). Agglutination occurred with immune sera collected from animals with 7–22-week-old infections but not with sera from mice with a 4-week-old infection. Sera from chronically-infected patients also agglutinated *Y* parasites. No agglutinated parasites were observed, however, after incubation of trypomastigotes from the *CL* strain with either homologous or heterologous immune sera (Fig. 1). Preincubation of the trypomastigotes from the *Y* strain with immune mouse sera, or with sera from chronic patients, significantly decreased their infectivity, inducing, when inoculated into normal mice, lower parasitaemias than those observed with untreated parasites. Parasitaemia levels in mice inoculated with immune serum-treated *CL* parasites were not affected.

The passive protective effect of transfer of serum has also been investigated for both strains. Mice that received undiluted homologous and heterologous immune sera 1 hour before a bloodstream-form challenge with *Y* strain presented significantly lower parasitaemias and mortalities than those injected with normal mouse serum (Fig. 2). The course of parasitaemia and the mortality rates were, however, not altered in animals that received immune sera, and were inoculated with the *CL* strain.

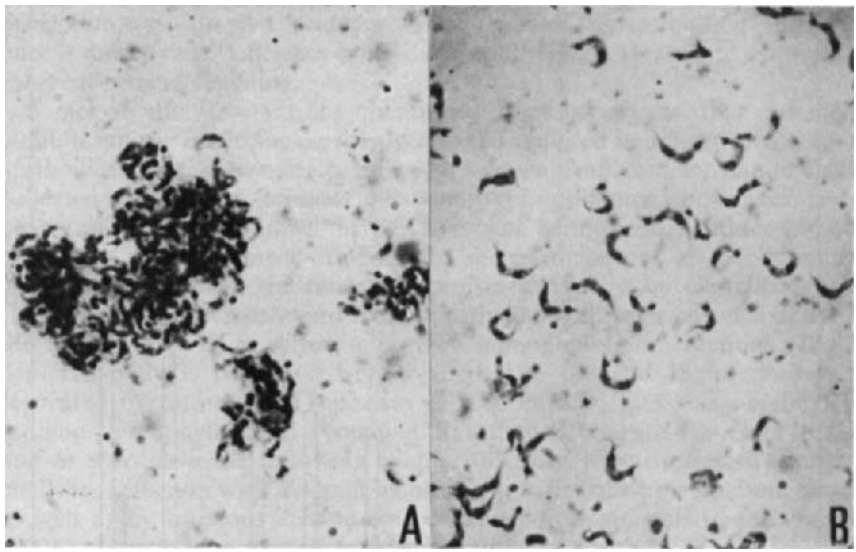

FIG. 1. Effect of immune serum on bloodstream forms of *T. cruzi*. A, Clumps of agglutinated parasites from the *Y* strain; B, non-agglutinated parasites from the *CL* strain. (After Krettli and Brener, 1976.)

The reasons for the impressive differences between the *Y* and *CL* strains as regards the effects of immune sera are not yet fully understood. They are likely to be related to the wide range of differences observed *in vitro* and in the living host among those 'polar' types of *T. cruzi* populations (Brener, 1977). As mentioned by Krettli and Brener (1976), the differences described in their paper as regards the *Y* and *CL* strains closely parallel previous experiments showing that slender bloodstream trypomastigotes, which predominate in the *Y* strain, inoculated intravenously into immune mice, are very soon cleared from the blood, whereas stout forms, predominant in the *CL* strain, are able to keep circulating for many days (Brener, 1969; Howells and Chiari, 1975). It is conceivable that similar intraspecific variations may account for the discrepancies reported in the literature in relation to humoral mechanisms of immunity. This suggestion is at least partially supported by McHardy (1977). He performed cross, passive immunization experiments with sera from mice convalescent from infections with strains *Y* and *Tulahuen*. Sera from mice inoculated with the *Y* strain conferred strong protection against the homologous infection but only slight protection against the *Tulahuen* strain; *Tulahuen* strain immune sera afforded protection against *Y* but little against the homologous strain. The author suggested that the variability of results may be due to differences in behaviour between *T. cruzi* of various stocks.

These results strongly suggest that *T. cruzi* can no longer be considered, from an immunological point of view, as an homogeneous species but rather as a complex of parasite populations. Morphology, susceptibility to antibodies

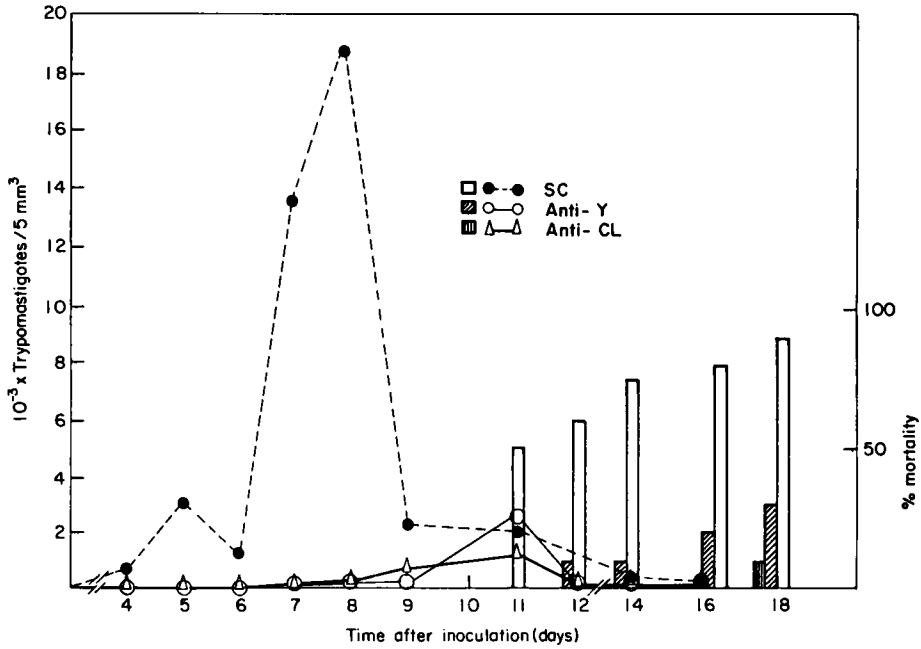

FIG. 2. Effect of passive transfer of serum on the course of infection and mortality in mice inoculated with *T. cruzi* (*Y* strain). SC, Normal mouse serum; anti-*Y* and anti-*CL*, respectively, homologous and heterologous sera. (After Krettli and Brener, 1976).

and other biological characteristics should be described in any study related to this parasite in order to permit comparison of results.

Further evidence of the importance of antibodies in host resistance was provided by Hanson (1977) who observed that in mice the increase in total serum gammaglobulins and in IgG is related to the decrease of circulating and intracellular parasites. The maximum concentration of those serum factors occurred about 6 weeks after inoculation. Serum collected from donor mice at this time and passively transferred to recipient mice induced a marked decrease in parasitaemia and mortality after challenge. The density of intracellular stages was correspondingly lower in the recipient mice.

The specific role of antibodies in the host resistance was investigated by Kierszenbaum and Howard (1976) using high (Ab/H) and low (Ab/L) responder mice. These two genetically-selected lines of animals differ in their ability to produce all classes of immunoglobulins but show negligible differences as regards cell-mediated immune response. The low responder mice were more susceptible to inoculation of two *T. cruzi* strains than the high responder animals, as evaluated by maximal parasitaemia and minimal mean survival time. The effects of passive antibody transfer were determined in Ab/L mice by injecting immune sera collected from animals with chronic Chagas' disease and challenging them with a highly virulent strain. Clear

protection was observed, evidence that the greater susceptibility of the Ab/L mice is due to their deficiency in antibody synthesis rather than to some other inherent genetic disability.

Some of the observations mentioned already suggest that immunoglobulins of the IgG class are involved in the acquired immunity mediated by antibodies: passive transfer of immunity was not affected by immune sera collected at the early stages of infection; IgG antibodies in mice reached a peak just when the number of parasites was being greatly reduced in the blood and organs. Direct evidence for the participation of IgG immunoglobulins in the host resistance mechanism was given by Castello Branco (1978). Immune sera collected from mice in the acute and chronic stages of the disease, as well as different purified immunoglobulin fractions (7S, 7S deprived of IgG, 19S, IgG) were studied. The 19S and 7S fractions were separated by filtration in Sephadex G-200. Purified IgG was obtained by binding to staphylococcal Protein A linked to Sepharose-4B. Tests *in vivo* and *in vitro* gave the following results: (a) mice were protected against a challenge infection with *Y* strain by passive transfer of whole immune serum, as well as by fractions 7S and IgG obtained from animals in the chronic phase; no protection was observed in groups of mice that received fractions 19S and 7S deprived of IgG; (b) no protection was provided by passive transfer of total immune serum, 19S and 7S from animals in the acute phase of the disease; (c) agglutination of bloodstream forms was observed with whole immune sera, and with 7S and IgG fractions from mice in the chronic phase; fraction 7S deprived of IgG had no agglutinating effect. These data clearly established that IgG is the immunoglobulin inducing resistance during the chronic phase. Fractionation of immune sera from chronically infected animals, using absorption with Protein A, and monospecific antisera, suggests that the protective effect depends on IgG2a and IgG2b but not on IgG1 (Takehara *et al.*, 1978).

3. *The spleen and the host resistance*

Despite its function as an immunological effector organ, the role of the spleen in the host immune response to parasites is still obscure. Removal of the spleen probably affects not only antibody production but also interferes with the regulation of other important immune functions, such as the traffic and sequestration of lymphocytes, as well as the clearance of parasites from the bloodstream. The complexity of the situation is illustrated by studies on the role of the spleen in malaria recently reviewed by Wyler *et al.* (1978). Despite some well-known processes in which the spleen participates, such as production of antibodies, opsonin-mediated clearance of parasites and 'pitting', the ways in which this organ influences the immune response are probably still uncertain.

In relation to Chagas' disease the bulk of evidence suggests that in spite of the clear participation of antibodies in the resistance to *T. cruzi*, the spleen has a minor role in host resistance. A recent review of the subject (Brener *et al.*, 1978) concluded that splenectomy, carried out by many authors in

many animals (mice, rats, guinea-pigs, dogs) did not significantly affect the course of infection when performed either before inoculation or during established infections. Brener *et al.* (1978) reinvestigated this problem by inoculating *T. cruzi* into groups of splenectomized, sham-splenectomized and intact mice. The general course of infection was similar in all three groups. Splenectomy has also been performed in mice chronically infected with *T. cruzi Y* and *CL* strains; in neither group were new episodes of high parasitaemia observed and the antibody titres, as evaluated by indirect immunofluorescence technique, were unchanged by spleen removal. No acute phase occurred in splenectomized animals after a challenge with the homologous strains. In summary, these data confirm that the spleen does not play a critical role in the protective immunity against *T. cruzi.*

Some *T. cruzi* strains display a selective parasitism of phagocytic cells from the spleen (Melo and Brener, 1978) and induce some local phenomena which may have immunological implications. For instance, a sudden and extensive destruction of intracellular parasites *in situ* occurs in the spleen together with a massive depletion of lymphoid cells. Melo and Brener (1978) suggested that infected macrophages might act as target cells for sensitized lymphocytes, an explanation that would agree with the fact that cell depletion occurs in spleen regions that are highly parasitized. The immunological consequences of this selective tropism of some strains for the spleen are not clear, but they are likely to be related to the immunosuppression that occurs in acute infections of *T. cruzi.*

4. *Complement and* T. cruzi

T. cruzi epimastigote culture forms are lysed by some normal sera from susceptible and naturally resistant hosts (Muniz and Borrielo, 1945; Rubio, 1956). Metacyclic trypomastigotes from cultures, however, are lysed only by sera from birds and frogs. Nogueira *et al.* (1975) investigated the role of complement in the selective lysis of epimastigotes by normal mammalian sera as well as the relative importance of the alternative and classical pathways for the activation of complement in this process. These authors concluded that it is the alternative pathway of complement activation which generates the lytic activity, on the basis of the following findings: the process depends on Mg^{2+} and not Ca^{2+}; the lytic effect is abolished by heating the serum at 50°C for 30 min; lysis is inhibited by removal of properdin; lytic activity is present in C4 deficient guinea-pig serum. Antibodies are not involved in the phenomenon, as is demonstrated by the lysis of flagellates cultivated in serum-free medium.

Bloodstream trypomastigotes of *T. cruzi* collected from acutely-infected mice are lysed by chicken, frog and toad sera (Rubio, 1956; Kierszenbaum *et al.*, 1976). These forms of the parasite had been considered resistant to the lytic effect of normal human, mouse and guinea-pig sera (Rubio, 1956; Budzko *et al.*, 1975). Lysis of bloodstream forms, however, was observed when they were incubated with sera of patients with chronic Chagas' disease, sera of immunized mice or immune mouse gammaglobulin plus guinea-pig-

derived complement (Budzko *et al.*, 1975; Kierszenbaum *et al.*, 1976). The lysis was clearly dependent on the presence of complement, which was activated in the human sera by both the classical and alternative pathways. Normal human sera, however, failed to induce lysis of the parasites.

More recently, Krettli and Nussenzweig (1977) and Krettli *et al.* (1979) investigated the effects of fresh human serum as a source of complement (HuC) on bloodstream forms of different *T. cruzi* strains collected from acutely infected mice. Parasites from the *Y* and *Berenice* strains were readily lysed on incubation with HuC, but those from the *CL* and *Gilmar* strains were not affected. Extensive studies performed with bloodstream stages from the *Y* strain demonstrated that the complement-mediated lysis depends on the presence of antibodies on the surface membrane of the trypomastigotes. The following evidence has been provided to support this suggestion: (a) antibodies bound to the membrane have been directly demonstrated by immunofluorescence and with radiolabelled anti-mouse immunoglobulins; (b) bloodstream forms collected from lethally X-irradiated mice, and lacking membrane-bound immunoglobulins, could not be lysed by HuC; these parasites, however, were lysed when exposed to HuC after being incubated with sera from acutely or chronically infected mice; (c) parasites failed to be lysed when the membrane-bound antibodies were eluted by previous incubation at 37°C; (d) trypomastigotes released from macrophages infected *in vitro* were not lysed by HuC. Experiments involving coated trypomastigotes, collected from X-irradiated mice, with different classes of immunoglobulin demonstrated that IgG participates strongly in the immune lysis mediated by complement (Castelo Branco, 1978).

The lysis of bloodstream stages is mainly related to complement activation by the alternative pathway, as shown by the inhibition of the lytic effect of HuC depleted of factor B or properdin (Krettli *et al.*, 1979). Nevertheless, the fact that a significant decrease of lytic activity was observed with a C2-deficient serum or in the absence of Ca^{2+} suggests that lysis results from a combined participation of both the alternative and classical pathways (Krettli *et al.*, 1979).

The reason for the absence of complement-mediated lysis of bloodstream forms of the *CL* strain, despite the presence of membrane-bound antibodies, remains obscure. It is unlikely to depend on the anti-*CL* antibodies being of a different nature, since incubation of *Y* trypomastigotes collected from X-irradiated mice with anti-*CL* immune sera renders them susceptible to lysis by complement. O the other hand, *CL* trypomastigotes are not basically resistant to complement-mediated lysis, since incubation with sera from chronically-infected mice plus HuC induces their lysis. Apparently, the parasites are equipped with evasion mechanisms that prevent complement-mediated lysis. Whether these mechanisms involve modulation of surface antigens as described in other systems, or membrane peculiarities, is not yet clear. There is some evidence that *CL* parasites release bound ^{125}I-labelled antibodies much more rapidly than the *Y* parasites (Krettli *et al.*, 1979).

The part played by immune-lysis mediated by complement in the host's resistance to trypanosomes remains controversial. In *Trypanosoma musculi*

infections in mice, the control of parasitaemia depends on C3 factor, but in *Trypanosoma lewisi* infections complement is not important in the host's resistance (Jarvinen and Dalmasso, 1977). Host factors may apparently be influential since, for instance, *Trypanosoma congolense* activates bovine, but not guinea pig, complement (Nielsen and Sheppard, 1977). As regards *T. cruzi*, Budzko *et al.* (1975) reported that depletion of complement by cobra venom factor exacerbated the course of infection in experimentally infected mice and suggested that lysis of bloodstream parasites *in vivo* was responsible for the control of parasitaemia. Krettli (1978), however, demonstrated that the patterns of parasitaemia and mortality in C5 deficient mice were similar to those in mice with normal C5 levels, evidence that immune lysis was not playing a significant role in the control of the infection (Fig. 3). An explanation for this apparent discrepancy is that complement factor C3 may act as an opsonizing agent, increasing the uptake and destruction of the parasites by activated macrophages. If this is the case, only depletion of C3 by cobra venom factor and not deficiency of C5 would influence the course of infection. Moreover, according to Jarvinen (1976) a C4-dependent process is important in the resistance of guinea pigs to *T. cruzi* infections.

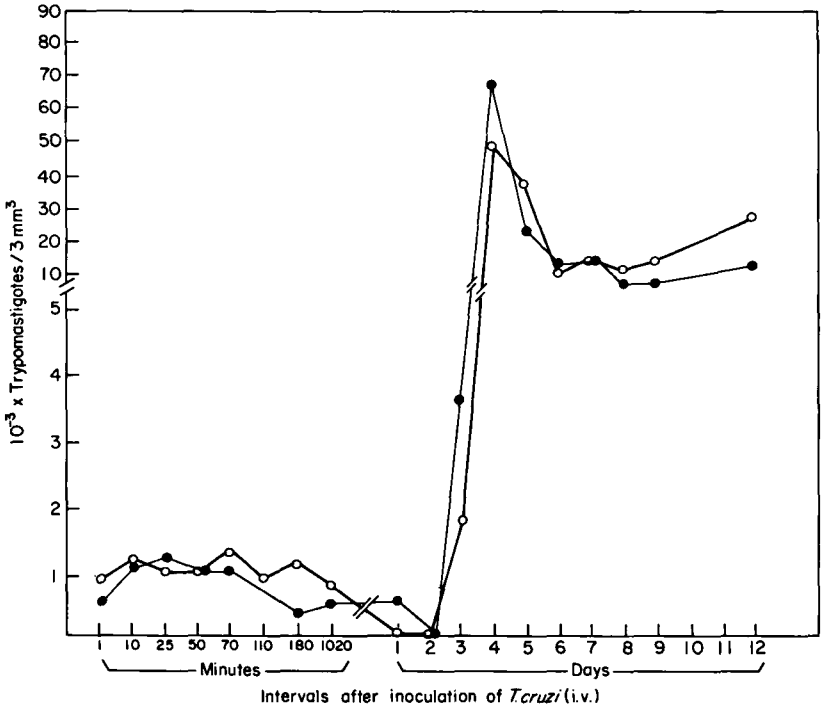

FIG. 3. Curves of parasitaemia in groups of C5-deficient mice (○, DBA/2J) and groups of mice with normal levels of C5 (●, DBA/1J); inoculated with *T. cruzi* (*Y* strain). (After Krettli, 1978.)

5. *Interferon*

Interferon and its inducers affect the development in tissue culture of some intracellular protozoa such as *Toxoplasma* (Remington and Merigan, 1968) and *Eimeria* (Fayer and Baron, 1971). A protective effect of interferon has been described in sporozoite-induced *Plasmodium berghei* infection in mice (Jahiel *et al.*, 1968). As an obligatory intracellular protozoan, *T. cruzi* also could be affected by this known anti-viral agent. Such an effect could be very important when we take into consideration that *T. cruzi* infection might itself stimulate the release *in vivo* of an interferon-like substance which could then have a regulatory role in the acute phase.

Rytel and Marsden (1970) reported that *T. cruzi* infection in mice was associated with an anti-viral factor that showed the characteristics of interferon. The amount of this substance reached a peak in the blood several days after inoculation, a finding that agrees with the kinetics of interferon release in other protozoan infections and is probably related to their slower generation time. Schmunis *et al.* (1977b) investigated the production of interferon by different *T. cruzi* strains in mice, evaluating its inhibitory effect by using 'L' cells line and GD7 virus. Interferon was apparently released as a consequence of cell penetration by the parasite but no correlation between parasitaemia levels and the amounts of interferon released could be established.

In the vertebrate host, interferon inducers are unable to afford protection against *T. cruzi* and there is evidence that they even enhance the infection. Martinez-Silva *et al.* (1970) studied the effect of the interferon stimulator polyinosinic-polycytidylic acid (poly I-C) and observed that parasitaemia and mortality were increased in the treated mice. Similar results have been reported by Kumar *et al.* (1971) in mice treated with the poly I-C interferon inducer. However, it is not yet clear whether interferon itself influences the lethality of *T. cruzi* in infected animals. In groups of infected mice which showed large amounts of interferon in the blood, the mortality rates were not higher than in groups in which the substance was not detectable (Schmunis *et al.*, 1977b).

The double-stranded poly I-C interferon inducer does not affect the intracellular development of *T. cruzi* in tissue culture (Martinez-Silva *et al.*, 1970). More direct evidence that interferon does not hinder the *T. cruzi* intracellular cycle in tissue culture has been provided by Golgher *et al.* (1976). Interferon of human origin was obtained by infecting primary human amniotic cells with inactivated Newcastle disease virus. This antiviral agent was then added to 'Vero' cell monolayers before and after inoculation with *T. cruzi* culture trypomastigotes. Interferon neither prevented the infection of the 'Vero' cells, nor affected the intracellular amastigote–trypomastigote transformation.

As discussed earlier (Brener, 1973), the failure of interferon to protect against *T. cruzi* infection may be explained by the fact that interferon apparently disturbs the synthesis of viral proteins by interfering with the translation of mRNA from the host cell ribosomes, whereas in *T. cruzi*, protein synthesis is carred out by the parasite's own biochemical machinery.

B. CELL-MEDIATED IMMUNITY

1. *Tests* in vitro

Some tests *in vitro* correlate with delayed hypersensitivity and provide sound evidence for the existence of cell-mediated immunity *in vivo*. Products of activated T lymphocytes may be detected indirectly, for instance by tests that evaluate inhibition of phagocytic cell migration by specific antigens. As regards Chagas' disease, Yanovsky and Albado (1972) reported a significant inhibition by soluble *T. cruzi* antigens of peripheral leukocytes collected from chronic patients compared with those from normal donors. Lelchuk *et al.* (1974) obtained similar results with gluteraldehyde-treated antigens. Inhibition of migration of mononuclear cells (MIF test) was observed by Schmunis *et al.* (1973) when specific antigen was added to peritoneal cells from *T. cruzi*-inoculated mice.

Cell-mediated immunity has been studied also in rabbits immunized with *T. cruzi* subcellular fractions and in rabbits with chronic infections. An interesting correlation was found between the degree to which the migration of blood mononuclear cells was inhibited and the nature of the subcellular fraction used to immunize the animals. Among eight fractions, two particulate antigens (F1 and the membrane-rich fraction F5) elicited a much greater inhibition than, for instance, the cytosol fraction; also in the chronically infected animals, the F5 fraction induced greater inhibition of mononuclear cell migration (Teixeira and Santos-Buch, 1975).

The response of peripheral lymphocytes from patients with chronic Chagas' disease to *T. cruzi* antigens and phytohaemagglutinin was investigated *in vitro* (Tschudi *et al.*, 1972). The specific antigens yielded about 5 to 30% lymphocyte transformation in the infected patients, whereas no significant transformation was elicited in normal controls. The response to phytohaemagglutinin was similar in both groups.

A comprehensive study of cellular immunity in patients with chronic Chagas' disease was published by Montufar *et al.* (1977). The evaluation of the thymus-dependent immune competence showed that cellular immunity is operative in Chagas' disease: migration of peripheral leukocytes was inhibited by *T. cruzi* antigens; the percentage and absolute numbers of peripheral T-lymphocytes were not different between infected patients and normal controls; the rates of sensitization by chlorodinitrobenzene (CDNB) were similar in patients and in normal controls and, finally, lymphocyte transformation by phytohaemagglutinin was alike in both groups. An important aspect which would deserve further investigation was the observation that in a few patients with the digestive form of the disease (megacolon) a slight decrease in the cellular immunity response was detected.

Lelchuk *et al.* (1977) investigated the effects of specific treatment with a nitrofuran compound on cell-mediated immunity in patients with chronic Chagas' disease. In the presence of *T. cruzi* antigens, the peripheral leucocyte migration index was significantly lower in untreated patients than in normal controls; in treated patients the migration index was similar to that found in the normal group. Since changes in the humoral response were not detected

in treated patients, the authors suggest that the nitrofuran exerts a particular effect on cell-mediated immunity, not apparently related to parasite eradication.

2. *Delayed hypersensitivity*

The existence of a delayed type of skin reaction in Chagas' disease remains controversial. Analysis of discrepancies is complicated by the diversity of antigens used, which include whole homogenates of culture forms, different subcellular fractions and tissue-culture infected-cell extracts. Earlier papers have been reviewed by Goble (1970) and most of them report negative results in both the human and the experimental disease. Positive delayed skin reactions, however, have been described by Amato Neto *et al.* (1964) in chronic patients using a soluble fraction extracted from infected cells and released trypomastigotes obtained from tissue-culture infected with *T. cruzi*. An insoluble fraction prepared from a suspension of culture forms disrupted under pressure gave delayed positive skin reactions in infected guinea-pigs (Gonzalez-Cappa *et al.*, 1968). Skin tests with culture form antigen were performed in two immunized and one 3-month-infected rhesus monkeys. The three animals presented delayed tuberculin type of reaction (Seah *et al.*, 1974). The skin response to an initial homogenate and to different subcellular fractions prepared from tissue culture parasites were studied in immunized rabbits and in chronically-infected rabbits (Teixeira and Santos-Buch, 1975) Both immediate and delayed-type reactions were detected with the homogenate and with the homologous antigens. Nevertheless, particulate antigens elicited more intense delayed rather than immediate reactions. Zeledón and Ponce (1974) devised a skin test for the diagnosis of Chagas' disease using culture forms submitted to repeated freezing and thawing at $-70°C$ and then centrifuged. In chronic patients the soluble fraction gave clear immediate positive reactions within 10–15 minutes, whereas the insoluble antigen induced weak delayed reactions that could not be used for diagnostic purposes.

3. *Cell-mediated immunity and resistance*

Some experimental manipulations that interfere mainly with the host cell-mediated immune mechanisms have been shown to enhance the severity of *T. cruzi* infection. Neonatal thymectomy in mice induced infections with higher parasitaemia and mortality than in intact hosts (Schmunis *et al.*, 1971); antibody synthesis, as evaluated by a number of serological tests, was delayed in the thymectomized animal. Parasitaemia and mortality were significantly increased in neonatally thymectomized rats compared with sham-operated and intact control animals (Roberson *et al.*, 1973). The production of protective antibodies, however, was not affected, as was demonstrated by the fact that passive transfer of serum from thymectomized rats protected mice against a challenge infection. Antithymocyte serum (ATS) increased the number of bloodstream and tissue stages in acutely infected mice, but was unable to induce new episodes of parasitaemia when

administered to animals which had recovered from the acute phase and presented subpatent parasitaemia (Roberson *et al.*, 1973).

Transfer of resistance to syngeneic animals by inoculation of spleen cells from infected mice and rats has been described (Santos, 1973; Roberson and Hanson, 1974). The induction of protective immunity against *T. cruzi* in mice by adoptive immunization with spleen cells from infected donors was studied by Kuhn and Durum (1975), who demonstrated that resistance could be induced by splenocytes collected from mice after 9 days of infection.

These experiments do not characterize the effector mechanisms of resistance against *T. cruzi* in the living host. Since in the spleen cell transfer experiments, *T* lymphocytes as well as antibody-producing cells were inoculated into the recipient animals, we do not know whether T lymphocytes are the only cells involved in the control of the infection. Preliminary results with treatment of sensitized spleen cells with anti-θ serum suggest that T cells are not the sole elements involved in the transferred resistance and that B cells probably also participate (Hanson, 1977). The neonatally thymectomized and ATS-treated animals used by Roberson *et al.* (1973) showed a depletion of circulating lymphocytes but further investigation, to identify specific cells, was not carried out.

4. *Cytotoxicity mechanisms*

Direct cytotoxic mechanisms which may contribute to the host's resistance have been little investigated in experimental *T. cruzi* infections. The scanty research in this field has been insufficient to answer clearly some crucial questions such as: is cytotoxicity directed against both circulating and intracellular stages of the parasite?; what are the effector cells?; do antibodies mediate cytotoxic effects?; which classes of antibodies are involved?; what is the role of cytotoxicity in the resistance?

Antibody-dependent cell-mediated cytotoxicity has been investigated by Abrahmsohn and da Silva (1977) and Sanderson *et al.* (1978). Unfortunately, in both papers epimastigotes derived from acellular culture medium were used instead of bloodstream forms, a choice that restricts the implications of the results as regards the vertebrate host. In the first paper the effects of normal mouse splenic lymphocytes on epimastigotes was evaluated by observing reductions in the number of motile parasites. A significant decrease in the number of motile antibody-coated epimastigotes was observed on incubation with spleen cells. Sanderson *et al.* (1978) used, instead, release of ^{3}H-labelled macromolecules as the criterion of parasite destruction by normal mouse spleen cells. Previous observations had demonstrated that, with culture forms cultivated in a medium containing [^{3}H]uridine, the release of ^{3}H-labelled RNA was a more suitable measure than DNA or protein release; RNA release above control levels was detected only when labelled parasites were incubated with antibody and spleen lymphocytes, strongly indicative of parasite degeneration.

The nature of the spleen cells which exert direct cytotoxic activity on epimastigote stages was further investigated (Sanderson *et al.*, 1977). Spleen

cells from normal mice were fractionated by centrifugation with Ficoll–Hypaque into high-and low-density fractions. Both fractions were tested against antibody-coated tumour cells (as an assay for K cells) and antibody-coated *T. cruzi* epimastigotes. The high-density cell fraction displayed little activity against tumour cells but was very active against epimastigotes, as demonstrated by a high RNA release; the fraction active in killing tumour cells had a negligible effect on *T. cruzi*. These observations apparently excluded the participation of K cells in cell-mediated cytotoxicity. Preliminary experiments with rat peritoneal exudates presenting high proportion of granulocytes strongly suggested that eosinophils were the effector cells that killed epimastigotes in the presence of antibody. This is a rather interesting finding since antibody-dependent cell-mediated cytotoxicity has been usually related to populations of thymus-independent lymphocytes presenting receptors for the Fc component of IgG. More recently, an eosinophil-mediated antibody-dependent mechanism of damage to *Schistosoma mansoni* has been reported (Butterworth *et al.*, 1978).

Santos-Buch and Teixeira (1974) reported lymphocyte-mediated cytotoxicity directed to parasitized cells when lymphocytes of the peripheral blood from infected rabbits were added to cultures of allogeneic foetal rabbit heart cells. Most lymphocytes, which in the beginning of the experiments were unattached, small and round, changed their shape after 18 h incubation, became attached to the heart tissue and produced marked destruction of parasitized and non-parasitized target cells. Cytophilic antibodies and complement also may have participated in the allogeneic cell destruction but, according to the authors, the cytolysis depended basically on the effect of *T. cruzi*-sensitized lymphocytes. The importance of this mechanism in host resistance has not yet been demonstrated. The existence of cross-reacting antigens in subcellular fractions of *T. cruzi* and heart cells, their recognition by lymphocytes, and the implications of these phenomena in the pathogeny of the disease are, discussed in section VIII of this review.

Destruction of parasitized syngeneic fibroblasts *in vitro* by spleen cells from infected mice was reported by Kuhn and Murnane (1977). The rate of cytolysis was estimated by assay of ^{51}Cr release. Normal, uninfected fibroblasts were not affected but significant destruction of infected fibroblasts was observed, suggesting that an immunity against intracellular stages,independent of antibody, develops in the acute phase. These results could not be confirmed by Hanson (1977), who used the same method of ^{51}Cr release to assess lymphocyte-mediated cytotoxicity directed against *T. cruzi* infected kidney or in syngeneic macrophages. No significant killing of infected cells was demonstrated and, moreover, no parasite antigens were detected by immunofluorescence in the host cell membranes.

5. *Macrophages and* T. cruzi

Macrophages from the mononuclear phagocytic system (MPS) interact with *T. cruzi* either as host cells, in which the parasites readily multiply and differentiate, or as effector cells of the immune response which participate

in the control of infection. The two functions can often not be clearly distinguished in the living host and the exact role of macrophages in the resistance remains to be determined. More data are needed to fill the gap between the findings *in vitro* (which were successful in establishing the conditions in which the anti-*T. cruzi* microbicidal effect occurred) and the process in the vertebrate host. The part played by the macrophages in the immune response in the acute and chronic stages of the disease, the role of opsonizing antibodies *in vivo*, and the interaction of macrophages with different *T. cruzi* populations are some of the aspects that need further investigation.

(a) *Macrophage parasitism in the living host.* The existence of *T. cruzi* strains that show selective parasitism for different tissues or organs has been demonstrated (reviewed by Melo and Brener, 1978). Badinez (1945) studied the infection by five *T. cruzi* strains in dogs and guinea-pigs and was apparently the first clearly to define the 'reticulotropism' of parasite populations, which are localized preferentially in cells of the reticulo-endothelial system. These populations differ from the 'myotropic' strains that invade mostly smooth, skeletal and heart muscle. Taliaferro and Pizzi (1955) studied the distribution in C3H mice of a highly reticulotropic strain. According to the nomenclature current at that time, parasites were found in macrophages, reticular cells, littoral cells of the liver, skin histiocytes, inflammatory macrophages and 'much less often in muscle cells'. Large numbers of parasites were observed in the spleen, liver and bone marrow.

The distribution of intracellular stages in mice experimentally infected with four different *T. cruzi* strains has been studied in detail (Melo and Brener, 1978). The *Y* strain showed a clear and marked preference for cells of the spleen, liver and bone marrow, whereas these organs were very seldom parasitized by the *CL* strain. These peculiar distributions were considered as selective infection of macrophages from the mononuclear phagocytic system on the grounds: (a) spleen, liver and bone marrow are organs that present high numbers of cells from the MPS; (b) in the spleen the parasitism is predominant in the red pulp and marginal zone (areas where macrophages are more abundant), but not in the white pulp; (c) in the liver, parasites invade specifically the Küppfer cells, components of the MPS; (d) experiments *in vitro* with mouse macrophages and bloodstream forms of the *Y* and *CL* strains, confirm the higher affinity of *Y* parasites for the phagocytic cells (Alcantara and Brener, 1978a).

An interesting aspect of the spleen parasitism, which may have immunological implications, is the sudden destruction of spleen lymphoid cells in the animals inoculated with the 'reticulotropic' strains, described by Taliaferro and Pizzi (1945) and confirmed by Melo and Brener (1978). This lymphocyte depletion occurred in the regions where parasitism was the more intense, such as the marginal zone and the red pulp, but its mechanism is still not clear. It has been suggested that infected macrophages might serve as target cells for sensitized lymphocytes.

As the reticulo-endothelial system (RES) comprises a wide range of cells that do not share a common origin, morphology or function, there is at present a tendency to consider that this term is misleading. The concept of

the RES should accordingly be replaced by the mononuclear phagocytic systems (MPS) which includes the macrophages found in the spleen, liver, bone marrow, lymph nodes and other areas of the living organism (van Furth, 1975). The macrophages have a common origin in the bone marrow, circulate as monocytes in the peripheral blood and find their homes in different organs. On the basis of these concepts, Melo and Brener (1978) and Brener *et al.* (1978) discussed the nature and nomenclature of the strains that interact predominantly with macrophages, suggesting that the term 'macrophagotropic' instead of 'reticulotropic' would better express their selective parasitism.

Whether different *T. cruzi* tissue tropisms exist in humans has not yet been demonstrated. Nevertheless, Bittencourt (1976) described in congenital human cases of Chagas' disease two kinds of predominant parasitism: in one, most parasites were detected in muscle cells, in the other 'within the cells of the reticuloendothelial system.'

(b) *Interactions* in vitro. A number of studies have been made of the development of *T. cruzi* in macrophages(Muniz and Freitas, 1946; Zuckerman, 1953; Lamy, 1970; Behbehani, 1973). Most observations were concerned with morphological aspects of the parasite life cycle in this phagocytic cell. More detailed investigations of the mode of entry, and fate, of the parasite, of the development of different stages and of the influence of different *T. cruzi* strains, have been recently published. This new accession of interest to this particular aspect of the *T. cruzi* life cycle has been stimulated by the recognition of macrophages as effector cells of the immune response and by the new advances in studies with cells of the MPS *in vitro*.

Although some findings are controversial, most evidence favours the idea that *T. cruzi* culture forms enter macrophages by phagocytosis. Nogueira and Cohn (1976) were unable by electron microscopy to detect any evidence of fusion between parasite and host cell membranes or of dissolution of macrophage membranes which would suggest direct penetration; in cells fixed 30 minutes after infection, epimastigotes and trypomastigotes were observed within phagocytic vacuoles. Moreover, cytochalasin B (an agent that reversibly inhibits phagocytosis) blocked the uptake of epimastigote and trypomastigote stages. Different results were obtained by Kipnis *et al.* (1979) who reported that the uptake of trypomastigotes was not blocked by cytochalasin B. The fate of the epimastigotes and of the infective metacyclic trypomastigotes from culture, in mouse macrophages, was also investigated (Nogueira and Cohn, 1976). Purified populations of trypomastigotes were obtained by lysing epimastigotes with guinea-pig complement; populations with high percentages of epimastigotes were obtained by harvesting the flagellates from acellular media. Epimastigotes were soon destroyed within phagocytic vacuoles; experiments with thorotrast-labelled macrophages indicated that secondary lysosomes delivered their contents into the epimastigote-containing vacuoles. Trypomastigotes and transition forms escaped from the phagocytic vacuole and multiplied in the cell cytoplasm; the mechanism involved in the lysis of the phagosome membrane was not clear but the authors suggested that it could be related either to a parasite-released factor or to a

membrane–membrane interaction. It is not known whether phagosome-lysosome fusion occurs before the escape of the parasite.

A peculiar interaction between tissue-culture-derived trypomastigotes and epimastigotes grown in NNN cultures, with mouse peritoneal macrophages, has been described (Dvorak and Schmunis, 1972). Trypomastigotes and epimastigotes collected at the stationary culture phase were destroyed after being ingested; the death of the macrophages followed. Epimastigotes obtained during the exponential culture phase, however, survived for at least 8 days within macrophages without escaping from the cell or multiplying. This unusual interaction has been suggested by the authors to be unique to the 'Ernestina' strain which they used and which was probably different from other highly adapted 'reticulotropic' strains of *T. cruzi*.

Electron microscopy studies with hamster peritoneal macrophages and *T. cruzi* bloodstream forms (Milder *et al.*, 1973, 1977) showed that parasites were found within membrane-bound phagosomes 1 to 2 h after infection; after 24 h, however, most trypomastigotes and multiplying stages were found free in the cytoplasm, confirming that the stages in the vertebrate host must escape from the phagocytic vacuole in order to accomplish the intracellular cycle.

Alcantara and Brener (1978a) investigated the interaction of *T. cruzi* bloodstream forms of the *Y* and *CL* strains with mouse peritoneal macrophages *in vitro* (Fig. 4). Trypomastigotes of the *Y* strain were shown to be 20–30-fold more infective to normal macrophages than those of the *CL* strain, after 3 h exposure, with a parasite/cell ratio of 5 : 1. The low rates of macrophage infection with *CL* parasites was still observed when the parasite/cell ratio was increased to 30 : 1 and the exposure time to 24 h, suggesting that this insusceptibility is related to inherent characteristics of the parasites rather than to factors of contact with the phagocytic cells.

The percentage of macrophages infected with bloodstream forms of the *Y* strain remained constant during the first 24–48 h after inoculation; later, the number of intracellular parasites per 100 infected macrophages steadily increased. These data do not agree with the findings of Kierszenbaum *et al.* (1974), who described an early destruction of bloodstream forms by normal macrophages *in vitro*.

The reasons for the differential behaviour of bloodstream forms of *T. cruzi* of the *Y* and *CL* strains as regards macrophages are not yet known for certain. The possibility that macrophages present specific receptors for 'macrophagotropic' strains, such as the *Y* strain, has been suggested by Alcantara and Brener (1978b) and is discussed in more detail in the next section. The concept of 'induced or stimulated phagocytosis' of intracellular protozoans (Jones *et al.*, 1972) implies a mechanism in which parasite membrane components play a critical role in endocytosis. It is possible that the parasites of the two strains may differ at membrane level and that these differences may influence their uptake.

(c) *Macrophage receptors and phagocytosis.* Protease treatment of macrophage plasma membrane removes receptors for complement (Bianco *et al.*, 1975) and for glutaraldehyde-treated red blood cells (Rabinovich, 1967). In

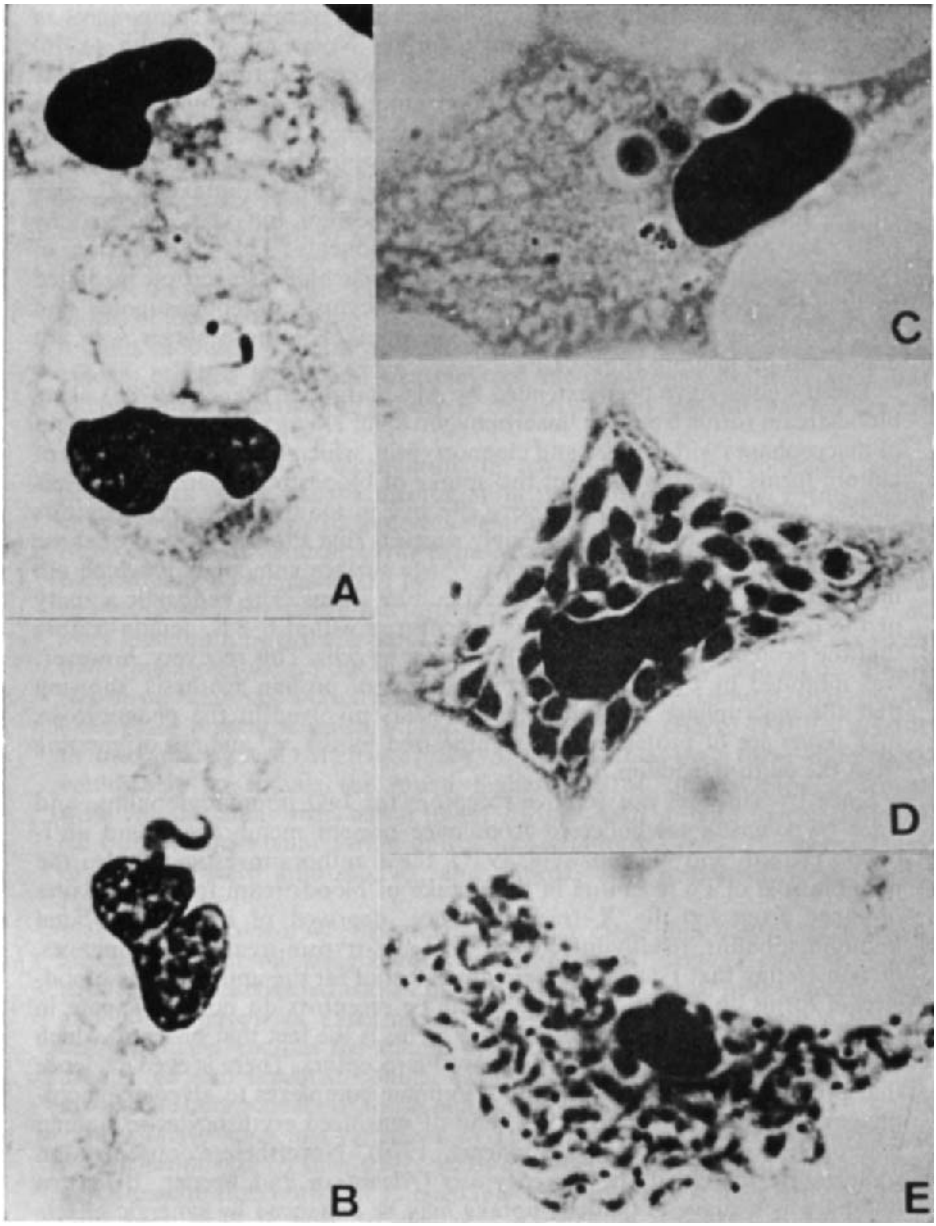

FIG. 4. Development of *T. cruzi* bloodstream forms in normal mouse peritoneal macrophages. A, *Y* strain, slender form; B, *CL* strain, opsonized stout form; C, D and E, development of organisms of the *Y* strain. (After Alcantara and Brener, 1978*a*).

an attempt to investigate the role of macrophage membrane components in the phagocytosis of *T. cruzi* culture forms, Nogueira and Cohn (1976) treated normal, and thioglycollate-induced, mouse peritoneal macrophages with trypsin and chymotrypsin. Both enzymes blocked the binding and the ingestion of epimastigote and trypomastigote stages. Since trypsin treatment does not remove Fc receptors and the C3 receptor is insensitive to chymotrypsin, Nogueira and Cohn (1976) established that the uptake of *T. cruzi* culture forms was not dependent on either receptor but was mediated by particular protease-sensitive membrane components. The IgG fraction of rabbit antiserum to mouse macrophages (which blocks ingestion mediated by the Fc receptors) did not inhibit phagocytosis of epimastigotes and trypomastigotes, showing again that the uptake of those stages was not dependent on binding sites for Fc.

These studies have been extended by Alcantara and Brener (1978b) using bloodstream forms from the 'macrophagotropic' *T. cruzi Y* strain. Treatment of macrophages with trypsin and chymotrypsin, which blocks phagocytosis of culture forms, does not prevent the uptake of bloodstream parasites. Nevertheless, pronase treatment was very effective in inhibiting the phagocytosis of bloodstream forms, which strongly suggests that the endocytosis of those parasitic stages is mediated by macrophage surface components, which are different from those required to ingest culture forms. The endocytic activity of the pronase-treated macrophages recovered within 6–8 h, demonstrating that the proteolytic enzyme did not damage the cells. This recovery, however, was inhibited by cycloheximide, an inhibitor of protein synthesis, showing that the macrophage membrane components involved in the phagocytosis of *T. cruzi* are of protein nature, synthesized by the cell and not originating from the culture medium.

Since trypsin does not remove receptors for IgG immunoglobulins, and since trypomastigotes collected from mice present membrane-bound antibodies (Krettli and Nussenzweig, 1977), these authors investigated also the possible role of Fc receptors in the uptake of bloodstream forms. Parasites obtained from lethally X-irradiated mice, deprived of membrane-bound immunoglobulins, readily infected normal and trypsin-treated macrophages, demonstrating that Fc receptors are not essential for the uptake of the bloodstream forms. A further indication that Fc receptors do not participate in the endocytosis of *T. cruzi* bloodstream forms is the fact that pronase, which blocks parasite uptake, does not remove Fc receptors. There is even evidence that pronase enhances the binding of immune complexes to alveolar macrophages (Arend and Mannik, 1972) and of sensitized erythrocytes to human macrophages (LoBuglio and Rinheart, 1970). Nevertheless, opsonization significantly increased the phagocytosis (Alcantara and Brener, 1978a), a finding which suggests that the uptake may be enhanced by synergic effects of different membrane components. Also, receptors for C3b are probably not involved in the phagocytosis of bloodstream forms: trypsin treatment, which removes such receptors (Lay and Nussenzweig, 1968), does not inhibit the uptake of these infective stages.

Although the concept of 'induced phagocytosis' (Jones *et al.*, 1972) applied

to obligatory intracellular protozoans implies active participation of the parasite, it is likely that a macrophage recognition mechanism might be important in the endocytic process. Czop *et al.* (1978) reported that human monocytes are able to recognize and ingest particles that activate the alternative C pathway; this mechanism is trypsin-sensitive and independent of Fc or C3b receptors. It is interesting to recall that *T. cruzi* epimastigote culture forms (which are largely phagocytosed by macrophages) are activators of the C alternative pathway (Nogueira *et al.*, 1975) and that their uptake is not mediated by Fc or C3b receptors (Nogueira and Cohn, 1976), which suggest an analogy with the phenomenon described by Czop *et al.* (1978). Since bloodstream forms do not activate C unless they are coated with immunoglobulins (Krettli and Nussenzweig, 1977) and since their phagocytosis blocked by trypsin treatment of the macrophages, it is very unlikely is not that their ingestion is related to this general mechanism of recognition.

Besides presumptive mechanisms of recognition by macrophages, some peculiarities of the plasma membrane in the different *T. cruzi* forms might also influence endocytosis. A comprehensive study of the membrane characteristics of *T. cruzi* (Souza, 1978) demonstrated that: (a) the surface coat of bloodstream trypomastigotes is at least 3-fold thicker than that of culture epimastigotes; (b) freeze-fracturing studies showed that the number of intra-membranous protein particles is significantly lower in trypomastigotes than epimastigotes; (c) the surface charge investigated by binding of cationized iron and microelectrophoretic (MEP) mobility showed that trypomastigotes present a much higher negative surface charge (MEP $= -1{\cdot}14$) than epimastigotes (MEP $= +0{\cdot}50$). It is possible that these differences could help to induce the much higher rate of phagocytosis observed with culture than with bloodstream forms: with a parasite/cell ratio of 6 : 1 for blood-stream forms and 2 : 1 for culture forms, the percentages of infection were approx. 10% and approx. 40% respectively (Alcantara, 1979).

It is not yet known whether the macrophage membrane components which are essential for the phagocytosis of bloodstream forms *in vitro* also contribute to the intense parasitism by the *Y* strain of *T. cruzi* in the spleen, liver and bone marrow of experimental hosts. It is, however, possible that in certain organs, such as the spleen, the trapping of parasites is facilitated by their close and repeated contact with macrophages as well as by specific membrane components of those phagocytic cells.

(d) *Macrophages and resistance to* T. cruzi. There is increasing evidence that macrophages by themselves, or by a cooperative effect with lymphocytes, participate in the control of *T. cruzi* infection. In many studies culture forms have been used *in vitro* to challenge normal or activated macrophages. Some authors working with this system have failed to recognize that epimastigote forms are often destroyed in phagolysosomes not by mechanisms related to the immune response, but just because they are incompatible with normal intracellular development. Nevertheless, despite this, macrophages emerge as an important effector component in the host resistance.

Observations in vivo. Participation of macrophages in the control of *T. cruzi* infections in the vertebrate host has often been reported. The destruction of attenuated culture forms as well as of virulent bloodstream forms 'in free and fixed macrophages' in immune mice was observed by Taliaferro and Pizzi (1955). Pizzi (1957) extended these observations and reported that macrophages have only a limited capacity to destroy intracellular parasites. Partial blockade of macrophage cells by thorium dioxide (Goble and Boyd, 1962) or by Indian ink (Andrade *et al.*, 1967), before infection, resulted in increased susceptibility to *T. cruzi* infection. On the other hand, administration of Freund's complete adjuvant induced in mice some degree of resistance to the parasite (Andrade and Carvalho, 1969).

Kierszenbaum *et al.* (1974) inoculated *T. cruzi* into mice previously treated with silica particles, a mineral agent that is a specific poison for macrophages and which has no adjuvant effect as regards antibody formation. Parasitaemia levels and mortality rates were significantly increased in the silica-treated animals compared with controls, following challenge with virulent bloodstream forms. Stimulation of macrophage activity by diethylstilboestrol induced a significant decrease in the severity of the infection. These authors suggested that phagocytosis of the parasites by normal macrophages could represent a defence mechanism against *T. cruzi*. Macrophages collected from the peritoneal cavity soon after intraperitoneal inoculation of virulent bloodstream forms showed degenerating parasites; cultivation of the macrophages *in vitro* showed gradual destruction of the ingested trypomastigotes. This experiment has not apparently been repeated, but no significant intracellular parasite destruction was observed in normal macrophages infected *in vitro* with bloodstream forms from a 'macrophagotropic' strain (Alcantara and Brener, 1978a).

The influence of living immunizing agents, which activate macrophages *in vivo*, on the course of *T. cruzi* infection has also been investigated. Inoculation of *Toxoplasma gondii* and *Besnoitia jellisoni* renders the animals more resistant to a challenge inoculation with *T. cruzi* (Williams *et al.*, 1976). Confirming these findings, mice chronically infected with *T. gondii* were demonstrated to be more resistant to a *T. cruzi* challenge infection than were uninfected controls (Araujo and Nascimento, 1977).

More controversial results have been reported from experiments with BCG vaccine. According to Hoff (1975), BCG failed to protect mice against a *T. cruzi* challenge despite clear evidence *in vitro* of macrophage activation in the treated animals. Kuhn *et al.* (1975) also were unable to detect any protection in groups of animals immunized with large doses of BCG. Ortiz-Ortiz *et al.* (1975), however, also explored the possibility of immunizing mice with BCG and reported that treated animals presented significantly lower parasitaemias and increased survival after challenge with *T. cruzi* bloodstream forms. Similar results were obtained by Alcantara (1979), who showed clearly that protection was induced by single doses of intravenous BCG. The reason for these conflicting results are not known.

Brener and Cardoso (1976) immunized mice with formalin-killed *Corynebacterium parvum* and reported that the mice so treated showed, during the

course of *T. cruzi* infection, a markedly lower parasitaemia than did controls. *C. parvum* increases resistance to transplanted tumours, bacteria and protozoa, and is known to enhance cell-mediated immunity.

An interesting aspect, related to the participation of macrophages in the immune response in Chagas' disease, is the enhanced mononuclear phagocytic activity that occurs in mice experimentally infected with *T. cruzi* (Ortiz-Ortiz *et al.*, 1976). Animals built up a nonspecific resistance against *Listeria monocytogenes*, which was associated with increased clearance rates of carbon particles.

Experiments in vitro. The *T. cruzi*-killing activity of macrophages *in vitro* from mice immunized with BCG was investigated by Hoff (1975), using culture forms. By 48 h after the exposure period the percentage of macrophages infected and the number of intracellular parasites declined markedly in immune compared with normal macrophages. Since the author was not working with purified populations of metacyclic trypomastigotes, this decline is likely to represent epimastigote destruction. After 48 h, the number of intra-cellular stages increased in the normal macrophages but remained low in those collected from the immunized animals. BCG- and specifically activated macrophages displayed the same degree of resistance to *T. cruzi* challenge. As enhanced killing activity appeared in macrophages during the 3rd week of *T. cruzi* infection in mice when parasitaemia was still rising, and since mice harbouring BCG-activated macrophages were not resistant to *T. cruzi*, the author suggested that additional humoral or cellular mechanisms must be operating in resistance to the parasite.

The interaction between *T. cruzi* culture forms and normal or activated mouse macrophages was studied in a series of papers from the same group of investigators (Tanowitz *et al.*, 1975; Kress *et al.*, 1975, 1977). Normal macrophages were able to control a 1 : 1 (parasite/cell ratio) infection but not a 10 : 1 infection, whereas BCG-activated macrophages could control a 10 : 1 but not a 100 : 1 infection. Thorium dioxide-labelled secondary lysosomes were seen fusing with phagosomes containing parasites, in both normal and activated macrophages. After 24 h no significant differences in the number of intracellular stages could be detected between normal and activated macrophages; within 96 h, however, most parasites in the activated cells had been destroyed but not those in the normal controls.

Mice macrophages activated by infection with *Toxoplasma gondii* and *Besnoitia jellisoni* inhibited the intracellular multiplication of tissue-culture-derived trypomastigotes. For unknown reasons macrophages from animals infected with *B. jellisoni* displayed the higher microbicidal effect (Williams *et al.*, 1976). Normal intracellular multiplication of *T. cruzi* was obtained in human monocytes and monocyte-derived macrophages (Williams and Remington, 1977). Incubation of the monolayers with lymphocytes and streptokinase–streptodornase resulted in an inhibition of parasite intracellular multiplication, which was similar to that observed in the resistant mouse macrophages.

The conversion of resting resident mouse peritoneal macrophages into activated 'killing' macrophages able to destroy *T. cruzi* intracellular stages

has been studied in a series of experiments in which this parasite was used as a tool for approaching more general problems related to macrophage microbicidal activity (Nogueira and Cohn, 1976, 1978; Nogueira *et al.*, 1977). As previously mentioned (Nogueira and Cohn, 1976), normal resident mouse peritoneal macrophages or thioglycollate-induced macrophages failed to kill *T. cruzi* culture trypomastigotes. These authors then investigated the ability of macrophages from mice infected with *T. cruzi* to destroy the parasites (Nogueira *et al.*, 1977). No killing activity was displayed by macrophages from infected mice exposed to purified trypomastigotes. When, however, the infected mice were challenged with heat-killed trypanosomes, killed *Mycobacterium tuberculosis*, PPD or protease-peptone, the macrophages became able to destroy a variable number of ingested trypomastigotes. Macrophages from *T. cruzi*- or BCG-inoculated animals collected without a secondary challenge present a trypanostatic rather than a trypanocidal effect on the endocytosed parasites. The cells obtained after intravenous administration of BCG followed by a challenge with mycobacterial antigens also displayed trypanocidal activity against *T. cruzi*. Nevertheless, in all experiments the remaining parasites were able to resume replication after 24 h maintenance of the macrophages *in vitro*.

The discrepancies between these results and those of the previous paper, which described killing effects of macrophages from infected animals that had not been submitted to a secondary challenge, could be explained, according to the authors, by the fact that they resulted from infection with non-purified trypomastigotes. In this case, the microbicidal effect was reflecting 'killing of epimastigotes and inhibition of growth of the few trypomastigotes' rather than a resistance mechanism.

The mechanisms responsible for the conversion of the trypanostatic activity into a trypanocidal effect by means of a secondary specific challenge was not clear at this stage and two hypotheses were raised: first, that the function of the macrophages would be changed by the influx of large numbers of sensitized lymphocytes caused by the challenge; second, that lymphocytes and macrophages could be influenced by the complex products generated by the local inflammation. In a further paper, Nogueira and Cohn (1978) investigated how macrophage microbicidal activity against *T. cruzi in vitro* could be introduced and sustained. Normal, resident and inflammatory mouse peritoneal macrophages were induced to exert microbicidal action against *T. cruzi* in a complete system *in vitro*. The effect was obtained by incubating macrophages with a spleen cell factor (SCF) released by lymphocytes collected from animals infected with *T. cruzi*. The SCF was generated when the lymphocytes were previously exposed to heat-killed *T. cruzi* antigen. The maintenance of the trypanocidal activity depended on daily addition of fresh SCF, which suggests that in the intact host a continuous stimulation of lymphocytes by parasite antigens may be essential for a steady activation of the macrophages.

T cells seem to be required for the origin and release of SCF, since pretreatment of the sensitized spleen cells with anti-θ serum and complement abolished the production of SCF. Trypanocidal activity was also induced by

incubating macrophages with supernatants of concanavalin-A- and LPS-treated lymphocytes, which again favours the participation of T cells in the biosynthesis of SCF. Since partially digested parasites are found within phagocytic vacuoles soon after treatment with SCF, the authors suggested that the lymphokine probably prevents the trypomastigotes escaping from the phagolysosomal system into the cytoplasm. A rather puzzling finding was that when the lymphokine was added after the parasites had already escaped from the phagosome, killed trypomastigotes were found in newly formed membrane-bound phagosomes.

The macrophages which show a microbicidal effect against *T. cruzi* also secrete high levels of plasminogen activator (Nogueira *et al.*, 1977). Nevertheless, secretion of plasminogen activator seems not to be related to trypanocidal activity.

In a recent review Cohn (1978) discussed the stepwise transformation of activated macrophages into microbicidal macrophages. According to the sequence outlined: nonspecific inflammatory events induce in blood monocytes and resident macrophages a series of properties or functions which include rapid spreading, metabolic changes, increase of lysosome hydrolases and plasma membrane changes; lymphokine-mediated events influence the development of microbicidal activity. At this stage intracellular production of hydrogen peroxide would probably be an important factor in the microbicidal effect.

The role of opsonization in the development of *T. cruzi* in macrophages was investigated by Alcantara and Brener (1978a). Significant increases in the uptake of antibody-coated *T. cruzi* (*Y* strain) blood stream forms were observed. Nevertheless, no microbicidal effect of macrophages was observed and the parasites apparently normally escape from the phagosomes to carry out their intracellular cycle. This outcome differs from that occurring with other microorganisms (such as opsonized virus) which are destroyed within phagolysosomes (Silverstein, 1975). Normal toxoplasms regularly develop in phagosomes, whereas antibody-coated parasites are not able to prevent the fusion of phagocytic vacuoles with lysosomes and are therefore destroyed (Jones *et al.*, 1975).

V. Effects of Immunosuppressors in Chagas' Disease

The vertebrate host immune response can be influenced by the administration of a number of immunosuppressive agents in both acute and chronic phases of Chagas' disease. Pizzi (1957) and Goble (1970) reviewed the effects of adrenocorticotropic (corticotropin) hormones and cortisone in *T. cruzi* infections, which effects had been first reported by Jarpa *et al.* (1951). Cortisone enhances *T. cruzi* acute infections in mice, increasing the number of circulating and intracellular parasites. Chronic infections in rats (Pizzi *et al.*, 1952) and mice (Brener and Chiari, 1963) are apparently unaffected by cortisone. Adrenocorticotropic hormone (corticotropin, ACTH) was reported to improve the course of infection in experimentally infected dogs (Robles Gil and Perrin, 1950).

Brener and Chiari (1971) studied the effects of four immunosuppressive agents (gamma radiation, cyclophosphamide, Imuran (azathioprine) and 6-mercaptopurine) in mice chronically infected with *T. cruzi*. Gamma radiation- and cyclophosphamide-induced in the animals inoculated with *T. cruzi CL* strain, new acute-phase episodes characterized by high parasitaemias and mortality rates. No such reactivation was observed in groups of animals inoculated with the other three *T. cruzi* strains (*Y*, *Berenice*, *PNM*). Imuran and mercaptopurine were unable to influence the course of the chronic infection. The reasons why only cyclophosphamide among the chemical immunosuppressive agents was able to induce a fresh acute phase are uncertain. This drug is a strong alkylating agent which affects both the early stages of the immune response and the ongoing antibody synthesis. As regards the absence of new acute-phase episodes in animals inoculated with the *Y* strain, the authors suggest that it might result from the higher susceptibility of the slender forms (which predominate in this strain) to specific antibodies, as already described in this review. As the circulating antibody molecules are radioresistant and unaffected by chemical immunosuppressive drugs, they are probably able to maintain the parasitaemia at a subpatent level despite the decay of the already synthesized immunoglobulins.

Krettli (1977) investigated the effect of *Plasmodium berghei* malaria on the chronic phase of Chagas' disease in mice. Recrudescences were observed in mice inoculated with *T. cruzi CL* and *Gilmar* strains, which present similar characteristics as regards morphology of blood stages, curves of parasitaemia and susceptibilities to antibodies. No acute phase occurred in mice inoculated with the *T. cruzi Y* strain and infected with *P. berghei*. Since malaria affects the humoral immune response by depleting lymphocyte populations bearing complement receptors (B cells), the author suggests that recrudescences are likely to result from decrease in antibody levels or to a macrophage defect.

There are a few reports of acute exacerbation of chronic Chagas' disease in humans. Acquired resistance against *T. cruzi* was suppressed in a case of Chagas' disease presenting concomitant chronic lymphatic leukemia (França *et al.*, 1969); post-mortem examination showed severe chronic chagasic myocarditis with large numbers of intracellular parasites in the heart muscle and nervous system. Almeida *et al.* (1974) reported a case of Hodgkin's disease treated with cyclophosphamide and corticosteroids which at the autopsy presented chronic chagasic myocarditis with a high prevalence of *T. cruzi* intracellular stages in the heart and oesophagus.

As suggested by Brener and Chiari (1971), the use of immunosuppressive drugs in human beings in endemic areas should take into consideration the reported harmful effects.

VI. Immunodepression in the Course of Chagas' Disease

Depression of the immune response during the active infection with *T. cruzi* in animals has been reported by several authors. Clinton *et al.* (1975) described a diminished humoral immune response to donkey erythrocytes in experimentally infected mice, which was related to the degree of the parasitism.

Haemolytic plaque assay was used to detect antibody-producing cells (PFC). Both direct PFC (19S) and indirect PFC (7S) assays were significantly reduced as parasitism increased.

Schmunis *et al.* (1977a) injected *T. cruzi* simultaneously with sheep red blood cells (SRBC) into mice and investigated with spleen cells the number of rosette-forming cells against SRBC by the immunocytoadherence test (ICA). The number of spleen cells forming rosettes was significantly decreased in heavily infected mice, whereas no depression of the immune response occurred in groups of animals with a low load of parasites. Although rosette-forming cells are considered essentially as antibody-producing cells, these authors seem unsure about the nature of the immunodepression and the cells involved. As the ICA test is apparently unable to discriminate among the effector spleen cells, they would accept that the observed immunodepression may be related to a decrease of T cells rather than of antibody-secreting cells.

The effects of an acute *T. cruzi* infection on the cellular immune response in mice was also studied by Reed *et al.* (1977). Delayed hypersensitivity was investigated in mice sensitized with Freund's complete adjuvant and challenged with BCG injected into the foot-pad and in mice sensitized with oxazolone and skin tested thereafter with the same drug. During the period of high parasitaemia a significant depression of the cellular immune response to BCG inoculation was detected. Moreover, mice responsive to oxazolone before the infection failed to react to this sensitizing agent as infection progressed.

Lima Pereira (1978) inoculated mice with the *T. cruzi Y* strain and investigated the following aspects: (a) the humoral and cellular immune response in mice sensitized to SRBC; (b) histological changes in the thymus, spleen and lymph nodes; (c) inflammatory reactions during the course of infection and their possible relationship to changes in immunity. Depression of the humoral and cellular immune response was demonstrated by significant decline in the number of antibody-producing cells and diminished intensity of the foot-pad reaction. Depletion of lymphocytes in the thymus, spleen and lymph nodes was observed after 8 days of infection, following the peak of parasitaemia at 7 days of infection. The inflammatory reaction in the heart and skeletal muscle was very mild until day 15 of infection despite the high tissue parasitism, probably an expression of the depression of the cellular immune response.

Immunodepression of the cell-mediated immune mechanism in human acute Chagas' disease was reported by Teixeira *et al.* (1978b). Working in an endemic area of Bahia, Brazil, these authors followed up, for 18 months, 544 individuals with negative serology for Chagas' disease. Within this period, 12 new cases were detected both by positive Chagas-latex test and by demonstration of *T. cruzi* by examination of fresh blood. A group of five patients showed an acute illness with high fever, enlarged spleen and signs of portal of entry (chagomas, Romaña sign); another group of seven patients presented as clinically inapparent Chagas' disease and were only detected because of the regular follow-up of the exposed population. The patients with apparent disease presented a positive delayed-type of skin response to a *T. cruzi*

antigen and a significant degree of inhibition of blood leucocyte migration in the presence of specific antigen. The patients with inapparent disease, however, showed negative skin tests, the migration of their blood leucocytes was not inhibited and they could not be sensitized to chlorodinitrobenzene, indicating a correlation between the severity of the clinical symptoms in the acute phase and the degree of delayed-type hypersensitivity developed to the parasite.

The mechanisms responsible for the immunodepression are not yet known. Depletion of lymphocytes in lymphoid organs, chiefly the spleen, which is a common phenomenon in the course of infection with some *T. cruzi* strains, has been considered as an important factor leading to alterations in the immune response (Schmunis *et al.*, 1977a; Reed *et al.*, 1977). This was not, however, the conclusion of Lima Pereira (1978) since in his experience the lymphocyte depletion in the spleen and lymph nodes occurred when the immunodepression was already established in the animals. Difficulty or failure of macrophages to process the test antigens has also been suggested as an element which could participate in the depression of the immune response. All authors who experimentally investigated the immunosuppressive effect of *T. cruzi* infection worked with 'reticulotropic' (=macrophagotropic) populations which elicit an extremely high macrophage parasitism. Reed *et al.* (1977) suggested that parasite-harbouring macrophages could present changes in their surface receptor sites which would interfere with the interaction with T lymphocytes. Nevertheless, phagocytic activity of the macrophages in *T. cruzi*-infected mice is increased as demonstrated by colloidal carbon clearance tests (Clinton *et al.*, 1975), evidence that 'blockade' of mononuclear phagocytic cells is not an essential factor in the process.

Some other mechanisms have been suggested, such as antigenic competition caused by the massive load of parasite antigens or suppressive effects of antigen–antibody complexes. Clinton *et al.* (1975), on the other hand, mentioned that interferon might be inducing immunodepression in *T. cruzi*-infected mice. As previously mentioned in this review, interferon inducers enhance *T. cruzi* infection; further, exogenous interferon preparations suppressed mouse antibody producing spleen cells in erythrocyte-immunized mice.

A decrease in the capability of spleen cells from *T. cruzi*-infected mice to respond to T- or B-cell mitogens such as concanavalin A or lipopolysaccharide has been described (Ramos *et al.*, 1979). Responses to the mitogens were normal in early infections but depressed after 2 weeks of infection. This finding demonstrates the existence of a suppressor cell population in the spleen of infected mice, which decreases the ability of lymphoid cells to respond to T- and B-mitogens. This cell population probably influences the cellular and humoral immunosuppression occurring at the late stages of infection. Further data indicate that those suppressor cells are either T-cells or a population influenced by T-cells.

Teixeira *et al.* (1978a) discussed the mechanisms that induce immunodepression in patients with the clinically-inapparent Chagas' disease. The results suggest that thymus-derived T lymphocyte functions are depressed.

T. cruzi antigens are likely to stimulate T cells, which would release factors suppressing T cell responses.

VII. Evasion of the Immune Response by *T. cruzi*

Despite the presence of humoral and cellular immunity against *T. cruzi*, Chagas' disease is a lifelong infection and no spontaneous cure has been reported. This suggests that the parasite may be equipped with mechanisms of escape which would prevent their destruction by the host. Nevertheless, very little work has been done to investigate these presumed mechanisms, and there is no evidence as yet that the parasite can change surface antigens as does *Trypanosoma brucei*. Siqueira *et al.* (1978) carried out agglutination and direct immunofluorescence tests with trypomastigotes recovered from rats in successive days of infection. The positivity of the tests fluctuated suggesting the occurrence of parasite populations with different antigenic characteristics; no further evidence of antigenic variation was provided.

Schmunis *et al.* (1978b) used immunofluorescence methods to investigate the effects of sera from human patients with Chagas' disease on the surface antigens of bloodstream forms of the *T. cruzi Y* strain. These authors were looking for antibody-induced capping of surface antigens already reported for *Leishmania* and *Toxoplasma*. The findings indicated that the parasite surface-membrane antigens moved laterally and formed multiple ('patch') or single ('cap') aggregations. Only limited shedding, presumably of immunocomplexes was observed. It has been suggested that 'capping' might represent a mechanism of evasion from the host's humoral response.

The extremely low susceptibility of stout forms from *CL* and other *T. cruzi* strains to antibody-induced agglutination, complement-mediated lysis and macrophage uptake already described in this review may also be considered as a mechanism whereby at least a part of the population evades the host's immune response. As previously suggested by Brener (1973) and by Howells and Chiari (1975) in a somewhat teleological way, those forms would represent parasites pre-adapted to the new metabolic conditions to be faced by the parasite in the vector.

VIII. Auto-immune Reactions and the Pathogeny of Chagas' Disease

> "The effector pathways recruited for host defence in both the nonimmune and the immune host are specific and generate an array of beneficial functions, but they can also be detrimental to the host if the response is misdirected or excessive."
>
> (Austen, 1978

An unexplained phenomenon in Chagas' disease is the occurrence, at the chronic stage, of severe myocarditis which cannot be related to parasitism of heart muscle cells. Actually, in the chronic phase, parasites are at a submicroscopical level both in the bloodstream and tissues. Heart lesions are therefore very unlikely to be produced by direct destruction of tissue.

Because of these circumstances, it has been often suggested that autoimmune mechanisms are involved in the pathogeny of Chagas' disease myocardiopathy.

Early authors (Torres, 1941; Muniz and Azevedo, 1947) have already suggested 'allergic' mechanisms to explain the chagasic myocarditis. Muniz and Azevedo (1947) tried to reproduce the myocarditis in rhesus monkeys by repeatedly injecting killed *T. cruzi* culture forms; they described mild focal inflammatory lesions.

Evidence for the participation of cell-mediated immune reactions in the origin of myocardial lesions has been presented. Santos-Buch and Teixeira (1974) described a destruction of allogeneic parasitized and non-parasitized rabbit heart cells when they were incubated with lymphocytes collected from rabbits chronically infected with *T. cruzi* or immunized with particulate parasite fractions. Scanning electron microscopy showed that 'rough-surfaced lymphocytes had crawled beneath heart cells' causing detachment and death of the heart cells. A cross-reacting antigen common to the subcellular particulate *T. cruzi* antigen and the allogeneic heart cells was detected by the macrophage migration inhibition test. According to the results, the fact that *T. cruzi*-sensitized lymphocytes can recognize and damage non-parasitized heart cells may explain the persistent infiltrates of mononuclear cells, chiefly lymphocytes, in the absence of patent tissue parasitism in the chronic chagasic myocardiopathy.

Teixeira *et al.* (1975) produced in rabbits, by a single inoculation of trypomastigote stages, heart lesions similar to the myocarditis found in human chronic Chagas' disease. Such microscopical lesions could also be induced by repeated injections of particulate subcellular fractions of *T. cruzi*, evidence that heart lesions can be generated by a delayed hypersensitivity to *T. cruzi* antigens.

Experiments with lymphocytes from chagasic individuals have been performed by Cossio *et al.* (1976). The authors selected three cases presenting positive macrophage migration inhibition tests with mycocardial antigens. Their lymphocytes strongly attached to, and destroyed, murine heart cells, as shown by light and electron microscopy. No such reactions were observed with liver cells. Mononuclear cells from patients with negative macrophage migration inhibition tests were unable to interact with heart cells. These data also suggest that *T. cruzi*-sensitized lymphocytes can operate as effector cells directed against non-parasitized mycocardial preparations.

Cytotoxicity of T-lymphocytes, collected from patients with Chagas' disease, to parasitized and non-parasitized human foetal heart cells labelled with ^{51}Cr was investigated by Teixeira *et al.* (1978a). The lytic effect of immune lymphocytes on the normal target cells was much higher than that obtained with normal non-sensitized lymphocytes. The release of ^{51}Cr from heart cells exposed to immune lymphocytes from patients with Chagas' disease was significantly higher when the lymphocytes were from acute-phase patients than it was when they were from patients with the chronic disease. The ^{51}Cr release was the same in heart cells exposed to lymphocytes obtained from patients with chagasic cardiomyopathy and from those in the

'indeterminate form' (patients without clinical evidence of heart injury). These observations are consistent with previous findings of Lopes *et al.* (1975) who detected scattered mild inflammatory lesions in 'asymptomatic' patients with chronic Chagas' disease.

Since allogeneic systems of lymphocytes and heart cells were used in the experiments reported, extending those conclusions to the events that pertain to the living host has been criticized. Teixeira *et al.* (1978a) used the following arguments to consolidate their concepts on auto-immune reactions in Chagas' disease: (a) cell destruction was not caused by sensitization of T lymphocytes *in vitro* by the antigenically incompatible target heart cells since the phenomenon of cell lysis was detected as early as 1 h after incubation; (b) the specificity of the heart cell destruction was demonstrated by the fact that 'Vero' and kidney cells were not affected by *T. cruzi*-sensitized lymphocytes; (c) the role of histocompatibility antigens in the allogeneic lymphocyte–heart cell system has been apparently ruled out because, whereas heart cells were extensively lysed, kidney cells from the same host were not destroyed by the immune lymphocytes. Since histocompatibility antigens are found in the membrane of all host cells, kidney cells would also be destroyed if histocompatibility antigens were involved in the cell lysis.

Andrade and Andrade (1979) included among the evidence that misdirected immune reactions can be injurious to the host, the occurrence in human patients and in animals, of necrotizing arteritis presumably caused by hypersensitivity mechanisms mediated by immune complexes (Brito and Vasconcelos, 1959; Andrade and Andrade, 1968).

As regards humoral factors which could participate in the pathogeny of the heart disease, Cossio *et al.* (1974a) used immunofluorescence techniques to demonstrate in human Chagas' disease a serum gammaglobulin element which reacted with endocardium, vascular structures and interstitium of striated muscle (EVI antibody). This antibody was present in 95% of patients with Chagas' cardiopathy and only in 45% of asymptomatic patients; it has not been detected in normal controls or in a number of sera from patients with different cardiopathies or auto-immune diseases. Ultrastructural immunochemical methods demonstrated that reactions were occurring in the plasma membrane of heart and skeletal muscle fibres as well as of endothelial cells of blood vessels. Absorption of positive sera with *T. cruzi* epimastigotes abolished the reaction. The presence of antigen in the plasma membrane of myocardial cells suggests that the functions of these structures are affected (Cossio *et al.*, 1974b).

Szarfman *et al.* (1975b) detected IgM circulating antibodies reacting by the immunofluorescence technique with endocardium, vascular structures and striated muscle cells in three congenital cases of Chagas' disease. In two cases the antibodies reacted against self-tissue; gammaglobulins bound to the plasma membrane of skeletal muscle and of endothelial cells were observed in two other cases with severe myositis. Since IgM–EVI antibodies were found in the three newborn congenital cases but not in their mothers, the authors suggested that *T. cruzi* may induce the synthesis of EVI antibodies during the foetal and perinatal stages.

The EVI antibody is probably the expression of antigens cross-reacting between *T. cruzi* and some tissues of the vertebrate. As discussed by Andrade and Andrade (1979), it presents three important characteristics: high prevalence in severe chagasic myocardiopathy and absence in other heart diseases; cross-reaction with *T. cruzi* antigens; high specificity as regards other parasitic diseases. Nevertheless, the exact role of the EVI factor in the pathogeny, the cardiac form of Chagas' disease, still remains to be established.

Destruction of neuronal cells from the autonomic nervous system of the heart and digestive tract seems to represent an important element in the pathogeny of Chagas' disease syndromes (Koeberle, 1961). Santos *et al.* (1976) suggested that damage of the neuronal cells could result from immunological reactions. Antibodies directed against neurons were detected in sera from human patients by immunofluorescent technique using cryostat sections of newborn rat cerebellum as well as fluorescein-conjugated anti-IgG, anti-IgM and anti-IgE. In 129 human sera studied, 98% presented IgG antibodies and 7% IgM antibodies to neurons. The presumed stepwise events were further described by Santos (1977), as follows: antigens released from disrupted infected cells would bind to neighbouring neuronal cells; the host immune response would trigger an auto-immune reaction which would lead to further destruction of normal neurons.

Khoury *et al.* (1979) recently described, in both acute and chronic cases of Chagas' disease, using immunofluorescent techniques, an antibody that reacted with the Schwann sheaths of somatic and autonomic peripheral nerves. No positive reactions were observed with central nervous tissues such as neurons, glial cells or periaxonal sheaths. Rabbit immune sera directed against guinea pig spinal cord and the chagasic anti-nerve antibodies provided positive reactions with rat sciatic nerve, suggesting that the reactive antigens are likely to be located on the Schwann cells. A significant correlation regarding prevalence and serological titres was detected between the anti-nerve antibody and EVI antibodies. The anti-nerve antibody was absorbed by *T. cruzi* culture forms, which is strong evidence for the existence of cross-reacting antigens between the parasite and nerve tissue. The authors suggested that antibodies against Schwann cells might participate in the intense denervation that often occurs in the cardiac and digestive-tract autonomic system of infected vertebrate hosts.

The occurrence of antinuclear antibodies in mice inoculated with *T. cruzi* culture forms was reported by Szarfman *et al.* (1975a). No explanation for the presence of such antibodies was offered; it has been suggested that, as in virus infections, T/B lymphocytes in balance stimulate B cells to synthesize antinuclear antibodies. Nodular deposits of gammaglobulin and circulating antinuclear antibodies were detected in the kidney mesangia suggesting the possibility of an auto-immune disease produced by nuclear antigens and specific antibodies.

IX. Vaccination Against *T. Cruzi*

Attempts to protect vertebrates against *T. cruzi* have been carried out with a number of living 'attenuated' flagellates, killed parasites and subcellular

fractions. Use of living flagellates presents an inherent risk of inducing active infections by the inoculation of residual virulent organisms which have escaped the attenuation process. Notwithstanding, such vaccines have been used. Menezes (1969, 1971) immunized vertebrate hosts with culture forms from a 'non-virulent' strain of *T. cruzi*, observing partial protection after challenge with homologous virulent bloodstream forms. Fernandes *et al.* (1965, 1966) showed that culture forms of *T. cruzi* treated with actinomycin D retained their motility but had their multiplication and differentiation irreversibly inhibited. Suspensions of the treated parasites were unable to infect mice but afforded only partial protection against a challenge infection. A similar approach was adopted by Hungerer *et al.* (1976) using ethidium bromide to abolish infectivity of culture forms. Results in animals were discouraging (Z. Brener, unpublished work; Brener, 1979). Irradiated *T. cruzi* tissue culture forms,which were non-infective to cell culture and mice, have been used as a living vaccine. Repeated weekly inoculations of parasites irradiated with 150 kR induced also only partial protection (Hanson, 1977).

A number of authors used culture forms killed by chemicals such as merthiolate (Muniz *et al.*, 1946), formaldehyde (Hauschka *et al.*, 1950), sodium perchlorate (Kierszenbaum and Budzko, 1975) as immunizing agents. No protection at all, or only partial protection, was reported. Better results are obtained with vaccines of flagellates killed by physical means. A significant increase of the survival time was detected in mice immunized with culture forms disintegrated by sonication (Goble, 1964). Partial protection represented by a decrease of parasitaemia and in mortality rates was achieved by inoculation of culture or bloodstream forms treated by freeze–thawing, freeze–drying and sonication (Neal and Johnson, 1977). Culture forms disrupted under pressure at low temperature in a Ribi Refrigerated Cell Fractionator were used by Gonzalez-Cappa *et al.* (1968) but again the immunity induced in mice was not total.

The presumed existence of cross-reacting antigens between *T. cruzi* components and heart tissue cells emphasizes the importance of investigating *T. cruzi* subcellular fractions which would protect the vertebrate without giving rise to auto-immune effects. Segura *et al.* (1977) compared the protective action of membrane, and flagellar, culture form fractions, and demonstrated that the flagellar fraction produced significantly higher resistance in mice challenged with virulent forms. Kaneta (1973) reported that the immunizing properties of culture form homogenates were related to a ribosomal fraction obtained by centrifugation at 150 000 ***g***.

Monogenetic trypanosomatids, which apparently present cross-reacting antigens with *T. cruzi*, also may protect mice. A slight immunity against *T. cruzi* was provided by homogenates of *Crithidia fasciculata* (Johnson *et al.*, 1963; Pereira *et al.*, 1977). The most interesting results, however, were obtained by inoculating mice with living suspensions of *Herpetomonas samuelpessoai*, a trypanosomatid isolated from a naturally-infected *Zelus leucogrammus* (Hemiptera). Partial protection was reported by Souza and Roitman (1971) and Souza *et al.* (1974).

References

Abrahmsohn, I. A. and da Silva, W. D. (1977). Antibody dependent cell-mediated cytotoxicity against *Trypanosoma cruzi*. *Parasitology*, **75**, 317–323.

Afchain, D. (1976). Le caractère antigenique des *Trypanosomatidae* hétéroxènes parasites de l'homme: *Trypanosoma* (*S.*) *cruzi*, *Trypanosoma* (*T.*) *B. gambiense* et *Leishmania donovani*. Thesis, Université des Sciences et Techniques de Lille, 162 pp.

Afchain, D., Fruit, J., Yarzabal, L. and Capron, A. (1978). Purification of a specific antigen of *Trypanosoma cruzi* from culture forms. *American Journal of Tropical Medicine and Hygiene*, **27**, 478–482.

Alcantara, A. (1979). Interação *Trypanosoma cruzi* e macrófagos peritoneais de camundongos 'in vitro'. Thesis, University of Minas Gerais, 126 pp.

Alcantara, A. and Brener, Z. (1978a). The *in vitro* interaction of *Trypanosoma cruzi* bloodstream forms and mouse peritoneal macrophages. *Acta Tropica*, **35**, 209–219.

Alcantara, A. and Brener, Z. (1978b). Role of macrophage membrane receptors on the uptake of *Trypanosoma cruzi:* removal by proteases, resynthesis and specificity for bloodstream and culture forms. Proceedings of Meeting on 'Basic Research on Chagas' Disease', Caxambu, Brazil.

Almeida, H. O., Tafuri, W. L., Bogliolo, L. and Cunha, J. C. (1974). Parasitismo incomum do miocardio e do esôfago em chagásico crônico, portador de Doença de Hodgkin e em uso de imunodepressores. *Revista da Sociedade Brasileira de Medicina Tropical*, **8**, 117–121.

Alves, M. J. M. and Colli, W. (1974). Agglutination of *Trypanosoma cruzi* by concanavalin A. *Journal of Protozoology*, **21**, 575–578.

Alves, M. J. M., Silveira, J. F., Paiva, C. H. R., Tanaka, C. T. and Colli, W. (1979). Evidence for the plasma membrane localization of carbohydrate-containing macromolecules from epimastigote forms of *Trypanosoma cruzi*. *Febs Letters*, **99**, 81–85.

Amato Neto, V., Magaldi, C. and Pessoa, S. B. (1964). Intradermorreação para o diagnóstico da doença de Chagas com antígeno de *Trypanosoma cruzi* obtido de cultura de tecido. *Revista Goiana de Medicina*, **10**, 121–126.

Andrade, S. G. and Andrade, Z. A. (1968). Patologia da doença de Chagas experimental de longa duração. *Revista do Instituto de Medicina Tropical de São Paulo*, **10**, 180–187.

Andrade, S. G. and Carvalho, M. L. (1969). Efeito da excitação do sistema reticulo-endotelial pelo adjuvante de Freund, na doença de Chagas experimental. *Revista do Instituto de Medicina Tropical de São Paulo*, **11**, 229–235.

Andrade, S. G., Silva, A. R. and Andrade, Z. A. (1967). Bloqueio e estimulação do S. R. E. na doença de Chagas. *Gazeta Médica da Bahia*, **67**, 19–30.

Andrade, Z. A. and Andrade, S. G. (1979). Patologia. *In Trypanosoma cruzi* e Doença de Chagas (Z. Brener and Z. A. Andrade, eds.), pp. 199–248. Guanabara, Rio de Janeiro.

Araujo, F. G. (1976). Immunology of Chagas' disease. I. Circulating antigens in mice experimentally infected with *Trypanosoma cruzi*. *Revista do Instituto de Medicina Tropical de São Paulo*, **18**, 433–439.

Araujo, F. G. and Nascimento, E. (1977). *Trypanosoma cruzi* infection in mice chronically infected with *Toxoplasma gondii*. *Journal of Parasitology*, **63**, 1120–1121.

Arend, W. P. and Mannik, M. (1972). *In vitro* adherence of soluble immune complexes to macrophages. *Journal of Experimental Medicine*, **136**, 514–531.

Austen, F. K. (1978). Homeostasis of effector systems which can also be recruited for immunological reactions. *Journal of Immunology*, **121**, 793–805.

Badinez, O. S. (1945). Contribucion a la anatomia patologica de lay enfermendad de Chagas experimental. *Biologica*, **3**, 3–52.

Behbehani, K. (1973). Developmental cycles of *Trypanosoma* (*Schizotrypanum*) *cruzi* (Chagas, 1909) in mouse peritoneal macrophages *in vitro*. *Parasitology*, **66**, 343–353.

Bergendi, L., Knierim, F. and Apt, W. (1970). *Trypanosoma cruzi:* immunological properties of a soluble extract of culture forms. *Experimental Parasitology*, **28**, 258–262.

Bianco, C., Griffin, F. M. and Silverstein, S. C. (1975). Studies on the macrophage complement receptor: alteration of receptor function upon macrophage activation. *Journal of Experimental Medicine*, **141**, 1278–1284.

Bittencourt, A. L. (1976). Congenital Chagas' disease. *American Journal of Diseases of Children*, **130**, 97–103.

Brener, Z. (1969). The behaviour of slender and stout forms of *Trypanosoma cruzi* in the bloodstream of normal and immune mice. *Annals of Tropical Medicine and Parasitology*, **63**, 215–220.

Brener, Z. (1973). Biology of *Trypanosoma cruzi*. *Annual Review of Microbiology*, **27**, 347–383.

Brener, Z. (1977). Intraspecific variation in *Trypanosoma cruzi:* two types of parasite populations presenting distinct features. Pan American Health Organization, Scientific Publication No. 347, 11–21.

Brener, Z. (1979). Vacinação. In *Trypanosoma cruzi* e Doença de Chagas (Z. Brener and Z. A. Andrade, eds.), pp. 450–455. Guanabara, Rio de Janeiro.

Brener, Z. and Cardoso, J. E. (1976). Nonspecific resistance against *Trypanosoma cruzi* enhanced by *Corynebacterium parvum*. *Journal of Parasitology*, **62**, 645–646.

Brener, Z. and Chiari, E. (1963). Observações sobre a fase crônica da doença de Chagas experimental no camundongo. *Revista do Instituto de Medicina Tropical de São Paulo*, **5**, 128–132.

Brener, Z. and Chiari, E. (1971). The effects of some immunosuppressive agents in experimental chronic Chagas' disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **65**, 629–636.

Brener, Z., Krettli, A. U. and Alcantara, A. (1978). Role of the spleen in Chagas' disease. Proceedings of Meeting on "The role of the spleen in the immunology of parasitic diseases", World Health Organization, Geneva.

Brito, T. and Vasconcelos, E. (1959). Necrotizing arteritis in megaesophagus. Histopathology of ninety-one biopsies taken from the cardia. *Revista do Instituto de Medicina Tropical de São Paulo*, **1**, 195–206.

Budzko, D. B., Pizzimenti, M. C. and Kierszenbaum, F. (1975). Effects of complement depletion in experimental Chagas' disease: Immune lysis of virulent blood forms of *Trypanosoma cruzi*. *Infection and Immunity*, **11**, 86–90.

Butterworth, A. E., David, J. R. Franks, D., Mahmond, A. A. F., David, P. H., Sturrock, R. F. and Houba, V. (1977). Antibody-dependent eosinophil-mediated damage to ^{51}Cr-labelled schistosomula of *Schistosoma mansoni*: damage by purified eosinophils. *Journal of Experimental Medicine*, **145**, 136–146.

Camargo, M. E. and Amato Neto, V. (1974). Anti-*T. cruzi* antibodies as serological evidence of recent infection. *Revista do Instituto de Medicina Tropical de São Paulo*, **16**, 200–202.

Castelo Branco, A. Z. C. L. (1978). Proteção mediada por imunoglobulina G em camundongos infectados com *Trypanosoma cruzi*. Thesis, University of Minas Gerais, 79 pp.

Castro, A. C. L. and Ribeiro dos Santos, R. (1977). Imunopatologia do rim na forma crônica da moléstia de Chagas experimental. *Revista Goiana de Medicina*, **23**, 1–13.

Clinton, B. A., Ortiz-Ortiz, L., Garcia, W., Martinez, T. and Capin, R. (1975). *Trypanosoma cruzi:* early immune response in infected mice. *Experimental Parasitology*, **37**, 417–425.

Cohn, Z. (1978). The activation of mononuclear phagocytes: fact, fancy and future. *Journal of Immunology*, **12**, 813–816.

Cossio, P. M., Diez, C., Szarfman, A., Krentzer, E., Candiolo, B. and Anara, R. M. (1974a). Chagasic cardiopathy. Demonstration of a serum gammaglobulin factor which reacts with endocardium and vascular structures. *Circulation*, **49**, 13–21.

Cossio, P. M., Laguens, R. P., Diez, C., Szarfman, A., Segal, A. and Arana, R. M. (1974b). Chagasic cardiopathy. Antibodies reacting with plasma membrane of striated muscle and endothelial cells. *Circulation*, **50**, 1252–1259.

Cossio, P. M., Damilano, G., La Vega, M. T., Laguens, R. P., Meckert, P. C., Diez, C. and Arana, R. M. (1976). *In vitro* interaction between lymphocytes of chagasic individuals and heart tissue. *Medicina*, **36**, 287–293.

Culbertson, J. T. and Kolodny, M. H. (1938). Acquired immunity in rats against *Trypanosoma cruzi*. *Journal of Parasitology*, **24**, 83–90.

Czop, J., Fearon, D. T. and Austen, K. F. (1978). Opsonin-independent phagocytosis of activators of the alternative pathway by human monocytes. *Journal of Immunology*, **120**, 1132–1138.

Dias, E. (1933). Immunité naturelle des animaux à sang froid vis-à-vis de l'infection par le *Trypanosoma cruzi*. *Comptes Rendus des Sociétés de Biologie*,**112**,1474–1475.

Dias, E. (1944). Não receptividade do pombo doméstico à infecção por *Schizotrypanum*. *Memórias do Instituto Owasldo Cruz*, **40**, 191–193.

Dvorak, J. A. and Schmunis, G. A. (1972). *Trypanosoma cruzi:* interaction with mouse peritoneal macrophages. *Experimental Parasitology*, **32**, 289–300.

Dzbenski, T. H. (1974). Exoantigens of *Trypanosoma cruzi* 'in vivo'. *Tropenmedizin und Parasitologie*, **25**, 485–491.

Ebert, F., Schudnagis, R. and Mühlpfordt, H. (1978). Protein typing by disc electrophoresis of some species of trypanosomes with special emphasis to *Trypanosoma cruzi*. *Tropenmedizin und Parasitologie*, **29**, 115–118.

Fayer, R. and Baron, S. (1971). Activity of interferon and an inducer against development of *Eimeria tenella* in cell culture. *Journal of Protozoology*, **18**, 12–17.

Fernandes, J. F., Halsman, M. and Castellani, O. (1965). Effect of actinomycin D on the infectivity of *Trypanosoma cruzi*. *Nature* (*London*), **207**, 1004–1005.

Fernandes, J. F., Halsman, M. and Castellani, O. (1966). Effect of Mitomycin C, Actinomycin D and pyrimidine analogs on the growth rate, protein and nucleic acid synthesis, and on the viability of *Trypanosoma cruzi*. *Experimental Parasitology*, **18**, 203–210.

França, L. C. M., Lemos, S., Fleury, S. N., Melaragu Filho, R., Ramos, Jr., H. A. and Pasternak, J. (1969). Moléstia de Chagas crônica associada a leucemia linfática: ocorrência de encefalite aguda como alteração do estado imunitário. *Arquivos de Neuro-Psiquiatria*, **27**, 60–66.

Goble, F. C. (1964). Vaccination against experimental Chagas' disease with homogenates of culture forms of *Trypanosoma cruzi*. *Journal of Parasitology*, **50** (Section 2), 19.

Goble, F. C. (1970). South American Trypanosomiasis. *In* "Immunity to Parasitic Animals" (G. J. Jackson, R. Herman and I. Singer, eds.), pp. 597–689. Appleton-Century-Crofts, New York.

Goble, F. C. and Boyd, J. L. (1962). Reticulo-endothelial blockade in experimental Chagas' disease. *Journal of Parasitology*, **48**, 223–228.

Golgher, R. R., Bertelli, M. S. M., Peixoto, M. L. and Brener, Z. (1976). Effect of interferon on the development of *Trypanosoma cruzi* in tissue culture 'Vero' cells. Proceedings of Meeting, on "Basic Research on Chagas' Disease", Caxambu, Brazil.

Gonzalez-Cappa, S. M., Schmunis, G. A., Traversa, O. C., Yanofski, J. F. and Parodi, A. S. (1968). Complement fixation tests, skin tests, and experimental immunization with antigens of *Trypanosoma cruzi* prepared under pressure. *American Journal of Tropical Medicine and Hygiene*, **17**, 709–715.

Gottlieb, M. (1977). A carbohydrate-containing antigen from *Trypanosoma cruzi* and its detection in the circulation of infected mice. *Journal of Immunology*,**119**, 465–470.

Guerreiro, C. and Machado, A. (1913). Da reação de Bordet e Gengou na molés tia de Carlos Chagas como elemento de diagnóstico. *Brasil Médico*, **27**, 225–226.

Hanson, W. L. (1977). Immune response and mechanisms of resistance in *Trypanosoma cruzi*. Pan American Health Organization, Scientific Publication No. 347, 22–34.

Hanson, W. L., Devlin, R. F. and Roberson, E. L. (1974). Immunoglobulin levels in a laboratory acquired case of human Chagas' disease. *Journal of Parasitology*, **60**, 532–533.

Hauschka, T. S., Godwin, N. B., Palmquist, J. and Brown, E. (1950). Immunological relationship between seven strains of *Trypanosoma cruzi* and its application in the diagnosis of Chagas' disease. *American Journal of Tropical Medicine and Hygiene*, **30**, 1–16.

Hoff, R. (1975). Killing *in vitro* of *Trypanosoma cruzi* by macrophages from mice immunized with *T. cruzi* or BCG, and absence of cross-immunity on challenge *in vivo*. *Journal of Experimental Medicine*, **142**, 299–311.

Howells, R. E. and Chiari, C. A. (1975). Observations on two strains of *T. cruzi* in laboratory mice. *Annals of Tropical Medicine and Parasitology*, **69**, 435–438.

Hungerer, K. D., Enders, B. and Zwisler, O. (1976). On the immunology of infection with *T. cruzi*. II. The preparation of an apathogenic living vaccine. *Behring Institute Mitteilungen*, **60**, 84–97.

Jahiel, R. I., Nussenzweig, R., Vilcek, J. and Vanderberg, J. (1968). Protective effect of interferon inducers on *Plasmodium berghei* malaria. *American Journal of Tropical Medicine and Hygiene*, **18**, 823–835.

Jarpa, A., Agosin, M., Christen, R. and Atias, A. (1951). Ensayos de quimioterapia de la enfermedad de Chagas experimental. VII. Cortisona y fosfato de pentaquina. *Boletin de Informaciones Parasitarias Chilenas*, **6**, 25–27.

Jarvinen, J. A. C. (1976). The role of complement in experimental rodent trypanosomiasis. Dissertation Abstracts International, University of Minnesota.

Jarvinen, J. A. C. and Dalmasso, A. P. (1977). *Trypanosoma musculi* infections in normocomplementemic, C5-deficient, and C3-depleted mice. *Infection and Immunity*, **16**, 557–563.

Johnson, P., Neal, R. A. and Gall, D. (1963). Protective effect of killed trypanosome vaccines with incorporated adjuvants. *Nature (London)*, **200**, 83.

Jones, C. J., Yeh, S. and Hirsch, J .G. (1972). The interaction between *Toxoplasma gondii* and mammalian cells. I. Mechanism of entry and intracellular fate of the parasite. *Journal of Experimental Medicine*, **136**, 1157–1172.

Jones, T. C., Len, L. and Hirsch, J. G. (1975). Assessment *in vitro* of immunity against *Toxoplasma gondii*. *Journal of Experimental Medicine*, **141**, 466–482.

Kagan, I. G. and Norman, L. (1961). Immunologic studies on *Trypanosoma cruzi*. III. Duration of acquired immunity in mice initially infected with a north American strain of *T. cruzi*. *Journal of Infectious Diseases*, **108**, 213–217.

Kaneda, Y. (1973). Protective effect of disintegrated culture forms of *Trypanosoma cruzi* on the mortality of mice after challenge. *Japan Journal of Parasitology*, **3**, 146–153.

Ketteridge, D. S. (1978). Lipopolysaccharide from *Trypanosoma cruzi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **72**, 101–102.

Khoury, E. L., Ritacco, V., Cossio, P. M., Laguens, R. P., Szarfman, A., Dez, C. and Arana, R. M. (1979). Circulating antibodies to peripheral nerve in American trypanosomiais (Chagas' disease). *Clinical and experimental Immunology*, **36**, 8–15.

Kierszenbaum, F. and Budzko, D. B. (1975). Immunization against experimental Chagas' disease by using culture forms of *Trypanosoma cruzi* killed with a solution of sodium perchlorate. *Infection and Immunity*, **12**, 461–465.

Kierszenbaum, F. and Howard, J. G. (1976). Mechanisms of resistance against experimental *Trypanosoma cruzi* infection: the importance of antibodies and antibody-forming capacity in the Biozzi high and low responder mice. *Journal of Immunology*, **116**, 1208–1211.

Kierszenbaum, F., Knecht, E., Budzko, D. B. and Pizziment, M. C. (1974). Phagocytosis: a defense mechanism against infection with *Trypanosoma cruzi*. *Journal of Immunology*, **112**, 1839–1844.

Kierszenbaum, F., Ivanyi, J. and Budzko, D. B. (1976). Mechanisms of natural resistance to trypanosomal infection. Role of complement in avian resistance to *Trypanosoma cruzi* infection. *Immunology*, **30**, 1–6.

Kipnis, T. C., Calich, V. L. G. and Dias da Silva, M. (1979). Active entry of bloodstream forms of *Trypanosoma cruzi* into macrophages. *Parasitology*, **78**, 89–98.

Koeberle, F. (1961). Patologia y anatomia patologica de la enfermedad de Chagas. *Boletin de la Oficina Sanitaria Panamericana*, **51**, 404–428.

Kolodny, M. H. (1940). Studies on age resistance against trypanosome infections. VII. The influence of age upon the immunological response of rats to infection with *Trypanosoma cruzi*. *American Journal of Hygiene*, **31**, 1–8.

Kress, Y., Bloom, B. R., Wittner, M., Rowen, A. and Tanowitz, H. (1975). Resistance of *Trypanosoma cruzi* to killing by macrophages. *Nature* (*London*), **257**, 394–396.

Kress, Y., Tanowitz, H., Bloom, B. and Wittner, M. (1977). *Trypanosoma cruzi:* infection of normal and activated mouse macrophages. *Experimental Parasitology*, **41**, 66–73.

Krettli, A. U. (1977). Exacerbation of experimental *Trypanosoma cruzi* infection in mice by concomitant malaria. *Journal of Protozoology*, **24**, 514–518.

Krettli, A. U. (1978). Efeito de anticorpos e do complemento sobre tripomastigotas sanguíneos de camundongos infectados com *Trypanosoma cruzi*. Thesis, University of Minas Gerais, 111 pp.

Krettli, A. U. and Brener, Z. (1976). Protective effects of specific antibodies in *Trypanosoma cruzi* infections. *Journal of Immunology*, **116**, 755–760.

Krettli, A. U. and Nussenzweig, R. S. (1977). Presence of immunoglobulins on the surface of circulating trypomastigotes of *T. cruzi* resulting in activation of the alternative pathway of complement and lysis. Washington, DC, USA; *PAHO Scientific Publication* no. 347, 71–73.

Krettli, A. U., Carrington, P. W. and Nussenzweig, R. S. (1979). Membrane-bound antibodies of bloodstream *Trypanosoma cruzi* in mice: strain differences in susceptibility to complement mediated lysis. *Clinical and experimental Immunology*, **3**, 1–8).

Kuhn, R. E. and Durum, S. K. (1975). The onset of immune protection in acute experimental Chagas' disease in C_3H (HE) mice. *International Journal of Parasitology*, **5**, 241–244.

Kuhn, R. W. and Murnane, J. E. (1977). *Trypanosoma cruzi:* immune destruction of parasitized mouse fibroblasts 'in vitro'. *Experimental Parasitology*, **41**, 66–73.

Kuhn, R. E., Vaughn, R. T. and Herbst, G. A. (1975). The effect of BCG on the course of experimental Chagas' disease in mice. *International Journal of Parasitology*, **5**, 557–560.

Kumar, R., Worthington, M., Tilles, J. G. and Abelmann, W. H. (1971). Effect of the interferon stimulator polyinosinic-polycytidylic acid on experimental *Trypanosoma cruzi* infection. *Proceedings of the Society of Experimental Biology and Medicine*, **137**, 884–888.

Lamy, L. H. (1970). Transformation intracellulaire des formes amastigotes de *Leishmania donovani* et *Trypanosoma cruzi* en formes mastigotes. *Protistologia*, **6**, 457–460.

Lay, W. H. and Nussenzweig, V. (1968). Receptors for complement on leukocytes. *Journal of Experimental Medicine*, **128**, 991–1009.

Lederkremer, R. M., Alves, M. J. M., Fonseca, G. C. and Colli, W. (1976). A lipopeptidophosphoglycan from *Trypanosoma cruzi* (epimastigote). Isolation, purification and carbohydrate composition. *Biochemica et Biophysica Acta*, **444**, 85–96.

Lederkremer, R. M., Tanaka, C. T., Alves, M. J. M. and Colli, W. (1977). Lipopeptidophosphoglycan from *Trypanosoma cruzi*. *European Journal of Biochemistry* **74**, 263–267.

Lelchuk, R., Dalmasso, A. P., Inglesini, C. L., Alvarez, M. and Cerisola, J. A. (1970). Immunoglobulin studies in serum of patients with American trypanosomiasis (Chagas' disease). *Clinical and Experimental Immunology*, **6**, 547–555.

Lelchuk, R., Patrucco, A. and Manni, J. A. (1974). Studies of cellular immunity in Chagas' disease: effect of glutaraldehyde-treated specific antigen on inhibition of leukocyte migration. *Journal of Immunology*, **112**, 1578–1581.

Lelchuk, R., Cardoni, R. L. and Fuks, A. S. (1977). Cell-mediated immunity in Chagas' disease. Alterations induced by treatment with a trypanocidal drug (nifurtimox). *Clinical and Experimental Immunology*, **30**, 434–438.

Leslie, G. A. and Martin, L. N. (1973). Modulation of immunoglobulin ontogeny in the chicken: effect of purified antibody specific for μ chain on IgM, IgY and IgA productiin. *Journal of Immunology*, **110**, 959–967.

Lima Pereira, F. E. (1978). Observações sobre a imunodepressão durante a fase aguda da infecção do camundongo albino pelo *Trypanosoma cruzi*. Thesis, University of Minas Gerais, 91 pp.

Lo Buglio, A. F. and Rinehart, J. (1970). *In vitro* and *in vivo* modification of human macrophage receptor for IgG globulin. *Clinical Research*, **18**, 409–416.

Lopes, E. R., Chapadeiro, F., Almeida, H. O. and Rocha, A. (1975). Contribuição ao estudo da anatomia patológica dos corações de chagásicos falecidos subitamente. *Revista da Sociedade Brasileira de Medicina Tropical*, **9**, 269–282.

Maria, T. A., Tafuri, W. L. and Brener, Z. (1972). The fine structure of different bloodstream forms of *Trypanosoma cruzi*. *Annals of Tropical Medicine and Parasitology*, **66**, 423–431.

Marsden, P. D., Seah, S. K. K., Mott, K. E., Prata, A. and Platt, H. (1970). Immunoglobulins in Chagas' disease. *American Journal of Tropical Medicine and Hygiene*, **73**, 157–161.

Martinez-Silva, R., Lopez, V. A. and Chiriboga, J. (1970). Effects of Poly I-C on the course of infection with *Trypanosoma cruzi*. *Proceedings of the Society of Experimental Biology and Medicine*, **134**, 885–888.

McHardy, N. (1977). Passive immunization of mice against *Trypanosoma cruzi* using convalescent mouse serum. *Tropenmedizin und Parasitologie*, **28**, 195–201.

Melo, R. C. and Brener, Z. (1978). Tissue tropism of different *Trypanosoma cruzi* strains. *Journal of Parasitology*, **64**, 475–482.

Menezes, H. (1969). Active immunization of dogs with a non-virulent strain of *Trypanosoma cruzi*. *Revista do Instituto de Medicina Tropical de São Paulo*, **11**, 258–263.

Menezes, H. (1971). Aplicação da vacina viva avirulenta de *Trypanosoma cruzi* em seres humanos (nota prévia). *Revista do Instituto de Medicina Tropical de São Paulo*, **13**, 144–154.

Milder, R. V., Kloetzel, J. and Deane, M. P. (1973). Observation on the interaction of peritoneal macrophages with *Trypanosoma cruzi*. I. Initial phase of the relationship with bloodstream and culture forms *in vitro*. *Revista do Instituto de Medicina Tropical de São Paulo*, **15**, 386–392.

Milder, R. V., Kloetzel, J. and Deane, M. P. (1973). Observations on the interaction of peritoneal macrophages with *Trypanosoma cruzi*. II. Intracellular fate of bloodstream forms. *Revista do Instituto de Medicina Tropical de São Paulo*, **19**, 313–322.

Montufar, O. M. B., Musatti, C. C., Mendes, E. and Mendes, N. F. (1977). Cellular immunity in chronic Chagas' disease. *Journal Clinical Microbiology*, **5**, 401–404.

Muniz, J. and Azevedo, A. P. (1947). Novo conceito da patogenia da doença de Chagas. *Hospital*, **32**, 165–183.

Muniz, J. and Borrielo, A. (1945). Estudo sobre a ação lítica de diferentes soros sobre as formas de cultura e sanguícolas do *Schizotrypanum cruzi*. *Revista Brasileira de Biologia*, **5**, 563–567.

Muniz, J. and Freitas, G. (1944a). Contribuição para o diagnóstico da doença de Chagas pelas reações de imunidade. I. Estudo comparativo entre as reações de aglutinação e de fixação de complemento. *Memórias do Instituto Iswaldo Cruz*, **41**, 303–333.

Muniz, J. and Freitas, G. (1944b). Contribuição para o diagnóstico da doença de Chagas pelas reações de imunidade. II. Isolamento de polisacarídeos de *Schizotrypanum cruzi* e de outros tripanosomideos, seu comportament nas reações de precipitação de fixação do complemento e de hipersensibilidade. Os testes de floculação (sublimado e formol-gel). *Revista Brasileira de Biologia*, **4**, 421–438.

Muniz, J. and Freitas, G. (1946). Realização 'in vitro' do ciclo do *S. cruzi* no vertebrado, em meios de caldo-líquido peritoneal. *Revista Brasileira de Biologia*, **6**, 467–484.

Muniz, J., Nobrega, G. and Cunha, M. (1946). Ensaios de vacinação preventiva e curativa nas infecções pelo *Schizotrypanum cruzi*. *Memórias do Instituto Oswaldo Cruz*, **44**, 529–541.

Neal, R. A. and Johnson, P. (1977). Immunization against *Trypanosoma cruzi* using killed antigens and with saponin as adjuvant. *Acta Tropica*, **34**, 87–96.

Nery-Guimarães, F. (1972). A refratariedade das aves ao *Trypanosoma* (*Schizo trypanum*) *cruzi*. I. Ausência de passagem para o sangue; duração da viabilidade e destruição dos parasitas na pele. *Memórias do Instituto Oswaldo Cruz.*, **70**, 37–48.

Nielsen, K. and Sheppard, J. (1977). Activation of complement by trypanosomes. *Experientia*, **33**, 769–771.

Nogueira, N. and Cohn, Z. (1976). *Trypanosoma cruzi:* mechanism of entry and intracellular fate in mammalian cells. *Journal of Experimental Medicine*, **143**, 1402–1420.

Nogueira, N. and Cohn, Z. (1978). *Trypanosoma cruzi:* in vitro induction of macrophage microbicidal activity. *Journal of Experimental Medicine* **148**, 288–300.

Nogueira, N., Bianco, C. and Cohn, Z. (1975). Studies of the selective lysis and purification of *Trypanosoma cruzi*. *Journal of Experimental Medicine*, **142**, 224–229.

Nogueira, N., Gordon, S. and Cohn, Z. (1977). *Trypanosoma cruzi:* modification of macrophage function during infection. *Journal of Experimental Medicine*, **146**, 157–171.

Nussenzweig, V. and Goble, F. C. (1966). Further studies on the antigenic constitution of strains of *Trypanosoma* (*Schizotrypanum*) *cruzi*. *Experimental Parasitology*, **18**, 224–230.

Nussenzweig, V., Deane, L. M. and Kloetzel, J. (1962). Diversidade na constituição antigênica de amostras de *Trypanosoma cruzi* isoladas do homen e de gambás (Nota preliminar). *Revista do Instituto de Medicina Tropical de São Paulo*, **4**, 409–410.

Nussenzweig, V., Deane, L. M. and Kloetzel, J. (1963a). Differences in antigenic constitution of strains of *Trypanosoma cruzi*. *Experimental Parasitology*, **14**, 221–232.

Nussenzweig, V., Kloetzel, J. and Deane, L. M. (1963b). Acquired immunity in mice infected with strains of immunological types A and B *Trypanosoma cruzi*. *Experimental Parasitology*, **14**, 233–239.

Ortiz-Ortiz, L., Gonzales-Mendoza, A. and Lamoyi, E. (1975). A vaccination procedure against *Trypanosoma cruzi* in mice by nonspecific immunization. *Journal of Immunology*, **114**, 1424–1425.

Ortiz-Ortiz, L., Ortega, T., Capin, R. and Martinez, T. (1976). Enhanced mononuclear phagocytic activity during *Trypanosoma cruzi* infection in mice. *International Archives of Allergy and applied Immunology*, **50**, 232–242.

Pereira, N. M., Souza, W., Machado, R. D. and Castro, F. D. (1977). Isolation and properties of flagella of Trypanosomatids. *Journal of Protozoology*, **24**, 511–514.

Pizzi, T. (1957). Immunologia de la enfermedad de Chagas. Universidad de Chile, 183 pp.

Pizzi, T., Rubio, M., Prager, R. and Silva, R. C. (1952). Acción de la cortisona en la infeccion experimental por *Trypanosoma cruzi* (Comunicacion preliminar). *Boletin de Informaciones Parasitarias Chilenas*, **7**, 22–24.

Rabinovitch, M. (1967). Attachment of modified erythrocytes to phagocytic cells in the absence of serum. *Proceedings of the Society of Experimental Biology and Medicine*, **124**, 396–400.

Ramos, C., Lamoyi, E., Feoli, M., Rodrigues, M., Perez, M. and Ortiz-Ortiz, L. (1978). *Trypanosoma cruzi:* immunosuppressed response to different antigens in the infected mouse. *Experimental Parasitology*, **45**, 190–199.

Reed, S. G., Larson, C. L. and Speer, C. A. (1977). Suppression of cell-mediated immunity in experimental Chagas' disease. *Zeitschrift für Parasitenkunde*, **52**, 11–17.

Remington, J. S. and Merigan, T. C. (1968). Interferon: protection of cells infected with an intracellular Protozoan (*Toxoplasma gondii*). *Science*, **161**, 804–806.

Roberson, E. L. and Hanson, W. L. (1974). Transfer of immunity to *Trypanosoma cruzi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **68**, 338.

Roberson, E. L., Hanson, W. L. and Chapman, W. L. (1973). *Trypanosoma cruzi:* effects of anti-lymphocyte serum in mice and neonatal thymectomy in rats. *Experimental Parasitology*, **34**, 168–180.

Robles Gil, J. and Perrin, M. (1950). Nota preliminar del estudio experimental sobre la acción de la hormona adrenocorticotrópica de la hipófisis en la enfermedad de Chagas. *Archivos del Instituto de Cardiologia de Mexico*, **20**, 314–326.

Rubio, M. (1956). Actividad litica de sueros normales sobre formas de cultivo y sanguineas de *Trypanosoma cruzi*. *Boletim Chileno de Parasitologia*, **11**, 62–69.

Rytel, M. W. and Marsden, P. D. (1970). Induction of an interferon-like inhibitor by *Trypanosoma cruzi* infection in mice. *American Journal of Tropical Medicine and Hygiene*, **19**, 929–931.

Sanderson, C. J., Lopez, A. F. and Moreno, M. M. B. (1977). Eosinophils and not lymphoid K cells kill *Trypanosoma cruzi* epimastigotes. *Nature* (*London*), **268**, 340–341.

Sanderson, C. J., Moreno, M. M. B. and Lopez, A. F. (1978). Antibody dependent cell-mediated cytotoxicity of *Trypanosoma cruzi:* the release of tritium-labelled RNA, DNA and protein. *Parasitology*, **76**, 299–307.

Santos, R. R. (1973). Contribuição ao estudo da imunidade na fase aguda da doença de Chagas experimental. *Revista de Patologia Tropical*, **2**, 433–463.

Santos, R. R. (1977). Imunopatologia da destruição neuronal da doença de Chagas experimental. Thesis, University of São Paulo, 94 pp.

Santos, R. R., Oliveira, J. C. R. and Rossi, M. A. (1975). Antibodies to neurones in chronic Chagas' disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **70**, 167.

Santos-Buch, C. A. and Teixeira, A. R. L. (1974). The immunology of experimental Chagas' disease. III. Rejection of allogeneic heart cells 'in vitro'. *Journal of Experimental Medicine*, **140**, 38–53.

Schmunis, G. A., Gonzalez-Cappa, S. M., Traversa, O. C. and Yanovsky, J. F. (1971). The effect of immuno-depression due to neonatal thymectomy on infections with *Trypanosoma cruzi* in mice. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **65**, 89–94.

Schmunis, G. A., Vattuone, H., Szarfman, A. and Pesce, U. J. (1973). Cell-mediated immunity in mice inoculated with epimastigotes or trypomastigotes of *Trypanosoma cruzi*. *Zeitschrift fur Tropenmedizin und Parasitologie*, **24**, 81–85.

Schmunis, G. A., Szarfman, A., Pesce, U. J. and Gonzalez-Cappa, S. M. (1977a). The effect of acute infection by *Trypanosoma cruzi* upon the response of mice to sheep erythrocytes. *Revista do Instituto de Medicina Tropical de São Paulo*, **19**, 323–331.

Schmunis, G. A., Barón, S., Gonzalez-Cappa, S. and Weissenbacher, M. C. (1977b). El *Trypanosoma cruzi* como inductor de interferon. *Medicina* **37**, 429–430.

Schmunis, G. A., Szarfman, A., Coarasa, L. and Vainstok, C. (1978a). Immunoglobulin concentration in treated human acute Chagas' disease. *American Journal of Tropical Medicine and Hygiene*, **27**, 473–477.

Schmunis, G. A., Szarfman, A., Langembach, T. and Souza, W. (1978b). Induction of capping in blood-stage trypomastigotes of *Trypanosoma cruzi* by human anti-*Trypanosoma cruzi* antibodies. *Infection and Immunity*, **20**, 567–569.

Seah, S. K. K., Marsden, P. D., Voller, A. and Pettit, L. E. (1974). Experimental *Trypanosoma cruzi* infection in rhesus monkeys—the acute phase. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **68**, 63–69.

Segura, E. L., Cura, E. N., Panlone, I., Vasquez, C. and Cerisola, J. E. (1974). Antigenic makeup of subcellular fractions of *Trypanosoma cruzi*. *Journal of Protozoology*, **21**, 571–574.

Segura, E. L., Vasquez, C., Bronsina, A., Campos, J. M., Cerisola, J. E. and Gonzalez-Cappa, S. M. (1977). Antigens of the subcellular fractions of *Trypanosoma cruzi*. II. Flagellar and membrane fraction. *Journal of Protozoology*, **24**, 540–543.

Silverstein, S. C. (1975). The role of mononuclear phagocytes in viral immunity. *In* 'Mononuclear phagocytes in immunity infection and pathology' (R. van Furth, ed.), pp. 557–573. Blackwell, Oxford.

Siqueira, A. F., Ribeiro, R. D. and Ferrioli Filho, F. (1978). Aspectos imunitários iniciais observados em ratos infectados por *Trypanosoma cruzi*. I. Reações de aglutinação e de imunofluorescência com antígeno de tripomastigotas recuperados em dias sucessivos da infecção. *Revista Brasileira de Pesquisas Médicas e Biológicas* **11**, 35–37.

Souza, W. (1978). Estudo ultra-estrutural, citoquímico e por criofratura da superfície celular do *Trypanosoma cruzi*. Thesis, University Federal of Rio de Janeiro, 90 pp.

Souza, M. C. and Roitman, I. (1971). Protective effects of *Leptomonas pessoai* against the infection of mice by *Trypanosoma cruzi*. *Revista do Instituto de Microbiologia*, **2**, 187–189.

Souza, M. C., Reis, A. P., Dias da Silva, W. and Brener, Z. (1974). Mechanisms of acquired immunity induced by *Leptomonas pessoai* against *Trypanosoma cruzi* in mice. *Journal of Protozoology*, **21**, 579–584.

Szarfman, A., Cossio, P. M., Laguens, R. P., Segal, A., de la Vega, M. T., Arana, R. M. and Schmunis, G. A. (1975a). Immunological studies in Rockland mice infected with *T. cruzi*. Development of antinuclear antibodies. *Biomedicine*, **22**, 489–495.

Szarfman, A., Cossio, P. M., Arana, R. M., Urman, J., Krentzer, E., Laguens, R. P., Segal, A. and Coarasa, L. (1975b). Immunological and immunopathological studies in congenital Chagas' disease. *Clinical Immunology and Immunopathology*, **4**, 489–499.

Takehara, H. A., Perini, A., Silva, M. H. and Mota, I. (1978). Imunidade humoral na doença de Chagas experimental. Proceedings Meeting on "Basic Research in Chagas' Disease", Caxambu, Brazil.

Taliaferro, W. H. and Pizzi, T. (1955). Connective tissue reactions in normal and immunized mice to a reticulotropic strain of *Trypanosoma cruzi*. *Journal of Infectious Diseases*, **96**, 199–226.

Tanowitz, H., Wittner, M., Kress, Y. and Bloom, B. (1975). Studies of *in vitro* infection by *Trypanosoma cruzi*. I. Ultrastructural studies on the invasion of macrophages and L-cells. *American Journal of Tropical Medicine and Hygiene*, **24**, 25–33.

Teixeira, A. R. L. and Santos-Buch, C. A. (1974). The immunology of experimental Chagas' disease. I. Preparation of *Trypanosoma cruzi* antigens and humoral antibody-response to these antigens. *Journal of Immunology*, **113**, 859–869.

Teixeira, A. R. L. and Santos-Buch, C. A. (1974). The immunology of experimental Chagas' disease. II. Delayed hypersensitivity to *Trypanosoma cruzi* antigens. *Immunology*, **28**, 401–410.

Teixeira, A. R. L., Teixeira, L. and Santos-Buch, C. A. (1975). The immunology of experimental Chagas' disease. IV. Production of lesions in rabbits similar to those of chronic Chagas' disease in man. *American Journal of Pathology*, **80**, 163–178.

Teixeira, A. R. L., Teixeira, G., Macedo, V. and Prata, A. (1978a). *Trypanosoma cruzi*-sensitized T-lymphocyte mediated ^{51}Cr release from human heart cells in Chagas' disease. *American Journal of Tropical Medicine and Hygiene*, **27**, 1097–1107.

Teixeira, A. R. L., Teixeira, G., Macedo, V. and Prata, A. (1978b). Acquired cell-mediated immunodepression in acute Chagas' disease. *Journal of Clinical Investigation*, **62**, 1132–1141.

Torres, C. M. (1941). Sobre a anatomia patológica da doença de Chagas. *Memorias do Instituto Oswaldo Cruz*, **36**, 391–404.

Tschudi, E. I., Anziano, D. F. and Dalmasso, A. P. (1972). Lymphocyte transformation in Chagas' disease. *Infection and Immunity*, **6**, 905–908.

van Furth, R. (1975). Mononuclear phagocytes in immunity, infection and pathology. Blackwell Scientific Publications, Oxford and Edinburgh, 1602 pp.

Vattuone, N. H., Szarfman, A. and Gonzalez-Cappa, S. M. (1973). Antibody response and immunoglobulin levels in humans with acute or chronic *Trypanosoma cruzi* infections (Chagas' disease). *American Journal of Tropical Medicine and Hygiene*, **76**, 45–47.

Voller, A. and Shaw, J. J. (1965). Immunological observations on an antiserum to *Trypanosoma cruzi*. *Zeitschrift für Tropenmedizin und Parasitologie*, **16**, 181–187.

Williams, D. M. and Remington, J. S. (1977). Effect of human monocytes and macrophages on *Trypanosoma cruzi*. *Immunology*, **32**, 19–23.

Williams, D. M., Sawyer, W. and Remington, J. S. (1976). Role of activated macrophages in resistance of mice to infection with *Trypanosoma cruzi*. *The Journal of Infectious Diseases*, **134**, 610–614.

Wyler, D. J., Oster, C. N. and Wuinn, T. C. (1978). Role of the spleen in malaria. Proceedings Meeting on "The role of the spleen in the immunology of parasitic diseases", World Health Organization, Geneva.

Yanovsky, J. F. and Albado, E. (1972). Humoral and cellular responses to *Trypanosoma cruzi* infection. *Journal of Immunology*, **109**, 1159–1161.

Zeledón, R. and Ponce, C. (1974). A skin test for the diagnosis of Chagas' disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **68**, 414–415.

Zuckerman, A. (1953). Culture or mononuclear cells from rat peritoneal exudate. *Proceedings of the Society of Experimental Biology and Medicine*, **82**, 469–472.

Immunity to Ticks

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I. Introduction

The investigation of acquired immunity to ticks has a long history. It was observed early this century that different breeds of cattle tend to carry different numbers of the tick *Boophilus microplus* and the first laboratory investigations of immunity were made in the 1930s. These early reports were sporadic and often inconclusive in their results. Fortunately, over the last decade or so there has been increasing interest in this aspect of parasite immunology, to the extent that a short review of current knowledge in the field is warranted. Some early results (to 1967) have been briefly discussed by Balashov (1971) and a more recent review of ectoparasites includes some of the work on ticks (Nelson *et al.*, 1977).

Despite the increasing literature in this field, no single host–parasite system is yet understood. At present then, it is probably more profitable not to catalogue research findings on a tick-by-tick and a host-by-host basis, but rather to consider the broader areas of immunological response which have been found in different systems in an attempt to see what generalizations can be made about the tick–host immunological relationship. In this review therefore I will discuss first the way in which immunity is expressed. This will be followed by a discussion of the various types of immune response: antibody formation and complement activation; delayed hypersensitivity; immediate hypersensitivity; and cellular reactions. Finally, the nature of the antigens involved and the possibility of artificial immunization will be mentioned.

In this review, 'immunity' will be used to denote any immunologically mediated response that is disadvantageous to the parasite. Frequently, this immunity is partial in that a proportion of parasites complete their life cycle successfully. In this case, the term 'resistance', often encountered in the literature on *Boophilus microplus*, is more accurate. The former term, however, will be mainly used since it does emphasize that the phenomena being discussed are immunological reactions.

II. Expression of Immunity

The way in which immunity is expressed varies greatly, depending on the host and tick species concerned. The effects range from simple rejection of the parasite, apparently with little or no damage to it, to interference with feeding, prolongation of feeding time, reduction in engorgement weights, inhibition of egg laying and decreased viability of eggs, to death of the parasite on the host.

The least dramatic immunity investigated in detail is that of cattle to *Boophilus microplus*. The main expression of resistance in *Bos taurus* (European cattle) is the rejection of larvae in the first 24 h of the parasitic life cycle (Roberts, 1968a) though the other new instars are similarly affected. The ability to reject ticks is an acquired one and starts to appear about 8 days after initial infestation of a previously unexposed animal with the parasite. This response was therefore presumed to be immunologically mediated (Roberts, 1968b). Observations of the same parasite on *Bos indicus* (Zebu or Brahman cattle) have given generally similar results (Wagland, 1979) though here it was found that a gradual loss of ticks occurred throughout the instars. The weights of fully engorged female ticks were reduced on immune cattle, for example by 30% on *Bos indicus* hosts (Wagland, 1978). Other effects of immunity are less important. There are reports of prolongation of feeding time and reduction of egg laying and egg viability in ticks fed on immune *Bos indicus* (Hewetson, 1971) but the effects were small on this host species, if they occurred at all (Wagland, 1975), and were apparently absent from those on *Bos taurus* or *Bos taurus* × *Bos indicus* crossbreds (Hewetson and Nolan, 1968; Roberts, 1968b). Of importance to our later consideration of the nature of the immune mechanism is the fact that there is little direct damage to the ticks. Although some may drown in serous exudate (Riek, 1962), this is rare (Roberts, 1971). During the first 24 h of the life cycle, the time of greatest susceptibility for the parasite, few die on the host (Koudstaal *et al.*, 1978). Three pieces of evidence show that damage to feeding larvae on immune hosts is minimal. Firstly, although larvae make repeated shorter attachments on hosts of high resistance relative to ones of low resistance (Kemp *et al.*, 1976), the fact that they are capable of detaching, moving and reattaching shows they have been little damaged over this time period. Secondly, larvae fed on both immune and non-immune hosts for 19 h, then removed and kept in an incubator had identical survival times (Roberts, 1971) again suggesting they were undamaged. Finally, it is known that if

an immune animal is physically restrained from grooming to remove parasites, the number of parasites successfully completing their life cycle can increase dramatically (Bennett, 1969; B. M. Wagland and L. A. Y. Johnston, personal communication), although the yields may eventually fall again. Again, this demonstrates that under normal circumstances a major expression of immunity in this system is the removal of ticks, rather than their destruction. One would hope to find an immunological mechanism consistent with this form of immunity.

Such undramatic immunity is the result of this particular host–parasite interaction, and not a necessary characteristic of either parasite or host separately. Attempts to rear *Boophilus miroplus* on rabbits were unsuccessful, as immunity was rapidly acquired and some ticks at least died on the host (Loomis, 1971). Cattle are capable of showing greater immunity to other ticks. *Bos taurus*, *Bos indicus* and crossbred cattle repeatedly infested with *Amblyomma americanum* acquired immunity, as shown by a reduction in the number of females engorging and a fall in the engorged weights. These effects were comparable with those found for *Boophilus microplus*. However, it was reported that many of the ticks which did not engorge fully died on the host at various ages and levels of engorgement (Strother *et al.*, 1974). Similarly, cattle on exposure to *Ixodes holocyclus* can acquire an immunity that results in removal of the ticks by grooming, death of the ticks *in situ*, or reduction in the engorgement weights (Doube and Kemp, 1975). Definite immunity was also expressed by cattle towards each instar of the three-host tick, *Haemaphysalis longicornis* (Sutherst *et al.*, 1979).

Rabbits have been used frequently in laboratory studies on immunity to ticks, and in many cases they were capable of mounting an effective response. Repeated infestation with *Ixodes ricinus* led to pronounced decreases in the percentage of ticks engorging, the engorged weights, the percentage of females laying eggs and the viability of the eggs and the length of feeding was substantially increased (Bowessidjaou *et al.*, 1977). Similar effects on the percentage of ticks engorging and engorgement weights have been observed for *Rhipicephalus appendiculatus* (Branagan, 1974), *Rhipicephalus sanguineus* (Garin and Grabarev, 1972) *Dermacentor variabilis* (Trager, 1939a) and *Hyalomma anatolicum excavatum* and (again) *Rhipicephalus sanguineus* (Köhler *et al.*, 1967). The last authors found the feeding times for both ticks significantly prolonged as well. Immunity in rabbits to *Haemaphysalis longicornis* was somewhat different in that, although the weights of engorged female ticks were reduced, the number of ticks engorging, length of engorgement and hatching rate of eggs were all unaltered (Fujisaki, 1978).

Three other systems have been investigated in detail. A single infestation of *Dermacentor andersoni* on guinea pigs was sufficient to produce almost complete immunity, as shown by a reduction in the percentage of larvae engorging to less than 20%, and frequently almost to zero (Allen, 1973; Wikel and Allen, 1976a). There was also a 6–7-fold reduction in the weight of larvae after 5 days of engorgement (Wikel and Allen, 1976a). These authors have provided additional evidence that the immunity was immunologically mediated by showing that its acquisition could be blocked by the prior or

concomitant treatment of the host with two immunosuppressants, methotrexate (Allen, 1973) and cyclophosphamide (Wikel and Allen, 1976b).

A single exposure of guinea pigs to larvae of *Ixodes holocyclus* conferred immunity, the percentage of larvae engorging falling from 40% on a primary infestation to only 1–2% on a secondary one (Bagnall, 1975a; Bagnall and Rothwell, 1974). The majority of the larvae died on the host (Bagnall, 1975b).

Finally, a number of amphibian species became immune to *Amblyomma testudinis*, with the result that engorgement weights, egg numbers and feeding rates were reduced (Schneider *et al.*, 1971).

This list is not exhaustive, but the tick–host systems mentioned so far are the ones in which information useful for an understanding of acquired immunity is available. Nevertheless, the acquisition of immunity has been observed with other ticks and other hosts by a variety of authors: Berdyev and Khudainazarova, 1976; Brumpt and Chabaud, 1947; Chabaud, 1950; Feldman-Muhsam, 1964; Musatov, 1967; 1970; Nikitina and Aristova, 1964; Pogorelyi, 1966; Stampa, 1959; Trager, 1939a. If these authors have contributed nothing directly to our understanding of the immunological basis for immunity, their reports at least confirm that the expressions of immunity described so far are general.

So far then there is evidence for at least two types of response, physical removal of the parasite, probably induced by the irritation of tick attachment, and an inhibition of feeding. These may, of course, be two manifestations of the same immunological response. Does the host ever cause actual damage to the tick, other than indirectly, by starvation? One is tempted to believe so, but evidence is lacking. The reduced engorgement weights, prolonged feeding times, reduced egg laying, even reduced egg viability could be solely due to poor nutrition. The best evidence for a direct effect on the tick has been with *Ixodes ricinus*. Although female ticks engorging on an immune rabbit were of low weight, these weights were insufficient to account for the observed reduction in egg laying (Bowessidjaou *et al.*, 1977), suggesting a toxic effect on ticks. This point is of interest since, although it is easy to see how the reactions that will be described in the following sections may produce irritation and impede feeding, it is less obvious how effectively they may cause physical damage to a parasite that is largely external to the host.

Finally, in this section, it is worth discussing the problem of 'innate resistance'. This term has been frequently and loosely used in the tick literature. What does it mean? If some single animals of a species that is normally a host for a particular parasite are found to be resistant to it before initial exposure to that parasite, then they can reasonably be described as 'innately resistant' (Roberts, 1968b; Riek, 1962). However, there has been a tendency to use the term to describe inter-species variation, in particular, in the immunity of *Bos indicus* and *Bos taurus* cattle to *Boophilus microplus* (for example, O'Kelly and Spiers, 1976). Three points can be made here. First, inter-species variation is to be expected in the response of any parasite to its hosts. A separate term for this variation seems unnecessary. Second, for the particular case mentioned, there is now very good evidence

that *Bos indicus* cattle before exposure to *Boophilus microplus* are as susceptible to the parasite as are *Bos taurus* (Hewetson, 1971; Wagland, 1975, 1978). Therefore, good evidence must be provided for any claim of 'innate resistance'. Third, the immunological response of an animal to ticks can be affected by its previous history and earlier parasitisms (Callow and Stewart, 1978). The term 'innate resistance' cannot be constructively applied unless such factors can be excluded.

III. Nature of the Immunological Response

A. Antibody and Complement in Immunity to Ticks

There is evidence in four cases that antibody is involved in immunity to ticks. In one of the first laboratory studies of immunity to ticks, Trager (1939a) transferred partial immunity to *Dermacentor variabilis* with serum. Although he was initially unable to demonstrate the presence of antibody, complement-fixing antibodies were reported subsequently (Trager, 1939b). Unfortunately, the transferred 'immunity' was very variable and no statistical analysis of significance was given, so it is difficult to draw conclusions from these experiments.

The immunity which rabbits acquire to *Ixodes ricinus* is at least partially antibody mediated. Brossard (1977) injected serum from immune rabbits into susceptible recipients at a rate of about 0·25 ml (100 g body weight)$^{-1}$ 4 h before an initial infestation with ticks, and then again 24 h later. The females engorging on these treated rabbits weighed significantly less than those on comparable control hosts, though the duration of engorgement was not affected. This serum transfer thus reproduced, though with reduced effectiveness, one of the expressions of immunity. Bowessidjaou *et al.* (1977) noted that antibody to *Ixodes ricinus* salivary gland, as measured by indirect immunofluorescence, appeared towards the end of a first infestation and reached high titres on a second, but did not increase thereafter, although the hosts became progressively more immune. This is not necessarily evidence that a further mechanism of resistance developed, since total antibody titres towards a whole organ (the salivary gland) need not reflect the concentration of antibody to a critical antigen, or the effectiveness of an antibody, for example, in inhibition of a feeding enzyme or complement activation.

Wikel and Allen (1976a) working with *Dermacentor andersoni* on guinea pigs, found that transfer of serum from immune donors into recipients at a rate of 1·5 ml (100 g body weight)$^{-1}$ did not confer immunity. The only effect seen was a slight fall in larval engorgement weights. It was not stated whether this was significant, but it seems unlikely since, in parallel experiments, transfer of lymph node cells from non-immune donors to non-immune recipients, effectively a control experiment, had a greater depressant effect on larval weights. Nevertheless, there is evidence that antibody is involved in this system. The same authors found that if cyclophosphamide was given to immune guinea pigs before an infestation with ticks, then the expression of

immunity was largely blocked (Wikel and Allen, 1976b). The concentration of cyclophosphamide used was supposed to be just sufficient specifically to block B cells and, although the authors noted some depletion of T cells, the main effect was in fact on B cells. This provides indirect evidence for an antibody requirement in immunity. Wikel and Allen (1977) also found that depletion of complement with cobra venom factor dramatically affected immunity. Complement depletion during a primary infestation did not affect the number of larvae engorging, or the acquisition of immunity to subsequent infestation. However, if cobra venom factor was given before and during a second infestation on immune guinea pigs, the number of larvae maturing increased almost to the level of the controls and the weights of engorged larvae increased by a factor of three, though they were still significantly less than those from control animals. Interestingly, a striking feature of immunity to this tick was the accumulation of basophils at the attachment site of the tick, and this was absent from complement-depleted animals. Although complement activation by parasites occurs in both immunologically mediated and non-immunological ways (Santoro *et al.*, 1979), the effect of complement on *Dermacentor andersoni* would seem to be an immunological reaction and so presumably antibody mediated.

The fourth system in which there is evidence for antibody involvement is *Boophilus microplus* on cattle. Antibodies have been demonstrated in two ways. Brossard (1976) found that serum γ-globulin concentration was significantly increased following tick infestation. Using indirect immunofluorescence, he showed the presence of specific and non-specific antibodies to the salivary gland of adult female ticks. Specific antibody appeared following initial infestation of cattle with ticks, reached high titres, which were maintained throughout infestation, and then declined over a period of months once infestation was finished. Although the appearance of antibody coincided with the acquisition of resistance to the parasite, a causal relationship was not established. Willadsen *et al.* (1978) measured antibody to a purified tick antigen by indirect haemagglutination; the antibody was specific, in that it was absent from unexposed animals, but its concentration did not correlate with the degree of immunity of an animal and, in fact, higher titres tended to be found in cattle of low immunity. Roberts and Kerr (1976) transferred large volumes of plasma (4 ml (100 g body wt)$^{-1}$) from highly immune, poorly immune or unexposed cattle to groups of unexposed calves, which were then exposed to ticks. The numbers of adult females engorging on the last two groups were not significantly different, but on the first group, only about half the number of ticks matured, and the difference was statistically significant.

The production of antibody in response to tick infestation has been reported in a number of other cases, although evidence has not been given that this was a causal factor in immunity. Thus Fujisaki (1978) found that rabbits developed an IgG antibody to *Haemaphysalis longicornis*. Precipitating antibodies have been found in a number of amphibia exposed to *Amblyomma testudinis* (Schneider *et al.*, 1971), and both precipitating and complement fixing antibodies have been found in rabbits with *Hyalomma anatolicium*

excavatum and *Rhipicephalus sanguineus* (Köhler *et al.*, 1967; Weiland and Emokpare, 1968). On the other hand, attempts to transfer immunity to *Ixodes holocyclus* in guinea pigs with serum were unsuccessful (Bagnall and Rothwell, 1974). Since this system is, in other respects, very like *Dermacentor andersoni* on the same host, this result must be taken as inconclusive evidence that antibody is not involved.

What effects could antibody have on a tick? Inhibition of feeding enzymes has often been proposed, though there is no evidence that this occurs. Recently, however, Tracey-Patte (1979) found that activity of an enzyme from *Boophilus microplus* which is secreted into the host's skin within 1 h of attachment can be removed by a host previously exposed to the tick. In unexposed hosts, removal does not occur. This reaction could be antibody-dependent. In any tick–host system, antibody-dependent activation of complement could occur and the evidence for this in *Dermacentor andersoni* has already been mentioned. *Boophilus microplus* larvae excrete into the host a protein which is capable of inhibiting bovine complement, though whether this occurs *in vivo* is unknown (Willadsen and Riding, 1980). Thus complement would seem to be a fruitful area for investigation in the cases where anti-tick antibody has been demonstrated. However, observation of complement effects must be interpreted with caution. A salivary gland extract of *Dermacentor variabilis* generated chemotactic activity for neutrophils on incubation with dog, human or mouse serum and purified human C5. No activity was generated from serum from C3 or C5 deficient mice (Berenberg *et al.*, 1972). Possibly the tissue destruction caused by the neutrophils after this non-immunological reaction is beneficial to the tick's feeding.

Reaginic antibodies have not been discussed here but they will be considered below.

B. DELAYED HYPERSENSITIVITY REACTIONS

Although there are several reports in the literature of delayed hypersensitivity in response to ticks, only two cases have been investigated in detail. Wikel *et al.* (1978) found that intradermal injection of 50 μg of antigenic material from *Dermacentor andersoni* into tick-resistant guinea pigs gave a significant delayed hypersensitivity reaction, a transient increase in skin thickness occurring between 24 and 96 h after injection and reaching a maximum at 48 h. At this time, the reactions were indurated and necrotic. Unexposed control animals showed little reaction. Although there was a slight immediate reaction 30 minutes after injection, this was non-specific. The antigen used was an extract of salivary glands from adult female ticks which had been allowed to engorge for 4 days on rabbits. Attachment cement from the tick gave no reaction, though the amount of material tested was not specified. As a further indicator of delayed hypersensitivity, lymphocyte blastogenesis stimulated by the same salivary gland antigen was measured. Sensitivity to the antigen appeared 2–4 days after the end of a primary tick infestation. When a second infestation was started 1 week

after the end of the first, peak lymphocyte response was observed 24 h after initiation of the infestation but this fell rapidly, so that lymphocyte transformation was just significant by the end of the tick engorgement period. The induced blastogenesis was specific, as lymphocytes from non-immune donors were unaffected by the antigen. The authors also noted that lymphocytes from immune donors had lower basal levels of methyl thymidine uptake, and were less stimulated by the non-specific mitogen, phytohaemagglutinin, than were control cells. They suggested that immunosuppression could be occurring. Regrettably, the data on this effect were not given and neither was a statistical analysis, so the significance of the effect cannot be judged. If immunosuppression does occur in this case, it is clearly ineffective in preventing the expression of immunity. Whether T or B lymphocytes were being stimulated by antigen is unknown.

In the first paper of the series on this tick–host system, Allen (1973) found that the striking characteristic of the feeding lesion on the guinea pig was the accumulation of basophils. These began to appear in the dermis and epidermis at the end of a primary infestation and again 1 day after the start of a secondary infestation. The numbers were greatly increased by the end of the infestation, particularly in vesicles underneath attached larvae. Eosinophils were also prominent in the dermis by this time.

Although, as described above, attempts to transfer immunity with serum from immune animals were unsuccessful, transfer of cervical lymph node cells from immune donors to unexposed recipients did successfully transfer immunity (Wikel and Allen, 1976a). A total of 10^8 cells were injected intraperitoneally into recipients, which were challenged 48 h later. Two indices of immunity were used—the percentage of larvae engorging and the weights of larvae removed from the host after 5 days. A comparison of these for passively and naturally immunized hosts showed the former group to be almost as resistant to the tick larvae as the latter—for example 19% of larvae engorged on the former group and 13% on the latter, whereas 75% engorged on the controls. In an unpublished observation from the paper cited above, the authors state that immunity can be passively transferred with a population of lymphocytes enriched in T cells.

Thus many of the reactions seen in guinea pigs immune to *Dermacentor andersoni* are typical of a Jones-Mote reaction or cutaneous basophil hypersensitivity. The timing of a basophil accumulation correlates with the peak sensitivity of lymphocytes to salivary gland antigen, suggesting the two are related. Immunity is transferred by lymph node cells, with an indication that T cells may be the functional agent. It would be of great interest to know if the histology of the tick attachment site is the same in both passively and actively immunized hosts, but the authors do not comment on this. Just as, in this case, it is not certain that T cells are operative, in the literature reports on cutaneous basophil hypersensitivity there is evidence that T cells are essential (Stadecker and Leskowitz, 1975), but antibodies may be involved too (Askenase *et al.*, 1975). The situation may not be so simple, however, since although eosinophils are rare in cutaneous basophil hypersensitivity reactions (Dvorak *et al.*, 1970) they are common in the tick lesion.

Bagnall (1975b) investigated the immunity developed by guinea pigs to *Ixodes holocyclus* and found many similar features. At 18 h after attachment of larvae to immune hosts, erythema developed and an infiltration of basophils, lymphocytes and neutrophils had started. By 48 h, large numbers of basophils had accumulated and partially degranulated. By 72 h, most larvae had died, eosinophils were present and by 96 h their numbers approximately equalled those of the basophils. The lesion was strikingly different in non-immune hosts and basophils were not present in significant numbers before about 96 h. Somewhat comparable effects were seen in nymphal and adult lesions, though more intense local inflammation was seen in lesions on non-immune hosts with these stages. Intradermal injection of a larval extract gave a local inflammatory reaction at 30 min in both immune and control animals, though in immune ones the reaction was more pronounced. There was a small delayed (48 h) skin response and this occurred only in the immune hosts. Histological examination of the injection sites at 48 h showed inflammation in all animals, with an accumulation of neutrophils, lymphocytes and macrophages but the immune animals also showed a specific accumulation of basophils and eosinophils.

Attempts were then made to transfer immunity (Bagnall, 1975b). An intravenous injection of $2{\cdot}5 \times 10^8$ cells from axillary and prescapular lymph nodes was given and followed 3 h later by infestation with *Ixodes holocyclus* larvae. In three experiments the mean rejection of larvae ranged from 38 to 65% and these differences between immunized and control animals were highly significant. The transferred immunity is less striking than that reported for *Dermacentor andersoni*, although the method was slightly different. Interestingly, although the results of attempts to transfer resistance with serum from immune animals were variable and usually not significant, in one experiment a combination of serum and cells was used. In this case, the transfer of $2{\cdot}5 \times 10^8$ lymph node cells produced a larval rejection rate of 38%, intravenous injection of 5 ml of serum from immune animals gave a significant rejection of 33%, and for both treatments combined, the rejection was 65%. Although this is the result of just one experiment it is the best evidence to date of cooperation between cells and serum.

These two examples of delayed hypersensitivity reactions to ticks are both from guinea pigs and they have many features in common. It is still not certain, however, that the striking basophil accumulation is responsible for immunity. Askenase and Worms (1979) reported qualitatively the same results for slow-feeding hard ticks on guinea pigs as have been described for *Dermacentor andersoni* and *Ixodes holocyclus*. Immunity and basophil accumulation were both transferred to non-immune animals with either serum or peritoneal exudate cells. Immunity was not developed to rapidly feeding soft ticks since although basophil abscesses and basophilia occurred in guinea pigs infested repeatedly with soft ticks, these responses occurred after feeding was complete. More surprisingly perhaps, if a third soft tick infestation was given at the peak of basophilia induced by the second infestation, the ticks were still unaffected.

Delayed reactions have been reported in two other instances. Gregson (1970) found he developed an irritating wheal reaction to *Dermacentor andersoni* 24 h after tick attachment, or after injection of cement extract or saliva. His peripheral blood lymphocytes were stimulated by saliva or cement extract, whereas those from a non-sensitive person were not. There was no evidence that this delayed reaction affected the tick adversely and the human and guinea pig were presumably responding to different antigens from the same tick, since Gregson reacted to the attachment cement, and guinea pigs did not (Wikel *et al.*, 1978).

Tritschler (1965) reported one case of allergy in a horse to *Amblyomma americanum*. A macerated tick extract gave a swelling 6 to 36 h after injection. The indications from the report are that this reaction did not prevent prolonged attachment of the ticks.

C. IMMEDIATE HYPERSENSITIVITY REACTIONS

Immediate hypersensitivity in response to tick infestation has been investigated in detail in only one case, the association between cattle and *Boophilus microplus*. Riek (1956, 1962) reported that cattle exposed to the tick were intensely irritated by larvae. Papular reactions were seen around nymphs and adults on resistant cattle and a transient increase in blood histamine levels was found in exposed cattle during tick infestation. Intradermal injection of extracts of eggs or larvae gave immediate oedematous dermal reactions and these reactions could be passively transferred locally by serum. That is, Prausnitz-Küstner reactions were demonstrated. Although Riek suggested that these reactions were responsible for resistance, no causal relationship was shown. Riek (1956) stated that there was no correlation between the intradermal test reaction and the observed hypersensitivity to ticks. Hence the importance of this type of immunological response was uncertain, particularly after Tatchell's suggestion that the oedema consequent on an allergic reaction was advantageous to the tick (Tatchell, 1969; Tatchell and Moorhouse, 1968). Although this suggestion is feasible, *Boophilus microplus* has alternative ways of producing oedema or increasing blood flow. Saliva from adult female ticks is capable of contracting rat fundus and increasing capillary permeability in unexposed cattle (Tatchell and Binnington, 1973). One of the active components of this saliva is a prostaglandin (Dickinson *et al.*, 1976; Higgs *et al.*, 1976). Hence immediate hypersensitivity as an aid to feeding seems less necessary than was previously thought.

Three allergens have now been separated from unfed tick larvae and two of them have been highly purified (Willadsen and Williams, 1976; Willadsen *et al.*, 1978; Willadesen and Riding, 1979). On intradermal injection into cattle, all three of them gave oedematous reactions which reached a maximum after 20 min and subsequently faded. There was no detectable delayed response. The reactions were immunologically specific, since they were observed only in cattle previously infected with the tick and Prausnitz-Küstner reactions could be shown for all three with serum from exposed but not from unexposed cattle. More importantly, the immediate hypersensitivity

could be quantified by injecting a series of antigen dilutions and measuring the size of the resultant oedematous reactions. Two of the allergens (allergens 1 and 2) were tested in cattle with differing degrees of resistance and the sensitivity to allergen was correlated with the level of immunity (Willadsen *et al.*, 1978). The higher the level of immunity, the more sensitive the animal was to small amounts of allergen. In *Bos taurus*, there was a range of more than 10^4 in responsiveness to allergen and the sensitivity was high, some cattle giving measurable reactions to 0·1 ng of allergen 1 or to 0·01 ng of allergen 2. These results suggest strongly that immediate hypersensitivity is responsible in part for the resistance of cattle to the tick and a series of other observations are consistent with this possibility. As has been stated earlier, a major expression of resistance in this tick–host system is the rejection of larvae in the first 24 h of the life cycle; within this time period they make shorter, repeated attachments on hosts of high resistance compared with less-resistant ones, but if removed from the host they seem to be undamaged; restriction of grooming activity greatly increases the yield of ticks. All this suggests a response which is irritating to the host and sufficiently unpleasant to the tick to make it move, without causing it significant damage. It is also known that grooming activity is greater the greater the immunity, and that it is tick-induced (Koudstaal *et al.*, 1978). Injection of histamine beneath an attached tick will make it detach; this effect is specific to histamine (Kemp, 1978; D. Kemp and A. Bourne, unpublished work). Further, the cellular responses to larval attachment are typical of an immediate hypersensitivity reaction. Schleger *et al.* (1976) compared cellular responses to larval attachments on cattle with various degrees of resistance, 3 h after the larvae had attached. The most striking features in highly resistant animals were eosinophil accumulation and mast cell degranulation. These were less pronounced in animals of low resistance and little of either occurred in unexposed animals. Neutrophils were found occasionally in 3 h lesions, more commonly in 5 h ones and they seemed to be more numerous in cattle of high resistance.

What limits the intensity of the immediate hypersensitivity reactions to ticks? Although these responses are acquired, immunologically mediated ones there is great variation between animals of a single breed in their ability to become immune to ticks. The difference between cattle of high and low resistance is not due to differing exposure to the parasite as cattle after an initial period of susceptibility acquired an individual, relatively stable resistance level (Roberts, 1968b). A number of factors that could potentially limit the immediate hypersensitivity reactions have been investigated. Attempts to show differing serum concentrations of IgE specific to allergens 1 and 2 by Prausnitz-Küstner reactions were unsuccessful (Willadsen *et al.*, 1978). Although histamine is a major mediator of these reactions in cattle skin, sensitivity to intradermal injection of histamine varied little from animal to animal. There was, however, a two-fold difference in the average total histamine content of skin from high- and low-resistance cattle (Willadsen *et al.*, 1979), just as there were differences in mast cells between the two groups (A. Schleger and D. Lincoln, unpublished work). Whether these factors are sufficient to explain the very large range of sensitivity to allergen seems

doubtful. In fact, two pieces of evidence suggest that the differences between animals are not due to such factors, which would affect all allergic reactions in common. Firstly, although the sensitivity to two tick allergens correlated with resistance (Willadsen *et al.*, 1978), the sensitivities to these two proteins did not correlate with each other particularly well, indicating that a more specific factor limits reactivity. Secondly, no significant difference in the response to intradermal injection of concanavalin A in animals of high and low resistance was found by Willadsen (1979). Concanavalin A mimics an anti-IgE antibody in several species and in cattle it gives an immediate wheal reaction over the same time scale as an allergen. It might then be expected to show between-animal variation, if factors such as mast cell pharmacology, concentrations of inflammatory compounds, sensitivity to these and so on limited the allergic response. Since these appear not to be critical factors, then a specific immunological response, such as the amount of mast-cell bound anti-allergen IgE, may be critical but direct evidence of this is lacking.

Although considerable evidence now indicates that immediate hypersensitivity is partially responsible for immunity to *Boophilus microplus*, it is unlikely to be the whole cause. A proportion of the loss of larvae during the first 24 h of the life cycle is not attributable to grooming (Koudstaal *et al.*, 1978). Both this and the slow loss of ticks during the attachment of each instar may be due to some other type of response. There is evidence for a second immunological response from the studies on grooming. Although restraining resistant cattle from grooming caused a sharp increase in the yield of ticks, the numbers on some hosts eventually fell again (Bennett, 1969). During this second period of acquisition of resistance, the weights of engorged female ticks fell significantly, suggesting that some new or enhanced response may have been producing tick removal (B. M. Wagland and L. A. Y. Johnston, unpublished work).

Immediate hypersensitivity may be involved in immunity to other ticks. This has been suggested several times for *Dermacentor andersoni* (Allen, 1973; Allen *et al.*, 1979) though, except for the observed eosinophil infiltration and basophil degranulation, which are typical of allergic reactions, evidence is lacking. Similarly, in a comparison of cattle on primary or subsequent infestations with *Ixodes holocyclus*, the latter group showed increased eosinophil and basophil infiltration, and basophil degranulation. There was no significant difference in mast cells (Allen *et al.*, 1979). Skin sensitizing antibody was shown by passive cutaneous anaphylaxis with the serum of guinea pigs infested more than four times with the same tick (Bagnall, 1978). Only a single exposure, however, is needed to confer immunity.

Boese (1974) found that rabbits acquired a persistent immunity to *Haemaphysalis leporispalustris*. The serum of most rabbits gave positive homologous passive cutaneous anaphylaxis (PCA) reactions but these were only demonstrable after resistance had already been acquired. Nevertheless, he found that high levels of resistance tended to be associated with high PCA titres. Finally, both *Rhipicephalus sanguineus* and *Haemaphysalis spinigera* contain histamine-like substances, and the former also has a compound in salivary glands

capable of blocking histamine action on guinea pig ileum (Chinery and Ayitey-Smith, 1977). Although this is probably a system for producing and controlling oedema as an aid to feeding, a histamine-blocking compound might also act as a defence against an allergic reaction.

D. CELLULAR REACTIONS

Considerable work has been done on the histology of tick attachment sites, but it is not intended to review it here. This has been done elsewhere, for example by Balashov (1971) and by Binnington and Kemp in this volume. Most of the papers with relevance to tick immunity have already been mentioned. Several points, however, should be made. The occurrence of a cellular reaction is no indication in itself that the reaction is relevant to immunity. The evidence that *Dermacentor variabilis* generates chemotactic activity for neutrophils, possibly as an aid to feeding, has been given before (Berenberg *et al.*, 1972). Tatchell and Moorhouse (1970) showed that the tissue damage caused by *Rhipicephalus sanguineus* in dogs was due to the host's neutrophils, not the tick. With the same tick and host, Theis and Budwiser (1974) found polymorph infiltration, oedema and mast cell breakdown, occurring identically in both previously exposed and unexposed hosts. Resistance was not acquired, so these events apparently did the tick no harm. Had such observations been made with a tick attachment on an immune host, immunity could well have been attributed to them. Unfortunately, comparative histology of immune and non-immune hosts has been done in only a few cases.

Not surprisingly, there are variations in the cellular reactions for the single host species with a variety of ticks and for a particular tick with a variety of host species. For example, the histology of the attachment sites of *Ixodes holocyclus* and *Boophilus microplus* on cattle are significantly different (Allen *et al.*, 1977; Tatchell and Moorhouse, 1968; Schleger *et al.*, 1976), although the comparison is confused by the use of different instars. The tick *Amblyomma testudinis* on a number of immune amphibia gave different types of lesion (Schneider *et al.*, 1971). It would be interesting to know if such differences affect the expression of immunity.

IV. ARTIFICIAL IMMUNIZATION AND THE NATURE OF PROTECTIVE ANTIGENS

Several attempts have been made to artificially immunize hosts with tick extracts. Trager (1939b) obtained partial immunity in guinea pigs with an injection of *Dermacentor variabilis* extracts and Gregson (1941) used an extract of *Dermacentor andersoni*, though with a total of two guinea pigs in the experiment, the significance of the results is questionable. Köhler *et al.* (1967) reduced the number of *Hyalomma anatolicum excavatum* maturing on one rabbit by prior injection with salivary gland extract, though the procedure killed the three other rabbits in the experiment. More convincingly, Schneider *et al.* (1971) found that immunization of tortoises with a homogenate of 25

unfed nymphs of *Amblyomma testudinis* prevented feeding. Garin and Grabarev (1972) reported success in immunizing with subcutaneous injections of *Rhipicephalus sanguineus* salivary glands. Two convincing series of experiments have been published to date. Bagnall (1975b) in a number of tests with a total of 60 guinea pigs consistently produced immunity by subcutaneous injection of 1·4 mg of larval extract of *Ixodes holocyclus*. The percentage rejection of larvae given as a challenge dose 3 weeks later varied from 29 to 68%, compared with unvaccinated controls, but all results were statistically significant. This is still less than the naturally acquired immunity. More recently, Allen and Humphreys (1979) immunized guinea pigs against *Dermacentor andersoni* with extracts of either midgut and reproductive organs (antigen I) or all internal organs (antigen II). Ticks from the hosts immunized with antigen I produced significantly fewer eggs than those from controls, and no larvae hatched from the eggs that were laid. The effects were more dramatic in the guinea pigs immunized with antigen II since the ticks failed to engorge and produced no eggs. The antigen extracts were prepared from female ticks that had been allowed to feed for 5 days on guinea pigs. Extracts from tissues of unfed ticks were ineffective, suggesting that important antigens were produced only during the late development of the tick. Calves immunized with antigen I in the same way gave ticks in which live weights, egg production and the hatching of larvae were all reduced, though the total recovery of ticks was not. Although immunized guinea pigs seemed to give ticks that laid non-viable eggs, this appeared not to be the case with immunized cattle, since the decreased production of larvae was generally accounted for by the decreased production of eggs. Interesting though these results are, their full significance cannot be judged until something is known about naturally acquired immunity of cattle to *Dermacentor andersoni*. Does the injection of tick extracts mimic the natural acquisition of immunity, or does it lead to enhanced immunity by enabling the host to develop an immune response to antigens not normally encountered in the natural situation?

Finally in the list of immunization experiments, Brossard (1976) subcutaneously injected two calves at birth with 100 salivary glands from partially engorged adult female *Boophilus microplus*. These calves were infested at 2 and 5 months and gave a lower yield of engorged ticks than did two controls. Although the number of animals was too small for the result to be convincing, it is consistent with the unpublished observations of J. A. Roberts, who found that attempted immunization with extracts of this tick could reduce the initial susceptibility of cattle to the tick, though the eventual resistance level was not affected.

To date then, attempts to immunize artificially against ticks have been at best partially successful. This lack of success has been, if anything, a stimulus to proposals about how one might immunize against ticks and an inducement to enthusiastic speculation about the potential efficacy of immunization in tick control (Allen and Humphreys, 1979; Smith, 1979). There is currently no reason for such optimism, not only because success in laboratory experiments to date has been limited but also because none of the methods of

immunization used so far is likely to be acceptable and economically sound in a practical situation.

What is known about the nature of the protective antigens? If immunity is induced with one instar and shown to be operative against the others, clearly some of the protective antigens are the same for all stages of the life cycle. Such cross immunity has been shown for *Ixodes holocyclus* (Bagnall, 1978). Schneider *et al.* (1971) found that tortoises infested with either nymphs or adults of *Amblyomma testudinis* developed precipitating antibody to the same antigen. Such cross-immunity cannot be taken for granted. One of the antigens characterized in *Boophilus microplus* is an inhibitor of proteolytic enzymes (Willadsen and Riding, 1979). This is present in unfed larvae but it disappears within 2 or 3 days of attachment on the host, presumably by injection into the host and it is not detectable in other instars (Willadsen and Riding, 1980). Thus the same antigens need not persist throughout the life cycle.

Virtually nothing is known about the number and location of antigens within the tick. Almost all of the experiments reported to date have used extracts either of whole, macerated ticks or of tick salivary glands as 'antigens' and the latter has been the most common source. Although it is reasonable to expect antigens to be in the salivary gland, this might not always be the case, nor need they be confined to this one organ. Immunofluorescence staining with serum from rabbits infested with both *Hyalomma anatolicum* and *Rhipicephalus sanguineus* showed antigen in the digestive system as well as the salivary gland (Köhler *et al.*, 1967). How many antigens may be involved in the immune reaction in any particular host–parasite system is unknown, although Fujisaki (1978) found up to four precipitin lines with serum from rabbits infested with *Haemaphysalis longicornis*. Several authors have reported the number of precipitin bands formed after injection of a tick extract into, for example, a rabbit, but it is difficult to see any value in this information.

It is possible that a tick could secrete an antigen into the host with the sole intention of eventually stimulating an immune reaction. Unless this is the case, however, any antigen should be characterized not only by its immunological reactivity but also by a biochemical function, for example, as a feeding enzyme. Characterization of tick antigens to which hosts react under natural infestation in terms of their biochemical function has been reported only for *Boophilus microplus*. Of the three antigens studied to date in this tick, one is a hydrolytic enzyme, a serine esterase (Willadsen and Williams, 1976; Willadsen, 1976) and another an inhibitor of proteolytic enzymes (Willadsen and Riding, 1979). In tests *in vitro*, this second antigen inhibited both blood coagulation and complement-induced cell lysis. The function of the third antigen is still unknown.

Nothing is known about the effect a tick antigen may have on the form of immunological response to it, though such effects could be important. The probable involvement of cutaneous basophil hypersensitivity in the immunity of guinea pigs to ticks has already been discussed. Leonard *et al.* (1979) injected guinea pigs intradermally with the Phipps strain of bacille Calmette-Guerin, a tumour cell line and keyhold limpet haemocyanin. On

subsequent skin testing with cells, haemocyanin and the purified protein derivative of tuberculin, all three produced delayed cutaneous hypersensitivity reactions. However, histological examination showed that the first of these antigens gave a reaction containing predominantly mononuclear cells and with few basophils or neutrophils, the second a reaction containing mostly basophils and with few neutrophils, that is, cutaneous basophil hypersensitivity, and the third a reaction containing mostly neutrophils. Thus presumably the nature of the cellular response is at least partly determined by the antigen.

Finally, in a quite different approach, Allen *et al.* (1979) have shown that Langerhans cells trap salivary gland antigens from *Dermacentor andersoni* on tick resistant guinea pigs. This is the first indication of the form of antigen processing by a host.

V. Conclusions

Despite the relatively scanty evidence available so far, a variety of immunological responses have been implicated in immunity to ticks—antibody and complement, lymphocyte and serum-dependent cutaneous basophil hypersensitivity, and immediate hypersensitivity. So far there is no evidence for the involvement of a classic T-cell-mediated, cell-mediated immunity but one suspects this is solely due to the small number of systems investigated, rather than to a characteristic of tick immunity. In those ticks studied most intensively, there is either definite evidence, or at least an indication, that more than one type of response is involved. This is not surprising since most of the work done on helminth parasites has led to a similar conclusion. At the beginning of this chapter, it was said that the object of the review was to see what generalizations could be made about the nature of immunity to ticks. If the only conclusion is that all types of immunological responses are likely. this is hardly helpful.

All ticks, and probably all ectoparasites with extended feeding periods, are likely to have similar biochemical requirements for successful feeding. That is, they are likely to secrete a similar collection of feeding enzymes, coagulation inhibitors, complement activators and inhibitors and pharmacologically active compounds and inhibitors of such compounds. Further, it is reasonable to assume that many of these will be important antigens. Although there will naturally be interspecies variation, recognition that a particular type of activity is likely to be present in a tick feeding lesion would be a great aid in the investigation of immunity to a tick species. The presence or absence of a molecule with a particular biochemical function can usually be determined with much less effort than is involved in the study of an immunological reaction. The purification of, for example, a protein on the basis of its enzymic activity is much easier than purification on the basis of its antigenic activity, and the availability of characterized antigens could only be a stimulus to further understanding of the immunological responses induced by the tick. Such characterization of antigens has hardly been started.

This review has been concerned with immunity to ticks and has ignored the problem of non-immunity. Why do some hosts remain susceptible to a

particular tick species? One can imagine it would be difficult to express efficient immunity to rapidly feeding soft ticks but other cases are more puzzling. Dogs do not become immune to *Rhipicephalus sanguineus* (Theis and Budwiser, 1974), neither do rabbits nor sheep become immune to *Amblyomma hebraeum* (Norval, 1978). In the latter case at least, the lack of response cannot be due to an evolutionary adaption of parasite to the host, since the host range of this parasite is wide. Is an ineffective immunological response mounted or simply no response at all? An answer to such questions might help us to understand the factors limiting the expression of immunity in all tick–host systems.

Finally it must be stressed that most of the information discussed here has come from *Boophilus microplus*, *Dermacentor andersoni*, *Ixodes holocyclus* and *Ixodes ricinus*. The first of these is a one-host tick with a very restricted host range whereas the other three, being quite catholic in their tastes, have been studied on laboratory animals. Although there are experimental advantages in using such laboratory animals, there are potential dangers as well. It is a commonplace statement in parasitology that parasites adapt to their hosts and that, in so doing, they manage to evade or to minimize the normal immunological reactions that might otherwise lead to their rejection. Of the four ticks listed, *Boophilus microplus* shows the most subtle immunological adaptation to the host which might be expected as it is the most host-specific parasite. Certainly the other three seem to provoke, in general, greater immunity in the hosts studied and more pronounced immunological reactions. It may be unwise to generalize too much from such studies.

References

Allen, J. R. (1973). Tick resistance: basophils in skin reactions of resistant guinea pigs. *International Journal for Parasitology*, **3**, 195–200.

Allen, J. R. and Humphreys, S. J. (1979). Immunisation of guinea pigs and cattle against ticks. *Nature* (*London*), **280**, 491–493.

Allen, J. R., Doube, B. M. and Kemp, D. H. (1977). Histology of bovine skin reactions to *Ixodes holocyclus* Neumann. *Canadian Journal of Comparative Medicine*, **41**, 26–35.

Allen, J. R., Khalil, H. M. and Wikel, S. K. (1979). Langerhans cells trap tick salivary gland antigens in tick-resistant guinea pigs. *Journal of Immunology*, **122**, 563–565.

Askenase, P. W. and Worms, M. J. (1979). Immune cutaneous basophil resistance to parasite ticks. *Federation Proceedings*, **38**, 1220.

Askenase, P. W., Haynes, J. D., Tauben, D. and de Bernardo, R. (1975). Specific basophil hypersensitivity induced by skin testing and transferred using immune serum. *Nature* (*London*), **256**, 52–54.

Bagnall, B. G. (1975a). The Australian paralysis tick *Ixodes holocyclus*. *Australian Veterinary Journal*, **51**, 159–160.

Bagnall, B. G. (1975b). Cutaneous immunity to the tick *Ixodes holocyclus*. Ph.D. Thesis, University of Sydney.

Bagnall, B. G. (1978). Cutaneous immunity to the tick *Ixodes holocyclus*. *In* "Tick-Borne Diseases and their Vectors" (J. K. H. Wilde, ed.), pp. 79–81. Centre for Tropical Veterinary Medicine, University of Edinburgh.

Bagnall, B. G. and Rothwell, T. L. W. (1974). Responses in guinea pigs to larvae of the tick *Ixodes holocyclus*. *Proceedings of the 3rd International Congress of Parasitology*, **2**, 1082–1083.

Balashov, Yu. S. (1971). Bloodsucking ticks (Ixodoidea)—vectors of diseases of men and animals. *Miscellaneous Publications of the Entomological Society of America*, **8**, 159–376.

Bennett, G. F. (1969). *Boophilus microplus* (Acarina : Ixodidae): experimental infestations on cattle restrained from grooming. *Experimental Parasitology*, **26**, 323–328.

Berdyev, A. and Khudainazarova, S. N. (1976). A study of the acquired resistance to mature ticks of *Hyalomma asiaticum asiaticum* (Experiments on lambs). *Parasitologiya*, **10**, 519–535.

Berenberg, J. L., Ward, P. A. and Sonenshine, D. E. (1972). Tick-bite injury: mediation by a complement-derived chemotictic chemotactic factor. *Journal of Immunology*, **109**, 451–456.

Boese, J. L. (1974). Rabbit immunity to the rabbit tick, *Haemaphysalis leporispalustris* (Acari : Ixodidae). I. The development of resistance. *Journal of Medical Entomology*, **11**, 503–512.

Bowessidjaou, J., Brossard, M. and Aeschlimann, A. (1977). Effects and duration of resistance acquired by rabbits on feeding and egg laying in *Ixodes ricinus* L. *Experientia*, **33**, 528–530.

Branagan, D. (1974). The feeding performance of the Ixodid *Rhipicephalus appendiculatus* Neum. on rabbits, cattle and other hosts. *Bulletin of Entomological Research*, **64**, 387–400.

Brossard, M. (1976). Relations immunologiques entre bovins et tiques, plus particulièrement entre bovins et *Boophilus microplus*. *Acta Tropica*, **33**, 15–36.

Brossard, M. (1977). Rabbits infested with the adults of *Ixodes ricinus* L.: passive transfer of resistence with immune serum. *Bulletin de la Société de Pathologie Exotique*, **70**, 289–294.

Brumpt, E. and Chabaud, A. G. (1947). L'infestation par des ixodines, provoque-t-ellè une immunité chez l'hote? I. Note préliminaire. *Annales de Parasitologie Humaine et Comparée*, **22**, 348–356.

Callow, L. L. and Stewart, N. P. (1978). Immunosuppression by *Babesia bovis* against its tick vector, *Boophilus microplus*. *Nature, London*, **272**, 818–819.

Chabaud, A. G. (1950). L'infestation par des ixodines, provoque-t-elle une immunité chez l'hote? (2me note). *Annales de Parasitologie Humaine et Comparée*, **25**, 474–479.

Chinery, W. A. and Ayitey-Smith, E. (1977). Histamine blocking agent in the salivary gland homogenate of the tick *Rhipicephalus sanguineus sanguineus*. *Nature* (*London*), **265**, 366–367.

Dickinson, R. G., O'Hagan, J. E., Schotz, M., Binnington, K. C. and Hegarty, M. P. (1976). Prostaglandin in the saliva of the cattle tick *Boophilus microplus*. *Australian Journal of Experimental Biology and Medical Science*, **54**, 475–486.

Doube, B. M. and Kemp, D. H. (1975). Paralysis of cattle by *Ixodes holocyclus* Neumann. *Australian Journal of Agricultural Research*, **26**, 635–640.

Dvorak, H. F., Dvorak, A. M., Simpson, B. A., Richerson, H. B., Leskowitz, S. and Karnovsky, M. J. (1970). Cutaneous basophil hypersensitivity. II. A light and electron microscopic description. *Journal of Experimental Medicine*, **132**, 558–582.

Feldman-Muhsam, B. (1964). Laboratory colonies of *Rhipicephalus*. *Bulletin of the World Health Organization*, **31**, 587–589.

Fujisaki, R. (1978). Development of acquired resistance and precipitating antibody in rabbits experimentally infested with females of *Haemaphysalis longicornis* (Ixodoidea: Ixodidae). *National Institute of Animal Health Quarterly*, **18**, 27–38.

Garin, N. S. and Grabarev, P. A. (1972). Protective reactions in rabbits and guinea pigs when repeatedly exposed to *Rhipicephalus sanguineus* (Latr. 1806) ticks. *Meditsinskaya Parazitologiya i Parazitarnye Bolezni*, **41**, 274–279.

Gregson, J. D. (1941). Host immunity to ticks (Acarina). *Proceedings of the Entomological Society of British Columbia*, **38**, 12–13.

Gregson, J. D. (1970). Antigenic properties of tick secretions. *Journal of Parasitology* **56**, 1038–1039.

Hewetson, R. W. (1971). Resistance by cattle to the cattle tick, *Boophilus microplus*. III. The development of resistance to experimental infestations by purebred Sahiwal and Australian Illawarra Shorthorn Cattle. *Australian Journal of Agricultural Research*, **22**, 331–342.

Hewetson, R. W. and Nolan, J. (1968). Resistance of cattle to cattle tick, *Boophilus microplus*. I. The development of resistance to experimental infestation. *Australian Journal of Agricultural Research*, **19**, 323–333.

Higgs, G. A., Vane, J. R., Hart, R. J., Potter, C. and Wilson, R. G. (1976). Prostaglandins in the saliva of the cattle tick, *Boophilus microplus* (Canestrini) (Acarina, Ixodidae). *Bulletin of Entomological Research*, **66**, 665–670.

Kemp, D. H. (1978). *In vitro* culture of *Boophilus microplus* in relation to host resistance and tick feeding. *In* "Tick-Borne Diseases and their Vectors" (J. H. K. Wilde, ed.), pp. 95–99. Centre for Tropical Veterinary Medicine, University of Edinburgh.

Kemp, D. H., Koudstaal, D., Roberts, J. A. and Kerr, J. D. (1976). *Boophilus microplus:* the effect of host resistance on larval attachments and growth. *Parasitology*, **73**, 123–136.

Köhler, G., Hoffmann, G., Hörchner, F. and Weiland, G. (1967). Immunbiologische Untersuchungen an Kaninchen mit Ixodiden-Infestationen. *Berliner und Münchener Tierärztliche Wochenschrift*, **80**, 396–400.

Koudstaal, D., Kemp, D. H. and Kerr, J. D. (1978). *Boophilus microplus:* rejection of larvae from British breed cattle. *Parasitology*, **76**, 379–386.

Leonard, E. J., Lett-Brown, M. A. and Askenase, P. W. (1979). Simultaneous generation of tuberculin-type and cutaneous basophilic hypersensitivity at separate sites in the guinea pig. *International Archives of Allergy and Applied Immunology*, **58**, 460–469.

Loomis, E. C. (1971). Rearing of *Boophilus microplus* (Acarina: Ixodidae) on the laboratory rabbit. *Annals of the Entomological Society of America*, **64**, 598–603.

Musatov, V. A. (1967). Physiological and size changes of ixodid ticks in their feeding on repeatedly used animals. *Parazitologiya*, **1**, 288–292.

Musatov, V. A. (1970). The reaction of animal skin to the repeated attachment and feeding of ixodid ticks (Ixodoidea, ixodidae). *Parazitologiya*, **4**, 66–70.

Nelson, W. A., Bell, J. F., Clifford, C. M. and Keirans, J. E. (1977). Interaction of ectoparasites and their hosts. *Journal of Medical Entomology*, **13**, 389–428.

Nikitina, N. A. and Aristova, V. A. (1964). Protective reaction to ticks in rodents. *Meditsinskaya Parazitologiya i Parazitarnye Bolezni*, **33**, 141–144.

Norval, R. A. I. (1978). Repeated feeding of *Amblyomma hebraeum* (Acarina: Ixodidae) immatures on laboratory hosts. Host effects on tick yield, engorged weight and engorgement period. *Journal of Parasitology*, **64**, 910–917.

O'Kelly, J. C. and Spiers, W. G. (1976). Resistance to *Boophilus microplus* (Canestrini) in genetically different types of calves in early life. *Journal of Parasitology*, **62**, 312–317.

Pogorelyi, A. I. (1966). Studies on the immunity of animals to ectoparasitical disease. *Veterinariya (Kiev)*, **6**, 68–75.

Riek, R. F. (1956). Factors influencing the susceptibility of cattle to tick infestation. *Australian Veterinary Journal*, **32**, 204–208.

Riek, R. F. (1962). Studies on the reactions of animals to infestation with ticks. VI. Resistance of cattle to infestation with the tick *Boophilus microplus* (Canestrini). *Australian Journal of Agricultural Research*, **13**, 532–550.

Roberts, J. A. (1968a). Resistance of cattle to the tick *Boophilus microplus* (Canestrini). II. Stages of the life cycle of the parasite against which resistance is manifest. *Journal of Parasitology*, **54**, 667–673.

Roberts, J. A. (1968b). Acquisition by the host of resistance to the cattle tick, *Boophilus microplus* (Canestrini). *Journal of Parasitology*, **54**, 657–662.

Roberts, J. A. (1971). Behaviour of larvae of the cattle tick, *Boophilus microplus* (Canestrini), on cattle of differing degrees of resistance. *Journal of Parasitology*, **57**, 651–656.

Roberts, J. A. and Kerr, J. D. (1976). *Boophilus microplus:* passive transfer of resistance in cattle. *Journal of Parasitology*, **62**, 485–488.

Santoro, F., Bernal, J., Capron, A. (1979). Complement activation by parasites. A review. *Acta Tropica*, **36**, 5–14.

Schleger, A. V., Lincoln, D. T., McKenna, R. V., Kemp, D. H. and Roberts, J. A. (1976). *Boophilus microplus:* cellular responses to larval attachment and their relationship to host resistance. *Australian Journal of Biological Sciences*, **29**, 499–512.

Schneider, C. C., Roth, B. and Lehmann, H. D. (1971). Untersuchungen zum Parasit-Wirt-Verhältnis der Zecke *Amblyomma testudinis* (Conil, 1877). *Zeitschrift für Tropenmedizin und Parasitologie*, **22**, 1–17.

Smith, R. D. (1979). Current world research on ticks and tickborne diseases of food-producing animals. *Review of Applied Entomology Series B*, **67**, 118.

Stadecker, M. J. and Leskowitz, S. (1975). The inhibition of basophil-rich delayed skin reactions by a heterologous anti-guinea pig T cell serum. *Federation Proceedings*, **34**, 1040.

Stampa, S. (1959). Tick paralysis in the Karoo areas of South Africa. *Onderstepoort Journal of Veterinary Research*, **28**, 169–227.

Strother, G. R., Burns, E. C. and Smart, L. I. (1974). Resistance of purebred Brahman, Hereford and Brahman × Hereford crossbred cattle to the lone star tick, *Amblyomma americanum* (Acarina: Ixodidae). *Journal of Medical Entomology*, **11**, 559–563.

Sutherst, R. W., Roberts, J. A. and Wagland, B. M. (1979). Resistance in cattle to *Haemaphysalis* (Kaiseriana) *longicornis*. *International Journal for Parasitology*, **9**, 183–188.

Tatchell, R. J. (1969). The significance of host parasite relationships in the feeding of the cattle tick *Boophilus microplus* (Canestrini). *Proceedings of the 2nd International Congress of Acarology, 1967*, 341–345.

Tatchell, R. J. and Binnington, K. C. (1973). An active constituent of the saliva of the cattle tick, *Boophilus microplus*. *Proceedings of the 3rd International Congress of Acarology, Prague, 1971*, 745–748.

Tatchell, R. J. and Moorhouse, D. E. (1968). The feeding processes of the cattle tick *Boophilus microplus* (Canestrini). Part II. The sequence of host-tissue changes. *Parasitology*, **58**, 441–459.

Tatchell, R. J. and Moorhouse, D. E. (1970). Neutrophils: their role in the formation of a tick feeding lesion. *Science*, **167**, 1002–1003.

Theis, J. H. and Budwiser, P. D. (1974). *Rhipicephalus sanguineus:* sequential histopathology at the host-arthropod interface. *Experimental Parasitology*, **36**, 77–105.

Tracey-Patte, P. D. (1979). Effect of the bovine immune system on esterase deposited by *Boophilus microplus* larvae. *Australian Advances in Veterinary Science*, 1979, p. 78.

Trager, W. (1939a). Acquired immunity to ticks. *Journal of Parasitology*, **25**, 57–81.

Trager, W. (1939b). Further observations on acquired immunity to the tick *Dermacentor variabilis* Say. *Journal of Parasitology*, **25**, 137–139.

Tritschler, L. G. (1965). Allergy in a horse due to *Amblyomma americanum*. *Veterinary Medicine and Small Animal Clinician* **60**, 219–220.

Wagland, B. M. (1975). Host resistance to cattle tick (*Boophilus microplus*) in Brahman (*Bos indicus*) cattle. I. Responses of previously unexposed cattle to four infestations with 20 000 larvae. *Australian Journal of Agricultural Research*, **26**, 1073–1080.

Wagland, B. M. (1978). Host resistance to cattle tick (*Boophilus microplus*) in Brahman (*Bos indicus*) cattle. II. The dynamics of resistance in previously unexposed and exposed cattle. *Australian Journal of Agricultural Research*, **29**, 395–400.

Wagland, B. M. (1979). Host resistance to cattle tick (*Boophilus microplus*) in Brahman (*Bos indicus*) cattle. IV. Ages of ticks rejected. *Australian Journal of Agricultural Research*, **30**, 211–218.

Weiland, G. and Emokpare, C. I. (1968). Zur Brauchbarkeit der Komplementbindungsreaktion zum Antikörpernachweis bei Zeckeninfestationen. *Berliner und Münchener Tierärzltiche Wochenschrift*, **81**, 15–16.

Wikel, S. K. and Allen, J. R. (1976a). Acquired resistance to ticks. I. Passive transfer of resistance. *Immunology*, **30**, 311–316.

Wikel, S. K. and Allen, J. R. (1976b). Acquired resistance to ticks. II. Effects of cyclophosphamide on resistance. *Immunology*, **30**, 479–484.

Wikel, S. K. and Allen, J. R. (1977). Acquired resistance to ticks. III. Cobra venom factor and the resistance response. *Immunology*, **32**, 457–465.

Wikel, S. K., Graham, J. E. and Allen, J. R. (1978). Acquired resistance to ticks. IV. Skin reactivity and *in vitro* lymphocyte responsiveness to salivary gland antigen. *Immunology*, **34**, 257–263.

Willadsen, P. (1976). Allergenic activity of an esterase from *Boophilus microplus*. *FEBS Letters*, **72**, 346–349.

Willadsen, P. (1979). The relationship between immediate hypersensitivity reactions and the resistance of cattle to the tick *Boophilus microplus*. *Australian Advances in Veterinary Science*, 1979, p. 77.

Willadsen, P. and Riding, G. A. (1979). Characterization of a proteolytic enzyme inhibitor with allergenic activity. Multiple functions of a parasite-derived protein. *Biochemical Journal*, **177**, 41–47.

Willadsen, P. and Riding, G. A. (1980). On the biological role of a proteolytic enzyme inhibitor from the ectoparasite, *Boophilus microplus*. *Biochemical Journal* (in press).

Willadsen, P. and Williams, P. G. (1976). Isolation and partial characterization of an antigen from the cattle tick, *Boophilus microplus*. *Immunochemistry*, **13**, 591–597.

Willadsen, P., Williams, P. G., Roberts, J. A. and Kerr, J. D. (1978). Responses of cattle to allergens from *Boophilus microplus*. *International Journal for Parasitology*, **8**, 89–95.

Willadsen, P., Wood, G. M. and Riding, G. A. (1979). The relation between histamine concentration, histamine sensitivity and the resistance of cattle to the tick, *Boophilus microplus*. *Zeitschrift für Parasitenkunde*, **59**, 87–94.

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Role of Tick Salivary Glands in Feeding and Disease Transmission

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I. Introduction

Argasid ticks feed rapidly and pass through numerous moults to reach the engorged female stage. On the other hand, the feeding of ixodid ticks is characterized by the capacity of each instar to ingest large volumes of host blood and so avoid the frequent host-finding associated with argasids. Growth of the cuticle during slow feeding of ixodids and subsequent stretching of cuticle during a final rapid ingestion period (Lees, 1952) enables the intake of sufficient blood to give an increase in weight of 200 times. An adaptation to feeding on blood is the concentration of the meal by removal of excess water. Argasid ticks concentrate their meal with the coxal organ but ixodid ticks eliminate most excess water via the salivary glands, which have the capacity to secrete large volumes of water, particularly during the later stages of feeding (Gregson, 1967; Tatchell, 1967). During the earlier stages of feeding, transpiration through the cuticle (Lees, 1952) may also be a significant source of water loss.

In fact the salivary glands of ixodid ticks are remarkable for their diversity of function. In many species the salivary glands secrete a cement that attaches the tick to the host for several days (Moorhouse and Tatchell, 1966; Chinery, 1965, 1973; Binnington, 1978). They synthesize and release a variety of enzymes and other chemicals which may assist in the development of a feeding lesion and provide an ample flow of blood for engorgement. In concentrating the

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blood meal, the salivary glands not only secrete excess water but also maintain the ionic balance of the haemolymph by selective secretion and retention of ions (Tatchell, 1967, 1969a; Gregson, 1967; Kaufman and Phillips, 1973). The presence of an additional type of salivary cell in males and the fact that males salivate during copulation suggest a role for salivary secretions in reproductive physiology (Feldman-Musham *et al.*, 1970).

Any discussion on the role of ixodid salivary glands in feeding must also take into account the host response to the salivary secretions. The immunological response to salivary antigens may be detrimental to the ticks, particularly the slow feeding stages (Roberts, 1968; Willadsen *et al.*, 1978); on the other hand, it has been suggested that ticks have become adapted to take advantage of these host reactions to obtain a blood meal (Tatchell, 1969b). While aspects of the host response which are likely to affect tick feeding directly are discussed in this review, the immune response is dealt with in a companion review (Willadsen, 1980). Also reviewed here will be studies on the dependence on tick salivary glands of the disease-causing organisms *Theileria* and *Babesia* for their development and transmission. The physiology of ixodid salivary glands in relation to excretion of excess water has been reviewed (Sauer, 1977; Kaufman and Sauer, 1980) and will not be covered here.

II. Salivary Gland Functions During Attachment and Feeding

A. Secretion of Attachment Cement

The short feeding period of argasid ticks requires rapid and deep penetration of the host skin to release sufficient blood for engorgement. To accomplish this the chelicerae and cheliceral digits of argasids are well developed compared with those of ixodids. In contrast, the hold-fast organ (hypostome) is relatively delicate (Balashov, 1965). The slow-feeding ixodid ticks are considered to be more specialized (Balashov, 1972), with less need for well-developed cutting organs but greater need for a stout hypostome and more secure attachment for a prolonged sojourn on the host. However, there is considerable variation among the Ixodidae, in the length of the hypostome, depth of penetration and presence of an attachment cement (Moorhouse, 1969). Generally, ixodids with relatively long mouthparts penetrate the skin deeply and have a sheath-like tube of cement around the mouthparts like the feeding tube of mites (Jones, 1950) and it has been suggested that this sheath prevents the adjacent dermal tissues being damaged and loosening the attachment of the tick (Moorhouse, 1969). Ixodids with short mouthparts rely on the more copious secretion of a cement cone which initially spreads over the skin and between the keratinized layers, and later extends into the lesion to secure the shallow attachment (Moorhouse and Tatchell, 1966).

Cement production and hardening has been observed in *Dermacentor andersoni* (Gregson, 1960), *Hyalomma asiaticum* (Balashov, 1972) and *Boophilus microplus* (B. F. Stone, K. C. Binnington and R. Court, unpublished work). In *D. andersoni* the cement precursor material is secreted as a milky white liquid which hardens rapidly (Gregson, 1960). In *B. microplus* cement

secretion may be very rapid, some larvae having secreted a substantial cone within 5 minutes of being placed on the host (Tracey-Patte, 1979). Moorhouse and Tatchell (1966) introduced the term "internum" for the cone of cement laid down by *B. microplus* from 5 minutes to 24 h after attachment. Adult females secrete this cement in small drops which immediately harden into rings that are first formed around the distal part of the hypostome and then, with further secretion, coalesce to form a sheath that completely encloses the hypostome (B. F. Stone, K. C. Binnington and R. Court, unpublished work). "Cortical" cement is then deposited around the internum and between the layers of the stratum corneum; its deposition may continue until about 96 h after attachment. During this time cement also flows into the lesion to form an internal flange of "secondary cement" (Moorhouse and Tatchell, 1966; B. F. Stone, K. C. Binnington and R. Court, unpublished work). During the deposition of cement there is a loss of cytoplasmic granules and pycnosis of nuclei within certain cell types of the salivary gland of *B. microplus* (cell *a* of acinus II; cells *d*, *e* of acinus III; see Fig. 1). The suggestion that these are cement precursor cells is supported by comparative histochemical data on salivary glands and cement of *Haemaphysalis spinigera* (Chinery, 1965, 1973) and *B. microplus* (Moorhouse and Tatchell, 1966; Binnington, 1978; B. F. Stone, K. C. Binnington and R. Court, unpublished work). In *Amblyomma hebraeum* electron micrographs have revealed the presence of spiral structures in both the cement and in one particular cell type, which is believed therefore to be involved in cement secretion (P. Diehl, unpublished work).

The internum of the cement cone of *B. microplus* has been shown histochemically to contain lipoprotein, and the cortex protein, which contains carbohydrate (Moorhouse and Tatchell, 1966; B. F. Stone, K. C. Binnington and R. Court, unpublished work). The internal and cortical zones are more clearly different in cement deposited around the mouthparts (primary cement) than in that secreted into the feeding lesion (secondary cement). The terminology applied by Chinery (1973) to the cement of *H. spinigera* differs from that used by Moorhouse and Tatchell (1966) in that cement deposited on the outside of the skin is called external cement and that within the lesion, internal cement; these two zones are similar histochemically in *H. spinigera*, except for a negative response of the internal cement to reactions for disulphide groups. Histochemical evidence suggests that external cement may be secreted by cell *a* of acinus II and cell *d* of acinus III (using the nomenclature of Coons and Roshdy, 1973; see Binnington, 1978). However, in *B. microplus* it was suggested on the basis of coincidence of lipoprotein staining, that cell type *a* is the precursor cell of the internal cement and that cells *d* and *e* are the precursor cells of cortical cement (Binnington, 1978; B. F. Stone, K. C. Binnington and R. Court, unpublished work).

Biochemical analysis of the cement cones of *B. microplus* shows that some lipid and carbohydrate is present in the form of lipoprotein and glycoprotein respectively (B. F. Stone, K. C. Binnington and R. Court, unpublished work). Moorhouse and Tatchell (1966) suggested that the cement of *B. microplus* was hardened by quinone tanning of disulphide bonds. Although this possibility is supported by the presence of phenol oxidase activity (which

could have a role in the conversion of phenols into orthoquinones) in the salivary glands (Binnington, 1978) and cement cone of *B. microplus*, the presence of only a low percentage of sulphur-containing proteins in the cement makes it unlikely that quinone tanning of disulphide bonds is the mechanism for hardening (B. F. Stone, K. C. Binnington and R. Court, unpublished work). Other enzymes have been demonstrated histochemically in cement of *B. microplus;* these include an esterase and an aminopeptidase (Schleger and Lincoln, 1976; D. T. Lincoln, unpublished work). The purpose of these enzymes is unknown; it is possible that the cement cone becomes contaminated with enzymes secreted in non-cement-producing saliva. The composition and hardening of cement may be governed to some extent by the need for the tick to isolate its mouthparts from direct contact with the host. Tatchell and Moorhouse (1968) found that cement of *B. microplus* did not provoke a reaction from the host (see Willadsen, 1980).

Further understanding of the relationships between the structure of tick salivary glands and cement secretion can be obtained by comparing the structure of the salivary glands of ticks that secrete cement with those that do not. With the possible exception of *Argas pusillus* larvae (Stiller and Ramchitham, 1975), argasids are not known to secrete cement and their salivary glands are much less complex than those of cement-secreting ixodids and lack cells with properties characteristic of cement-secreting cells. All ixodids studied have two types of granular acini in females and two or three in males in addition to the non-granular acinus I (Till, 1961; Chinery, 1965; Kirkland, 1971; Coons and Roshdy, 1973; Meredith and Kaufman, 1973; Megaw, 1976; Binnington, 1978), whereas argasids have only one granular acinus type (Sonenshine and Gregson, 1970; Balashov, 1972; Guirgis, 1971; Roshdy, 1972; Chinery, 1974). The salivary gland of *Ixodes holocyclus* which does not secrete cement, contains only four granule-secreting cell types in the female and five in the male (K. C. Binnington and B. F. Stone, unpublished work), whereas those of the cement-secreting ixodid, *B. microplus* (Binnington, 1978), contains nine granule secreting cell types in the female and ten in the male. It is noteworthy that the granular cells (*a* and *b* of acinus II; *d* and *e* of acinus III) of *I. holocyclus* stain for glycoproteins and therefore differ from the putative cement precursor cells of *B. microplus* (Binnington, 1978), which stain for protein and lipoprotein but not for glycoproteins.

B. SALIVARY SECRETIONS AND TICK FEEDING

Histological studies of the tick feeding lesion prior to the work of Tatchell and Moorhouse (1968, 1970) led to the assertion that tick saliva contained a variety of lytic agents, anticoagulants and a fibrinolysin to aid tick feeding. This evidence has been reviewed by Arthur (1965, 1970), Balashov (1972) and Nelson *et al.* (1977). Although it is a reasonable assumption that ticks need to secrete such chemicals, there are a number of reasons why these claims must be questioned. Firstly, as reported by Tatchell and Moorhouse (1968, 1970) the immunological response of cattle to the attachment of *B. microplus*

causes much more dramatic histolytic and pharmacological effects than those caused by tick saliva. For example, the visible oedematous reaction in sensitized hosts to the injection of less than 1 ng of antigen (Willadsen, 1980) emphasizes the need for unsensitized hosts as controls, which were lacking in many earlier experiments. The validity of relying on histological evidence is further called into question by the presence of a factor in salivary gland extracts of *Dermacentor variabilis* which acts on complement to produce a chemotactic substance and leads to infiltration and degranulation of host neutrophils; thus a lesion is formed by host autolysis and not by a tissue-destructive enzyme in saliva (Berenberg *et al.*, 1972).

There are other examples where tick attachment triggers host-mediated reactions that mimic postulated salivary functions or where histological information may have been misinterpreted. For instance, haemorrhage in the lesion may not result from a salivary cytolytic agent, but rather from mechanical damage by cheliceral teeth, followed by secretion of prostaglandin, a mediator identified in saliva and salivary glands of *B. microplus* (Dickinson *et al.*, 1976; Higgs *et al.*, 1976) and which is known to stimulate an increase in cutaneous blood flow (Vane, 1976). A more productive approach has been the demonstration in whole tick extracts, salivary glands or saliva, of biochemical or pharmacological activity which might help tick feeding, but in some of these experiments there are still difficulties in drawing valid conclusions. As mentioned, prostaglandin occurs in the saliva and salivary glands of *B. microplus* and is known to stimulate cutaneous blood flow as does the feeding of tick larvae (R. Hales, A. V. Schleger, D. H. Kemp and A. A. Fawcett, unpublished work). However, Dickinson *et al.* (1976) have pointed out that prostaglandin may have a physiological role within the tick. It is, for example, involved with reproduction in some insects (Destephano and Brady, 1977). It may also be suggested, on the basis of the information on salivary gland function in the previous sections, that prostaglandin is simply being excreted by the gland or leaking through it. Further evidence for a positive role in tick feeding is needed; for example, it may be possible to deplete prostaglandin in the tick or block the tick-induced elevation in blood flow, and observe effects on tick feeding.

One other example can be given to illustrate the care needed for the correct interpretation of such experiments. Willadsen and Riding (1979) have purified from larvae of *B. microplus* a proteolytic-enzyme inhibitor which can prevent blood coagulation but which also blocks the action of complement (Willadsen, 1980); since complement participates in most antigen–antibody reactions, the blocking of its activity could be a means of circumventing the adverse effects of host immunity (see Wikel and Allen, 1977). Several questions arise; is the inhibitor secreted into the host; is it introduced at a sufficient concentration and at an appropriate time to perform one or both of its known activities, or is it rapidly ingested to prevent blood clotting in the gut? The finding that the inhibitor is antigenic (Willadsen and Riding, 1979) is evidence that the inhibitor is secreted into the host.

From these examples it is clear that reactions which could assist tick feeding can be observed in the lesion and can be inferred from reactions

Ixodidae (esp. B. microplus)

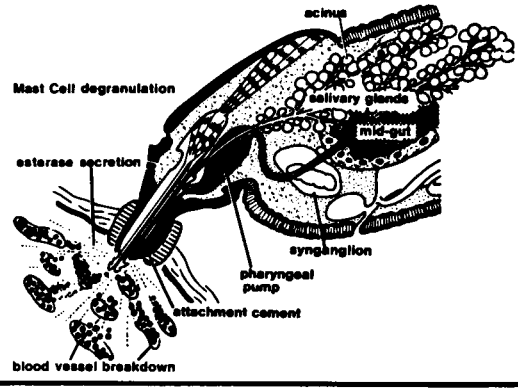
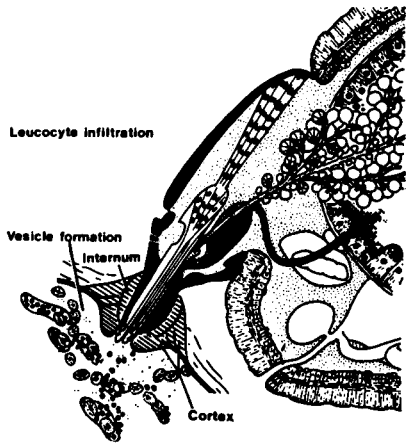
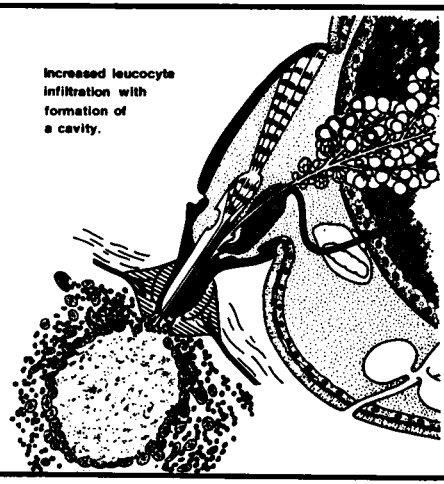
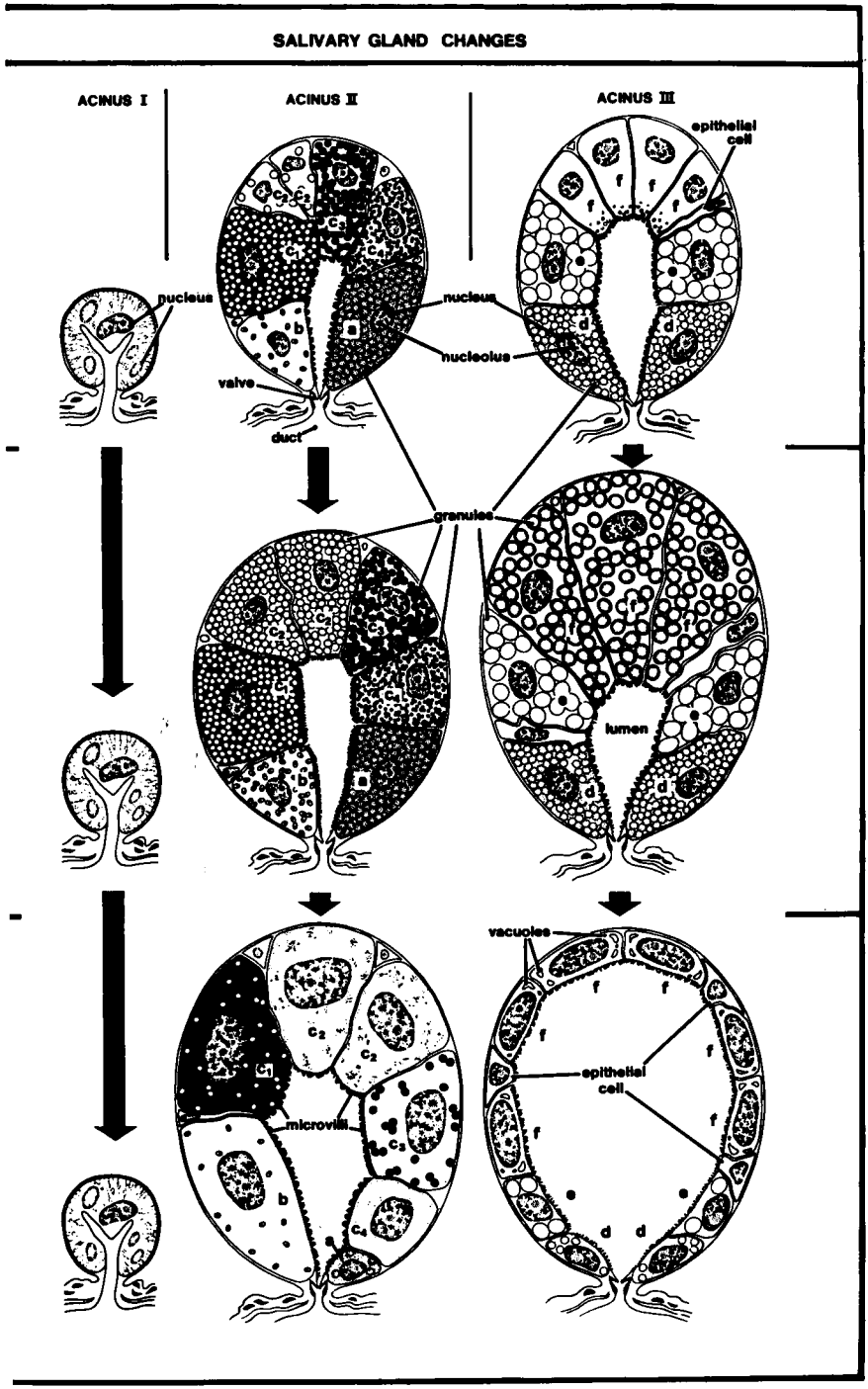
DEVELOPMENTAL STAGE OF FEMALE	FEEDING LESION CHANGES	TICK CHANGES
Newly-Attached Ca 2.4 mm		
1–3 Days <3 mm Slow feeding		
4 Days to engorgement about day 7 ⩾4.5 mm Rapid feeding in final 24 hours and concentration of blood meal		

FIG. 1. Simplified diagrammatic representation of changes in salivary glands and in the Binnington, 1978 and Kemp *et al.*, 1980).



host lesion during feeding of *B. microplus* on previously exposed cattle. (Adapted from

demonstrated *in vitro*, but it is difficult in many cases to prove that the appropriate chemical is secreted at the appropriate time.

Finally, the majority of ticks must feed from a previously exposed host and are likely to have adapted in some way to host immunity (Tatchell, 1969b). This may be more important for ixodid ticks which have a long period of attachment than for argasids (see end of Section IIb).

Tick feeding, salivation and host immunity are closely interrelated, and a simplified diagram (Fig. 1) shows the rate of tick growth, changes in salivary glands during feeding and formation of the feeding lesion, as epitomized by *B. microplus* feeding on cattle previously exposed to tick attack.

1. *Anticoagulant secretion*

Older references mention the continued outpouring of blood from the host lesion following detachment of ticks, particularly *Ornithodoros* species, and there are numerous demonstrations of anticoagulant activity in extracts of whole ticks, tick gut or tick salivary glands. Activity in whole tick, or gut extracts (Markwardt and Landmann, 1958, 1961) is not proof of anticoagulant secretion or of an anticoagulant role of saliva in feeding. Thus, Tatchell (1969c) found an anticoagulant in gut but not in salivary glands of *B. microplus* and Balashov (1972) noted that blood remains unclotted in the ixodid tick gut. However, salivary gland extracts from the following ticks have anticoagulant properties; *Argas persicus* (Nuttall and Strickland, 1908), *I. holocyclus* (Ross, 1926; Kaire, 1967), *Dermacentor sinicus* (Hoeppli and Feng, 1931), *O. moubata* (Hawkins and Hellmann, 1966; Hellman and Hawkins, 1967) and *I. ricinus* (Pavalovsky and Shtein, 1927). For example, in this last tick, extracts of 2·5 salivary glands from partially engorged females delayed clotting in 2 ml of sheep blood for at least 12 h (Foggie, 1959). Better evidence was obtained by Hellmann and Hawkins (1967), who identified two types of anticoagulant activity in extracts of *O. moubata* salivary glands, and noted that activity was weak after feeding but increased with starvation; they suggested that feeding is a stimulus for secretion of anticoagulant.

The evidence for an anticoagulant in saliva of ixodid ticks remains somewhat equivocal. The saliva and salivary glands of *B. microplus* have been reported to be without anticoagulant activity (Tatchell, 1969b). As mentioned previously, the protein extracted from *B. microplus* larvae, which has anticoagulant activity, is also antigenic and is therefore a likely component of the tick's saliva. However, even if a substance with anticoagulant activity is found in salivary glands and saliva, it may have some other role such as complement inhibition or proteolytic enzyme inhibition (Willadsen, 1980). In an addendum to their paper, Nuttall and Strickland (1908) noted that the coxal fluid of *Ornithodoros* species which is excreted after feeding, is able to prevent clotting, and this was found also by Hellmann and Hawkins (1967) and others. As Tatchell (1969b) has pointed out, some other blood-sucking arthropods apparently do not require anticoagulant for successful feeding.

However, although tsetse flies feed successfully when the salivary anticoagulant is withheld from the lesion by cutting the salivary duct their mouthparts are eventually blocked with clotted blood (Lester and Lloyd, 1928). It must also be considered that the introduction to the host of the anticoagulant protein of *B. microplus* may have been by regurgitation from the gut (Gregson, 1960) or with the faeces. Allergens of a house dust mite can be demonstrated in the intestine of the mite and are excreted as a strong antigen in the faeces (Mumcuoglu and Rufli, 1979).

In addition to that found in salivary glands, anticoagulant activity is found also in the saliva of some ticks. Y. S. Balashov, in a personal communication to Tatchell (1969b), stated that 10–50 μl of *H. asiaticum* saliva prevented clotting in 3–5 ml of blood, and C. J. Howell (again to Tatchell, 1969b), that 5 μl of saliva from *Ornithodoros savignyi* inhibited clotting of 10 ml of sheep blood. Chinery (1965, 1971) and Balashov (1972) believe that tick anticoagulants may be the mucoprotein or glycoprotein in granular salivary cells. More information is needed on tick anticoagulants and further investigation is required of their role in feeding.

2. *Enzymes*

Histological study of the tick feeding lesion has shown destruction of host tissue, which ranges in severity from pycnosis of adjacent cell nuclei and slight haemorrhage, to the production, by digestion, of a large cavity in the dermis (1·75 mm × 1·4 mm for *B. microplus*; Tatchell and Moorhouse, 1968) or development of a haematoma (Fig. 1). The existence of such tissue damage has been used as evidence for the presence of cytolytic enzymes in saliva, but as we mentioned earlier much of the destruction can be attributed to autolysis by infiltrating and degranulating host neutrophils (Tatchell and Moorhouse, 1970; Berenberg *et al.*, 1972; Theis and Budweiser, 1974). Even some of the less dramatic effects such as pycnosis of nuclei and capillary dilation, may result not from salivary enzymes, but from pharmacological agents, or other chemicals in oral secretions, which cause mast cell degranulation (Schleger *et al.*, 1976; Allen *et al.*, 1977). In fact, Tatchell (1969c) and J. D. Gregson (personal communication to Tatchell, 1969a) state that saliva and salivary glands of *B. microplus* and *D. andersoni* are without tissue destroying enzymes, and extracts of salivary glands from *I. ricinus*, though causing oedema when injected intradermally, did not contain hyaluronidase (Foggie, 1959). However, the saliva of ixodid ticks is not completely devoid of enzymes that could digest tissues. Although hydrolytic enzymes that rapidly break down tissues, such as hyaluronidase, phospholipase and proteinase, are absent from *B. microplus* saliva; there are 'weak' hydrolytic enzymes such as esterase and aminopeptidase (Tatchell, 1969c, 1971) which may help to create, over a period of hours or days, a small lesion with sufficient vascular damage to provide the ticks' bloodmeal. This is in keeping with Tatchells' argument (1969b) that too destructive an enzyme would endanger the tick attachment, and his evidence that a small lesion is adequate for feeding. Thus depletion of leucocytes in dogs, although preventing tissue destruction,

did not prevent female *Rhipicephalus sanguineus* from feeding normally on a negligible lesion (Tatchell and Moorhouse, 1970).

'Weak' hydrolytic enzymes have been identified histochemically in the granular salivary cells of *B. microplus* (Binnington, 1978) and *Rhipicephalus appendiculatus* (Martins, 1978) and some of these may be secreted into the host. Moderate to strong histochemical reactions for esterase, lipase and aminopeptidase were located in the dermis immediately adjacent to the mouthparts of *B. microplus* larvae that had been attached to the host for about 4 h (Schleger and Lincoln, 1976). The amount of esterase secreted by each larva is very variable but a substantial deposit has been made by 1 h and this changes little until some later more diffuse deposits are added (Tracey-Patte, 1979). There is no proof, as yet, that the enzyme, or enzymes, are digesting tissues or damaging blood vessels to aid feeding. Geczy *et al.* (1971) suggested that a salivary esterase might increase vascular permeability by promoting mast cell degranulation, but an esterase from *B. microplus* purified by Willadsen *et al.* (1978) did not cause a visible oedema when injected into non-immune hosts. However, the location of the esterase at the tick attachment site, the timing of its deposition, and its more rapid removal from hosts that are partially resistant to ticks (Tracey-Patte, 1979), are all supporting evidence for a role in feeding.

Argasid ticks may have more destructive salivary enzymes (Tatchell, 1969b), but the only information comes from a preliminary study (Howell *et al.*, 1975) on proteolytic enzyme activity in the saliva of *O. savignyi* and a similar identification by Tatchell (1971) of such an enzyme in the saliva of *A. persicus*.

3. *Pharmacological agents*

Even when care has been taken to ensure that a host animal has had no previous experience of ticks, an oedematous reaction, or dilation of capillary blood vessels at the tick attachment site may still occur (Tatchell and Moorhouse, 1968; Allen, 1973; Allen *et al.*, 1977). We have already suggested that a salivary chemical may cause mast cell degranulation, leading to vascular changes. An oedematous reaction on injection of salivary gland extract or saliva is not therefore sufficient proof of a pharmacologically active agent in tick saliva. However, in pursuit of such an agent, Tatchell and Binnington (1973), separated on sephadex a fraction from *B. microplus* saliva and salivary glands, which caused contraction of a rat fundus preparation and oedema in cattle skin. They suggested that the latter response would aid in tick feeding. The vasoactive agent was subsequently identified as prostaglandin E_2 (PGE_2) (Dickinson *et al.*, 1976; Higgs *et al.*, 1976) and the greatest activity was found in salivary glands of females just before final rapid engorgement (Dickinson *et al.*, 1980). We know that in cattle PGE_2 is more effective in promoting an increase in cutaneous blood flow than in promoting cutaneous oedema (Kemp *et al.*, 1980), and that the rapid feeding stage of the larvae stimulates a local increase in bovine capillary blood flow (R. Hales, A. V. Schleger, D. H. Kemp and A. A. Fawcett, unpublished work). The inference from these results is that *B. microplus* secretes PGE_2 to increase blood flow during periods of rapid engorgement (see also Binnington, 1978). Flaws in

this argument were pointed out in the introduction, and they stem from lack of information on when PGE_2 is secreted and on the necessity of elevated blood flow for tick feeding. Chinery and Aitey-Smith (1977) demonstrated histamine like activity and a histamine-blocking agent in extracts of *R. sanguineus* salivary glands. Another active agent, as yet unidentified, was isolated from *B. microplus* glands (Dickinson *et al.*, 1980). These agents appear to provide, in the slower feeding ixodid ticks, a less destructive mechanism of obtaining blood than that which results from the action of the salivary enzymes in argasid species.

In conclusion, there are obviously interesting and complex interactions between the host and the tick salivary anticoagulins, enzymes and pharmacological agents, but there is still the question, are they necessary for tick feeding?

4. *Salivary secretions, feeding and host immunity*

The comments that follow apply only to ixodid ticks. The slow progression of tick feeding over several days, followed by a short period of rapid engorgement (Fig. 1) is typical at least for adult females. The amounts ingested by larvae and by the nymphs and females during their slow phase, are so small relative to the total amount taken by that stage, that it is not surprising that only a very small amount of tissue disruption occurs during this phase of feeding. This was shown for the feeding lesion of larvae on previously unexposed hosts (Tatchell and Moorhouse, 1968; Allen, 1973; Bagnall, 1975; Schleger *et al.*, 1976). However, for nymphs and females, tissue destruction resulting from the host reaction, particularly that seen in the final stage of feeding, obscures the relatively minor tissue damage required for feeding. In *B. microplus*, this masking of minimal damage can occur even during primary infestation since the immunity of Brahman cattle (*Bos indicus*) to *B. microplus* can be expressed as early as 3 days after primary infestation (Wagland, 1978). The accumulated evidence outlined above suggests that secretion of anticoagulants, weak hydrolytic enzymes, and pharmacological agents, coupled with capillary blood pressure and some tissue damage by cheliceral teeth, can liberate enough host blood for tick engorgement.

It would be expected that the proposed salivary functions would be reflected in a diversity of granular cell types in the glands, as well as in depletion of granules, enzymes or other material at particular stages of feeding. The diversity of granular cell types, and the relationship between cell types and the ability or otherwise of a particular species to produce cement, have been discussed in Section II A. In ticks such as *B. microplus*, only cells not involved in cement production are likely to secrete the enzymes and pharmacological agents involved in the creation of the feeding lesion, whereas in argasids and *I. holocyclus* all cells present could secrete such agents. The smallest number of cell types occurs in argasids and the most in cement-secreting ixodids; *I. holocyclus*, an ixodid that does not secrete cement, has a gland with an intermediate complexity of structure. Thus it appears that for successful feeding the ixodid ticks may need to secrete a wider range of substances than the rapidly feeding argasids. Further, it seems that ixodids such as *B. microplus*

(Binnington, 1978) and *R. appendiculatus* (K. C. Binnington, A. S. Young and F. D. Obenchain, unpublished work) which form a shallow attachment and secrete copious cement (Moorhouse, 1969), secrete a more complex saliva than ticks that penetrate deeply and secrete either no cement or a relatively small volume of cement. A feature of the tick salivary cells not involved in cement secretion is that they react to the periodic acid–Schiff reaction for glycoproteins (Chinery, 1965; Balashov, 1972; Binnington, 1978). In *I. ricinus* and *H. asiaticum* (Balashov, 1972), metachromasia seen following Toluidine Blue staining and Alcian Blue staining at low pH have indicated that acid mucopolysaccharides may also be present. Chinery (1965) has suggested that sulphated mucopolysaccharides in the salivary gland of *H. spinigera* may act as anticoagulants.

Various authors have noted changes in salivary gland morphology during feeding; for example, although *I. holocyclus* does not produce cement, two of the four cell types lose their granules within 24 h of attachment (K. C. Binnington and B. Stone, unpublished work). Esterase depletion from cell type C_1 of *B. microplus* occurs during early feeding and there is also evidence of early deposition and accumulation of esterase within the lesion (Tracey-Patte, 1979). However, in no other study has the secreted material been identified; instead the timing of secretion of salivary gland material has been inferred from the depletion of granules from their cell of origin.

There seems little need to invoke tissue lysis by destructive enzymes or host neutrophils as a prerequisite for feeding since, at least in one case, ticks fed normally without it (Tatchell and Moorhouse, 1970). The suggestion by Tatchell and Moorhouse (1968) that *B. microplus* can take advantage of the increased availability of tissue fluids associated with a hypersensitivity reaction to tick antigens has yet to be supported by experimental evidence. It has been suggested that in another tick–host association (field mouse, *Apodemus sylvaticus–Ixodes trianguliceps*) the tick may take advantage of the host immune response (Randolph, 1979).

Most ticks in the natural situation must feed on animals that have already been exposed to ticks (Tatchell, 1969b), and the host reactions they provoke range from severe to mild (Willadsen, 1980). In the long association between *B. microplus* and Brahman cattle, the host has evolved an immunological response which is detrimental to the ticks. On average 99% are rejected, compared with 85% on a European breed (Utech *et al.*, 1978), and the weight of surviving ticks is much reduced (Wagland, 1978). In the accompanying review (Willadsen, 1980) it has been suggested that certain salivary secretions of ticks can block the expression of immunity. It is also apparent that we must take into account the type of lesion the tick encounters on the exposed host when considering the function and importance of the salivary agents described in this section. The topic is virtually unexplored.

C. PASSAGE OF MATERIAL THROUGH SALIVARY GLANDS DURING FEEDING

The role of tick salivary glands in the excretion of excess water has been reviewed (Sauer, 1977; Kaufman and Sauer, 1980), but the phvsiology of the

tick, rather than the host–parasite relationship has received most attention. In addition to material from granular cells, substances that may be 'excreted' by the glands along with excess water, could either assist the tick in obtaining a blood meal or, in addition, act as antigens. Saliva produced during final engorgement may in fact originate from the haemolymph and contain little material synthesized within the salivary gland cells, since many of these cells are relatively poor in cytoplasmic granules during this phase of feeding (Binnington, 1978). However, it must be emphasized that histological studies provide only a static picture and give therefore a poor indication of synthesis and secretion of material. Saliva obtained by pilocarpine stimulation of engorged *B. microplus* females was found by Tatchell (1971) to be extremely dilute; however, this is not necessarily the equivalent of naturally secreted saliva. By comparing electrophoretic patterns of pilocarpine-stimulated saliva and haemolymph, Tatchell (1971) concluded that proteins, including glycoproteins and haemoproteins present in the saliva were probably of haemolymph origin and that only the enzymes present originated within the salivary glands. There is also immunological evidence for transport of haemolymph proteins through the salivary glands to the host. Antibodies produced by guinea pigs exposed to *B. microplus*, reacted with antigens present in haemolymph (and also extracts of whole ticks), but not with homogenates of tick salivary gland or saliva (B. M. Wagland, personal communication). However, this may simply mean that immunologically identical antigens which are secreted in saliva and are present in haemolymph are more easily detected in haemolymph. Studies on other arthropods demonstrate that proteins can pass through the salivary glands; Miles (1967) and Miles and Sloviak (1970) showed that labelled glucose, glycerol and amino acids rapidly appear in the watery saliva of phytophagous Heteroptera and that peroxidase, with a molecular weight of about 40 000, was also transferred from the haemolymph to saliva. In the fly *Chironomus thummi*, labelled fly haemoglobin was selectively taken up by the salivary gland, most radioactivity being associated with the lumen (Schin and Laufer, 1974). It may also be relevant that in *Argas arboreus*, the fluid excreted through its coxal glands which, in respect to water excretion, are the argasid 'equivalent' of ixodid salivary glands, contains most of the proteins detectable by polyacrylamide-gel electrophoresis in the haemolymph (Dolp and Hamdy, 1971).

III. Toxicosis

The most important form of tick toxicosis is undoubtedly paralysis of the host and in a recent review (Murnaghan and O'Rourke, 1978) 31 species from seven genera of ixodid ticks and six species of argasids from three genera were listed as being implicated in tick paralysis; of these species, *I. holocyclus* and *D. andersoni* were considered to be the most important. Most studies on the mode of action of tick paralysis toxins have been done with material from these two ticks and two soft ticks, *A.* (*P.*) *persicus* and *Argas* (*P.*) *walkerae*. A detailed investigation into the biochemical nature of salivary toxin(s) has been made only on *I. holocyclus* (Stone *et al.*, 1979). For a

comprehensive coverage of present knowledge of tick paralysis, reviews by Gregson (1973), Gothe and Kunze (1974), Nelson *et al.* (1975), Murnaghan and O'Rourke (1978) and Stone *et al.* (1979) should be consulted.

Forms of tick toxicosis other than paralysis also occur (Neitz, 1962). Riek (1957) reported that cattle showed inappetence during the time of final rapid engorgement of female *B. microplus*, and a slow weight gain for some time after tick infestation had ended, and concluded that a toxic component may be present in the tick's saliva. It has also been shown that heavy infestations of *B. microplus* can interfere with some of the host's metabolic processes (O'Kelly *et al.*, 1971). These authors suggested that the metabolic changes were specific toxic effects of the tick rather than a consequence of blood loss or anorexia and that liver dysfunction caused by tick toxins could explain their results.

It is important to know whether tick salivary glands synthesize or merely transport toxin and for what purpose the toxin is produced. This can be approached in several ways, but only the changes in morphology of the gland cells and the development of toxicity in gland extracts have so far been studied in relation to these questions. In the case of *D. andersoni*, an ultrastructural study of salivary glands provided valuable information on the cells responsible for water and ion transport (Meredith and Kaufman, 1973). For this purpose, female ticks close to engorgement were used, and evidence from observations on other ixodid ticks (see Table 1 in Binnington, 1978) indicate that the variety of granular cell types detectable in the salivary gland of such female *D. andersoni* is probably limited. In the male of another species of *Dermacentor*, *D. variabilis*, eight granular cell types have been shown by electron microscopy to be present (Coons and Roshdy, 1973) and females of two other cement-producing ixodids, *B. microplus* (Binnington, 1978) and *R. appendiculatus* (K. C. Binnington, A. S. Young and F. D. Obenchain, unpublished work) are known to contain nine granular cell types. If as expected, the salivary gland of *D. andersoni* is similarly complex, it will be a challenging task to show which cell type is responsible for toxin production, especially since it is not yet known whether there is only one toxin or whether the toxin(s) is produced within the salivary gland, or merely transported through the glands by water-secreting cells (see Meredith and Kaufman, 1973; Kaufman and Sauer, 1980).

I. holocyclus does not secrete attachment cement and has a less complex gland than most other ixodid ticks. An attempt has been made to relate changes in salivary gland morphology in this tick to changes in the toxicity of homogenized glands and to the time after attachment at which host paralysis occurs (K. C. Binnington and B. F. Stone, unpublished work). Host paralysis does not occur until *I. holocyclus* females have been attached and feeding on mice for 4–5 days (Murray and Koch, 1969; Stone *et al.*, 1979) and greatest toxicity is seen in extracts of homogenized salivary glands taken from ticks removed from the host 4–5 days after attachment (Stone *et al.*, 1979). Paralysis was also produced in mice by the injection of extracts of salivary glands from unfed females, but this may be irrelevant to the normal paralysis syndrome since newly attached ticks do not cause paralysis, and

clinical and biochemical properties of the partially purified toxin from glands of unfed females differed in some respects from the toxin of females fed for 4–5 days (Stone *et al.*, 1979). Histological observations show that one salivary cell type is virtually devoid of granules until about the third day of feeding when granule numbers increase and reach a peak at 4–5 days, before declining again in fully engorged ticks. Within the limits of the techniques used, the results suggest that this cell is the most likely source of toxin (K. C. Binnington and B. F. Stone, unpublished work). Of the other three granular cell types present, two appear to be no longer synthetically active by the time of maximum toxin production and another is active in granule production throughout the feeding period and rich in granules both before and after the peak in toxicity in the gland homogenates. The data could be greatly improved by using electron microscopy, which would show more accurately the state of synthesizing activity of a cell at a particular time and might also show when granules were being released into the lumen of salivary acini. A further improvement would be the tagging of toxin with specific immunoglobulins labelled with fluorescein, but the application of this technique must await purification and characterization of the toxin. Dermal gland secretion, collected by cuticular washing of engorged nymphs and females of *I. holocyclus*, also causes paralysis when injected into mice (Kaire, 1967; B. Doub, B. V. Goodger and J. Nolan, unpublished work). These authors have suggested that the toxin may be a metabolic by-product which is excreted by the salivary and dermal glands.

The biological significance of tick toxicosis is enigmatic, since there is no apparent advantage to the tick in causing paralysis of the host. It has been shown that the bandicoot, which is the natural host of *I. holocyclus* (Doube, 1975), is susceptible to paralysis only when reared tick-free (Koch, 1967), and throughout much of the year exposure to larval and nymphal instars of *I. holocyclus* could stimulate the bandicoot's immunity to the toxin (Stone *et al.*, 1979). A better understanding of the nature of the toxic substances may show that they have some important function in tick feeding. The increase in toxicity, and presumably toxin secretion, just before rapid feeding supports this view. Such a function might be anticoagulation, fibrinolysis or increased blood flow.

IV. Disease Transmission

The probable role played by tick saliva in the transmission of viruses, rickettsiae (including *Anaplasma*) and bacteria (including spirochaetes) has been covered in reviews by Arthur (1962), Balashov (1972) and Hoogstraal (1966, 1967, 1973). Little appears to be known of the location and development of these organisms in the tick salivary gland which, in many cases, merely acts as organs of transmission rather than being involved in a cycle of development of the microorganism. A more complex relationship is known to exist between ticks and the piroplasms, *Babesia* (reviewed by Riek, 1968 and by Mahoney, 1977) and *Theileria* (Martin *et al.*, 1964; reviewed by Barnett, 1968; Purnell and Joyner, 1968; Schein and Friedhoff, 1978). Following ingestion of the piroplasm in host erythrocytes, development proceeds in the

tick gut followed by passage into the haemolymph and the salivary glands either by direct transfer or by transovarial transmission; further development then occurs in the salivary gland before the piroplasm is transmitted to the vertebrate host.

A. *THEILERIA*

Cowdry and Ham (1932) reported that *Theileria parva* entered the salivary glands of pharate adults and adult *R. appendiculatus* which has engorged as nymphs on infested cattle and that development of the parasite occurred within the adult glands before reinfestation and attachment of the adult. The first stage observed in the gland was a club-shaped ookinette which transformed into a larger and rounder body (sporont) that contained smaller masses (sporoblasts) and was surrounded by a halo of clear cytoplasm. After attachment and feeding by the adult for 3 days, the sporoblasts gave rise to sporozoites, considered to be the final infective forms. Martin *et al.* (1964) were unable to confirm that *T. parva* was present within the salivary glands before adult feeding. A form of the parasite found 4–5 days after the commencement of adult feeding and which contained varying numbers of chromatin fragments was described as a cytomere and may be analogous to the sporont described by Cowdry and Ham (1932). Martin *et al.* (1964) found that there was no invasion of acinus I, but that both acini II and III of adult salivary glands contained piroplasms. Nymphal glands which had been infected following engorgement, detachment and moulting of larvae contained parasites only within acinus type III. Type IV acinus of males also contained no parasites. Purnell and Joyner (1968) studied the timing and development of various stages of *T. parva* in the salivary gland of adult *R. appendiculatus*. They divided the types of organisms into young forms, intermediate forms (sporonts; cytomeres) and mature forms (infective forms; sporozoites) and found that the number of ticks containing mature forms reached a maximum 4–5 days after attachment, although infective forms could develop at an earlier stage of feeding. They also confirmed the findings of Cowdry and Ham (1932) that *T. parva* is present in the salivary glands before adult feeding commences. Both Martin *et al.* (1964) and Purnell and Joyner (1968) observed that nuclei in parasitized salivary cells become hypertrophied. The occurrence of an intermediate maturation stage or cytomere, suggested that multiplication of *T. parva* was not by simple binary fission (Cowdry and Ham, 1932; Martin *et al.*, 1964; Purnell and Joyner, 1968).

Studies on the life cycle of *T. parva* in *R. appendiculatus* have been hampered by relatively low infection rates in ticks and the limitations imposed by the use of paraffin embedding and light microscopy techniques (Cowdry and Ham, 1932; Martin *et al.*, 1964; Purnell and Joyner, 1968). In more recent studies on the life cycle of *Theileria* species in ixodid ticks, higher infection rates have been obtained using *Theileria annulata* in *Hyalomma anatolicum excavatum*. Methacrylate embedding followed by semi-thin sectioning (Weber, 1972) and electron microscopy has greatly assisted interpretation of sections through tick tissues and through the different forms of *Theileria* (Schein, 1975, 1976; Schein *et al.*, 1975; Schein and Friedhoff, 1978). The

life cycle of *T. annulata* in *H. a. excavatum* was described and shown diagramatically by Schein and Friedhoff (1978). Following ingestion of infective forms by nymphs, the organisms were released by lysis from the host erythrocytes and gametogony was thought to take place within the gut lumen. A developmental phase within the gut epithelium produced club-shaped kinetes (vermicules) which migrated to the nymphal salivary glands. The kinetes changed within the nymphal gland to form fission bodies of about 10 μm diameter which occurred mainly within cells of acinus III but also within acinus II. Nuclear division occurred within the fission body which reached a diameter of about 20 μm. There was then no further change until after tick attachment, when the salivary glands were seen to 'proliferate' and transformation of *T. annulata* from primary fission body (sporont) to secondary fission body (primary sporoblast) to tertiary fission body (secondary sporoblast) and finally to the infective particle stage (sporozoite) occurred. The speed of transformation depended on the time spent as a free-living, unfed adult, sporozoites being produced in young ticks within 2 days of attachment. Findings for *T. annulata* in *H. a. excavatum* (Schein and Friedhoff 1978) provide further evidence that *Theileria* is present in ixodid salivary glands before adult feeding begins (Cowdry and Ham, 1932; Purnell and Joyner, 1968).

A specific stimulus for parasite development during feeding has not so far been identified but it seems possible that salivary gland development may be stimulated by attachment of the adult and the parasite in turn may detect this change. Further, it is possible that the parasite relies on a 'switching on' of protein synthesis within the gland for the provision of nutrients. Hypertrophy of the salivary cell nucleus (Martin *et al.*, 1964; Purnell and Joyner, 1968; Schein and Friedhoff, 1978) indicated that the parasite may even stimulate the cell to increase its synthetic activity. A further indication that the parasite used salivary cell secretion for its own development was the marked depletion of granules in infected cells (Martin *et al.*, 1964; Schein and Friedhoff, 1978). However, ultrastructural studies are needed to eliminate the possibility that infection of a salivary cell by the parasite inactivates the synthesizing processes of the cell. In heavily infected ticks, *Theileria* can be widely distributed throughout the salivary gland but cell type *e* (nomenclature of Coons and Roshdy, 1973) appears to be preferentially selected. A similar cell type has been shown histochemically to contain protein in *H. spinigera* and lipoprotein in *B. microplus* and is thought to secrete precursor material for attachment cement (Chinery, 1965; Binnington, 1978).

Laboratory propagation of infective *Theileria* and *Babesia* particles would be valuable for harvesting antigenic material for immunological studies. One approach has been to maintain the infected salivary glands of *R. appendiculatus in vitro* in order to determine some of the pharmacological and biochemical relationships between *T. parva* and the glands. Salivary glands from unfed adults responded to treatment with 5-hydroxytryptamine (5-HT) by increasing acid phosphatase activity and to 5-HT and theophylline by swelling of the types II and III acini. Parasitized acini were particularly sensitive to treatment with these drugs, but over a period of days, it was not

certain that drug treatment had stimulated development of the parasite to maturation (McCall, 1978). Studies *in vivo* on the enzyme histochemistry of infected and non-infected acini also showed that the parasites caused an increase in acid phosphatase activity in glands from unfed females. From this, and other changes in enzyme activity acsociated with parasite development, Martins (1978) concluded that *T. parva* utilized host cell enzymes for synthesis of its nucleic acids. In further studies of this type it would seem desirable to test the effects of chemicals such as dopamine and noradrenaline since the salivary glands of *B. microplus* are known to have an adrenergic nerve supply (Binnington and Stone, 1977). Further, Kaufman and Phillips (1973) have shown that *D. andersoni* glands *in vitro* are stimulated most effectively by adrenergic drugs; 5-HT was a relatively weak stimulant. However, it should be noted that the concentrations of 5-HT used by McCall (1978) were relatively high (1 mM) and, as she has noted, cell types involved with parasite development were not primarily concerned with water secretion and may therefore require a different chemical stimulus. So far there is no information on the stimuli for granular cell development or secretion which is more relevant to parasite development.

B. *BABESIA*

Riek's (1964) study of *Babesia bigemina* in *B. microplus* showed that, following ingestion of erythrocytic forms by the female and transovarial transmission, *Babesia* were first seen in the nymphal salivary glands. From a more recent study using both light and electron microscopy, it is known that *B. bigemina* infects both granular and non-granular acini of the salivary glands of *Boophilus decoloratus* (Potgeiter and Els, 1977); their figures show a vermicule lying both in close apposition to, and within, cells that contain distinct electron-dense sub-units. Cells showing this feature have been identified as cells *a* of acinus II and *d* of acinus III in *B. microplus* (Binnington, 1978). Weber and Friedhoff (1979) also used electron microscopy to demonstrate *B. bigemina* in cytoplasmic infoldings of salivary gland cells of *B. microplus* which were free of secretory granules. However, it is not clear from the published photomicrographs whether the sections are through the non-granular acinus I or a part of either acinus II or III which, in a nymph attached for about 6 days after the larval moult, may have secreted most of their granules (Binnington, 1978). Riek (1964), and Potgeiter and Els (1977), differ in their accounts of transformation of the vermicules into immature schizonts in the salivary glands; Riek (1964) found that as the vermicules become large and more rounded the chromatin did not move to the periphery, as occurs in primary schizogony, but remained in a central position before breaking up into a large number of chromatin bodies with a thin cytoplasmic envelope. Potgeiter and Els (1977) found that central and peripheral chromatin existed at this stage and hence they could not distinguish between primary and secondary schizogony on this basis. The ultrastructural changes occurring during primary and secondary schizogony of *B. bigemina* were found to be similar (Potgeiter and Els, 1977); as the vermicule became rounded, the thick

inner membrane of the pellicle disappeared as did the microtubules thought to be involved in the formation of the anterior polar ring. The nucleus then began to divide and micronemes became scattered in the cytoplasm. Organelles, presumed to be mitochondria, increased in number. At completion of mitosis, daughter nuclei together with some cytoplasm, became enclosed within membranes, thus forming immature small spherical merozoites. The only organelles seen within the cytoplasm of this stage were thought to be mitochondria. The thick inner membrane of a pellicle then developed and rhoptries and micronemes were observed as the merozoite developed to the pear-shaped mature infective form of *B. bigemina*.

Babesia argentina is also transmitted transovarially in species of *Boophilus* but multiplies in larval rather than nymphal glands (Riek, 1966; Potgeiter and Els, 1976). Potgeiter and Els (1976) found one schizont of *Babesia bovis* (= *B. argentina*) by electron microscopy in an unidentified salivary gland cell of larval *B. microplus* and remarked on the difficulty of finding infective forms of this species of *Babesia* in *B. microplus*. K. C. Binnington and D. F. Mahoney (unpublished work) found only a small percentage of positive infected salivary acini in sections of *B. microplus* larvae infected with *B. argentina* but were able to show that the only cell type infected appeared to be cell *e* of acinus III and that acini I and II did not contain *Babesia*.

Development of *B. argentina* from vermicule to infective particle has been shown to take place in the salivary glands of unfed *B. microplus* larvae in response to increased temperature (Dalgliesh and Stewart, 1979). It would be interesting to know if the effect on the *Babesia* is a direct one or is mediated indirectly through an effect on the salivary glands, or even more indirectly through the release of tick neurohormones. Prolonged heat stimulation depleted the number of parasites in the salivary glands, but it was not known whether this was due to an effect on the *Babesia* or a consequence of ageing of the larvae (Dalgliesh and Stewart, 1979). The effect of temperature on development is not confined to *Babesia*, a recent study by Young *et al.* (1980) having shown a similar effect on *T. parva* in the salivary glands of unfed adult *R. appendiculatus*.

References

Allen, J. R. (1973). Tick resistance: basophils in skin reactions of resistant guinea pigs. *International Journal for Parasitology*, **3**, 195–200.

Allen, J. R., Doube, B. M. and Kemp, D. H. (1977). Histology of bovine skin reactions to *Ixodes holocyclus* Nuemann. *Canadian Journal of Comparative Medicine*, **41**, 26–35.

Arthur, D. R. (1962). "Ticks and Disease". Pergamon Press, Oxford.

Arthur, D. R. (1965). Feeding in ectoparasitic acari with special reference to ticks. *In* "Advances in Parasitology" (B. Dawes ed.), Vol. 3, pp. 249–298. Academic Press, New York and London.

Arthur, D. R. (1970). Tick feeding and its implications. *In* "Advances in Parasitology", (B. Dawes, ed.), Vol. 8, pp. 275–292. Academic Press, New York and London.

Bagnall, B. G. (1975). Cutaneous immunity to the tick *Ixodes holocyclus*. Ph.D. Thesis, University of Sydney.

Balashov, Yu S. (1965). Mechanism of salivation and morphologic-histochemical peculiarities of salivary glands in ixodid ticks (Acarina, Ixodoidea). *Entomologicheskoe Obozrenie*, **44**, 785–802.

Balashov, Y. S. (1972). 'Bloodsucking ticks (Ixodoidea)—vectors of disease of man and animals' (Translation from Russian.) *Miscellaneous Publications of the Entomological Society of America*, **8**, 161–376.

Barnett, S. F. (1968). *In* "Infectious Blood Diseases of Man and Animals" (D. Weinman and M. Ristec, eds.), Vol. 2. Academic Press, New York and London.

Berenberg, J. L., Ward, P. A. and Sonenshine, D. E. (1972). Tick-bite injury: mediation by a complement-derived chemotactic factor. *Journal of Immunology*, **109**, 451–456.

Binnington, K. C. (1978). Sequential changes in salivary gland structure during attachment and feeding of the cattle tick, *Boophilus microplus*. *International Journal for Parasitology*, **8**, 97–115.

Binnington, K. C. and Stone, B. F. (1977). Distribution of catecholamines in the cattle tick *Boophilus microplus*. *Comparative Biochemistry and Physiology*, **58c**, 21–28.

Chinery, W. A. (1965). Studies on the various glands of the tick *Haemaphysalis spinigera* Neumann 1897. Part III. The salivary glands. *Acta Tropica*, **22**, 321–349.

Chinery, W. A. (1971). The salivary glands of *Argas persicus* (Oken, 1818). *Proceedings of the 3rd International Congress of Acarology* 1971, pp. 711–715.

Chinery, W. A. (1973). The nature and origin of the 'cement' substance at the site of attachment and feeding of adult *Haemaphysalis spinigera* (Ixodidae). *Journal of Medical Entomology*, **10**, 355–362.

Chinery, W. A. (1974). Studies on the salivary glands of *Argas persicus* (Oken, 1818). *Journal of Medical Entomology*, **11**, 480–487.

Chinery, W. A. and Ayitey-Smith, E. (1977). Histamine blocking agent in the salivary gland homogenate of the tick *Rhipicephalus sanguineus sanguineus*. *Nature* (*London*), **265**, 366–367.

Coons, L. B. and Roshdy, M. A. (1973). Fine structure of the salivary glands of unfed male *Dermacentor variabilis* (Say) (Ixodoidea: Ixodidae). *Journal of Parasitology*, **59**, 900–912.

Cowdry, E. V. and Ham, A. W. (1932). Studies on East Coast fever. 1. The life-cycle of the parasite in ticks. *Parasitology*, **24**, 1–49.

Dalgliesh, R. J. and Stewart, N. P. (1979). Observations on the morphology and infectivity for cattle of *Babesia ovis* parasites in unfed *Boophilus microplus* larvae after incubation at various temperatures. *International Journal for Parasitology*, **9**, 115–120.

Destephano, D. B. and Brady, V. E. (1977). Prostaglandin and prostaglandin synthetase in the cricket, *Acheta domesticus*. *Journal of Insect Physiology*, **23**, 905–911.

Dickinson, R. G., O'Hagain, J. E., Schotz, M., Binnington, K. C. and Hegarty, M. P. (1976). Prostaglandin in the saliva of the cattle tick *Boophilus microplus*. *Australian Journal of Experimental Biology and Medical Science*, **54**, 475–486.

Dickinson, R. G., Binnington, K. C., Schotz, M. and O'Hagan, J. E. (1980). Studies on the significance of smooth muscle contracting substances in the cattle tick *Boophilus microplus*. *Journal of the Australian Entomological Society*, **18**, 199–210

Dolp, R. M. and Hamdy, B. H. (1971). Biochemical and physiological studies of certain ticks (Ixodoidea) Protein electrophoretic studies of certain biological fluids of *Argas* (Argasidae) and *Hyalomma* (Ixodidae). *Journal of Medical Entomology*, **8**, 636–642.

Doube, B. M. (1975). Cattle and the paralysis tick *Ixodes holocyclus*. *The Australian Veterinary Journal*, **51**, 511–515.

Feldman-Muhsam, B., Borut, S. and Saliternik-Givant, S. (1970). Salivary secretion of the male tick during couplation. *Journal of Insect Physiology*, **16**, 1945–1949.

Foggie, A. (1959). Studies on the relationship of the tick-bite to pyaemia of lambs. *Annals of Tropical Medicine and Parasitology*, **53**, 27–34.

Geczy, A. F., Naughton, M. A., Clegg, J. B. and Hewetson, R. W. (1971). Esterases and a carbohydrate-splitting enzyme in the saliva of the cattle tick, *Boophilus microplus*. *Journal of Parasitology*, **57**, 437–438.

Gothe, R. and Kunze, K. (1974). Neuropharmacological investigations on tick paralysis of chickens induced by larvae of *Argas* (*Persicargas*) *walkerae*. *In* "Parasitic Zoonoses" (E. S. Soulsby, ed.), pp. 369–382. Academic Press, London and New York.

Gregson, J. D. (1960). Morphology and functioning of the mouthparts of *Dermacentor andersoni* Stiles. Part II. The feeding mechanism in relation to the host. *Acta Tropica*, **17**, 72–79.

Gregson, J. D. (1967). Observations on the movement of fluids in the vicinity of the mouthparts of naturally feeding *Dermacentor andersoni* Stiles. *Parasitology*, **57**, 1–8.

Gregson, J. D. (1973). "Tick paralysis: an appraisal of natural and experimental data." Monograph No. 9, Canada Department of Agriculture.

Guirgis, S. S. (1971). The subgenus *Persicargas* (Ixodoides, Argasidae, *Argas*). B. Histological studies on *A.* (*P.*) *arboreus* Kaiser, Hoogstraal and Kohls. *Journal of Medical Entomology*, **8**, 648–667.

Hawkins, R. I. and Hellmann, K. (1966). Factors affecting blood clotting from the tick *Ornithodoros moubata*. *Journal of Physiology*, **185**, 70p.

Hellmann, K. and Hawkins, R. I. (1967). The action of tick extracts on blood coagulation and fibrinolysis. *Thrombosis et Diathesis Haemorrhagica* **18**, 617–625.

Higgs, G. A., Vane, J. R., Hart, R. J., Potter, C. and Wilson, R. G. (1976). Prostaglandins in the saliva of the cattle tick, *Boophilus microplus* (Canestrini) (Acarina, Ixodidae). *Bulletin of Entomological Research*, **66**, 665–607.

Hoeppli, R. J. C. and Feng, L. C. (1931). Histological reactions in the skin due to ecto-parasites, *Dermacentor sinicus* P. Schulze from hedgehog, *Haemaphysalis campanulata hoeppliana* P. Schulze from dog, *Cimex lectularius* and *Pediculus vestimenti* from man. *National Medical Journal of China*, **17**, 541–556.

Hoogstraal, H. (1966). Ticks in relation to human diseases caused by viruses. *Annual Review of Entomology*, **11**, 261–308.

Hoogstraal, H. (1967). Ticks in relation to human diseases caused by *Rickettsea* species. *Annual Review of Entomology*, **12**, 377–420.

Hoogstraal, H. (1973). Viruses and ticks. *In* "Viruses and invertebrates" (A. J. Gibbs, ed.), Chap. 18, pp. 349–390. North Holland Publishing Co., Amsterdam.

Howell, C. J., Neitz, A. W. H. and Potgieter, D. J. J. (1975). Some toxic, physical and chemical properties of the oral secretion of the sand tampan, *Ornithodoros savignyi* Audouin (1827). *Onderstepoort Journal of Veterinary Research* **42**, 99–102.

Jones, B. M. (1950). The penetration of host tissue by the harvest mite, *Trombicula*. *Parasitology*, **40**, 247–260.

Kaire, G. H. (1967). Isolation of tick paralysis toxin from *Ixodes holocyclus*. *Toxicon*, **4**, 91–97.

Kaufman, W. R. and Phillips, J. E. (1973). Ion and water balance in the ixodid tick *Dermacentor andersoni* I. Routes of ion and water excretion. *Journal of Experimental Biology*, **58**, 523–536.

Kaufman, W. R. and Sauer, J. R. (1980). *In* "Physiology of Ticks" (F. D. Obenchain and R. Galun, eds.), Pergamon Press, London (In press).

Kemp, D. H., Stone, B. F. and Binnington, K. C. (1980). Tick attachment and feeding: role of the mouthparts, feeding apparatus, salivary gland secretions and the host response. *In* "Physiology of Ticks" (F. D. Obenchain and R. Galun, eds.). Pergamon Press, London (in press).

Kirkland, W. L. (1971). Ultrastructural changes in the nymphal salivary glands of the rabbit tick, *Haemaphysalis leporispalustris*, during feeding. *Journal of Insect Physiology*, **17**, 1933–1946.

Koch, J. H. (1967). Some aspects of tick paralysis in dogs. *N.S.W. Veterinary Proceedings*, **3**, 34.

Lees, A. D. (1952). The role of cuticle growth in the feeding process of ticks. *Proceedings of the Zoological Society of London*, **121**, 759–772.

Lester, H. M. O. and Lloyd, L. (1928). Notes on the process of digestion in tsetse flies. *Bulletin of Entomological Research*, **19**, 39–60.

Mahoney, D. F. (1977). Babesia of domestic animals. *In* "Parasitic protozoa" (J. P. Kreier, ed.), Vol. IV, pp. 1–52. Academic Press, New York and London.

Markwardt, F. and Landmann, H. (1958). Untersuchungen über-den blutgerinnungshem-menden Wirkstoff der Zecke *Ixodes ricinus*. *Naturwissenschaften*, **45**, 398–399.

Markwardt, F. and Landmann, H. (1961). Über einen Hemmstoff der Thrombokinase aus der Lederzecke *Ornithodoros moubata*. *Naturwissenschaften*, **45**, 433.

Martin, H. M., Barnett, S. F. and Vidler, B. O. (1964). Cyclic development and longevity of *Theileria parva* in the tick *Rhipicephalus appendiculatus*. *Experimental Parasitology*, **15**, 527–555.

Martins, M. I. (1978). Histochemical studies on the salivary glands of unfed and feeding *Rhipicephalus appendiculatus* during the development of *Theileria parva*. *In* "Tick-Bourne Diseases and their Vectors" (J. K. H. Wilde, ed.), pp. 336–347. Centre for Tropical Veterinary Medicine, University of Edinburgh.

McCall, H. (1978). Effect of salivary stimulants on isolated preparations of *Theileria parva*—infected and non-infected salivary glands of *R. appendiculatus*. *In* "Tick-Borne Diseases and their Vectors" (J. K. H. Wilde, ed.), pp. 343–350. Centre for Tropical Veterinary Medicine, University of Edinburgh.

Megaw, M. W. J. (1976). Structure and function of the salivary gland of the tick, *Boophilus microplus* (Canestrini). Ph.D. Thesis, University of Cambridge.

Meredith, J. and Kaufman, W. R. (1973). A proposed site of fluid secretion in the salivary gland of the ixodid tick *Dermacentor andersoni*, *Parasitology*, **67**, 205–217.

Miles, P. W. (1967). Studies on the salivary physiology of plant-bugs: transport from haemolymph to saliva. *Journal of Insect Physiology*, **13**, 1787–1801.

Miles, P. W. and Sloviak, D. (1970). Transport of whole protein molecules from blood to saliva of a plant-bug. *Experientia*, **26**, 611.

Moorhouse, D. E. (1969). The attachment of some ixodid ticks to their natural hosts. *Proceedings of the 2nd International Congress of Acarology*, 1967, pp. 319–327.

Moorhouse, D. E. and Tatchell, R. J. (1966). The feeding processes of the cattle tick *Boophilus microplus* (Canestrini): a study in host–parasite relations. Part I. Attachment to the host. *Parasitology*, **56**, 623–632.

Mumcuoglu, Y. and Rufli, Th. (1979). Localization of the antigen in the body of the House-Dust mite *Dermatophagoides pteronyssinus* by means of the indirect immunofluorescence test. *Proceedings of the Vth International Congress of Acarology*, 1978, Vol. 2, pp. 205–210.

Murnaghan, M. F. and O'Rourke, F. J. (1978). Tick paralysis. *In* "Arthropod venoms" (S. Bettini, ed.). Handbook of Experimental Pharmacology, N. S., Vol. 48, pp. 419–464. Springer-Verlag, Berlin.

Murray, M. D. and Koch, J. H. (1969). The engorgement of *Ixodes holocyclus* Neumann (Acarina: Ixodoidea) in mice. *Journal of the Australian Entomological Society*, **8**, 187–188.

Neitz, W. O. (1962). The different forms of tick toxicosis: a review. *2nd meeting FAO/OIE expert panel of tick-borne diseases of livestock*. Cairo, U.A.R.: FAO/OIE 1962 (Dec. Working paper No. 2).

Nelson, W. A., Keirans, J. E., Bell, J. F. and Clifford, C. M. (1975). Host–ectoparasite relationships. *Journal of Medical Entomology*, **12**, 143–166.

Nelson, W. A., Bell, J. F., Clifford, C. M. and Keirans, J. E. (1977). Interaction of ectoparasites and their hosts. *Journal of Medical Entomology*, **13**, 389–428.

Nuttall, G. H. F. and Strickland, C. (1908). On the presence of an anticoagulin in the salivary glands and intestines of *Argas persicus*. *Parasitology*, **1**, 302–310.

O'Kelly, J. C., Seebeck, R. M. and Springell, P. H. (1971). Alterations in host metabolism by the specific and anorectec effects of the cattle-tick (*Boophilus microplus*). II. Changes in blood composition. *Australian Journal of Biological Sciences*, **24**, 381–389.

Pavlovsky, E. N. and Shtein, A. K. (1927). Experimentelle Untersuchungen über die Wirkung von *Ixodes ricinus* (Ixodidae) auf die Menschenhaut. *Archiv. für Schiffs-und Tropenhygien*, **31**, 574–586.

Potgeiter, F. T. and Els, H. J. (1976). Light and electron microscopic observations on the development of small merozoites of *Babesia bovis* in *Boophilus microplus* larvae. *Onderstepoort Journal of Veterinary Research*, **43**, 123–128.

Potgeiter, F. T. and Els, H. J. (1977). Light and electron microscopic observations on the development of *Babesia begemina* in larvae, nymphae and non-replete females of *Boophilus decoloratus*. *Onderstepoort Journal of Veterinary Research*, **44**, 213–232.

Purnell, R. E. and Joyner, L. P. (1968). The development of *Theileria parva* in the salivary glands of the tick *Rhipicephalus appendiculatus*. *Parasitology*, **58**, 725–732.

Randolph, S. E. (1979). Population regulation in ticks: the role of acquired resistance in natural and unnatural hosts. *Parasitology*, **79**, 141–156.

Riek, R. F. (1957). Studies on the reactions of animals to infestation with ticks. I. Tick anaemia. *Australian Journal of Agricultural Research*, **8**, 209–214.

Riek, R. F. (1964). The life cycle of *Babesia bigemina* (Smith and Kilborne, 1893) in the tick vector *Boophilus microplus* (Canestrini). *Australian Journal of Agricultural Research*, **15**, 802–821.

Riek, R. F. (1966). Life cycle of *Babesia argentina* (Ligniéres, 1903) (Sporozoa: Piroplasmidea) in the tick vector *Boophilus microplus* (Canestrin:) *Australian Journal of Agricultural Research*, **17**, 247–254.

Riek, R. F. (1968). Babesiosis. *In* "Infectious blood diseases of man and animals" (D. Weiman and M. Ristic, eds.), Vol. 2, pp. 219–268. Academic Press, New York and London.

Roberts, J. A. (1968). Acquisition by the host of resistance to the cattle tick, *Boophilus microplus* (Canestrini). *Journal of Parasitology*, **54**, 657–662.

Roshdy, M. A. (1972). The subgenus *Persicargas* (Ixodoidea, Argasidae, *Argas*). 15. Histology and histochemistry of the salivary glands of *A.* (*P.*) *persicus* (Oken). *Journal of Medical Entomology*, **9**, 143–148.

Ross, I. C. (1926). An experimental study of tick paralysis in Australia. *Parasitology*, **18**, 410–429.

Sauer, J. R. (1977). Acarine salivary glands—physiological relationships. *Journal of Medical Entomology*, **14**, 1–9.

Schein, E. (1975). and Dschunkowsky Luhs (1904). On the life cycle of *Theileria annulata* in the midgut and hemolymph of *Hyalomma anatolicum excavatum* (Koch, 1844). *Zeitschrift für Parasitenkunde*, **47**, 165–167.

Schein, E. (1976). Zum Entwicklungszyklus von *Theileria annulata* (Dschunkowsky, Luhs, 1904) in der Überträgerzecke *Hyalomma anatolicum excavatum* (Koch, (1844). *Habil. Schrift, FU Berlin.*

Schein, E. and Friedhoff, K. T. (1978). Lichtmikroskopische untersuchungen über die entwicklung von *Theileria annulata* (Dschunkowsky and Luhs, 1904) in *Hyalomma anatolicum excavatum* (Koch, 1844). II Die entwicklung in Hämolymphe und Speicheldrüsen. *Zeitschrift für Parasitenkunde*, **56**, 287–303.

Schein, E., Buscher, G. and Friedhoff, K. T. (1975). Light-mikroskopische untersuchungen über die entwicklung von *Theileria annulata* (Dsclunkowsky und Luhs, 1904) in *Hyalomma anatolicum excavatum* (Koch, 1844). I. Die entwicklung im darm vollgesogener Nymphen. *Zeitschrift für Parasitenkunde*, **48**, 123–136.

Schin, K. and Laufer, H. (1974). Uptake of homologous haemolymph protein by salivary glands of *Chironomus thummi*. *Journal of Insect Physiology*, **20**, 405–411.

Schleger, A. V. and Lincoln, D. T. (1976). *Boophilus microplus:* characterization of enzymes introduced into the host. *Australian Journal of Biological Sciences*, **29**, 482–497.

Schleger, A. V., Lincoln, D. T., McKenna, R. V., Kemp, D. H. and Roberts, J. A. (1976). *Boophilus microplus:* Cellular responses to larval attachment and their relationship to host resistance. *Australian Journal of Biological Sciences*, **29**, 499–512.

Sonenshine, D. E. and Gregson, J. D. (1970). A contribution to the international anatomy and histology of the bat tick *Ornithodoros kelleyi* Cooley and Kohls, 1941. I. The alimentary system, with notes on the food channel in *Ornithodoros denmarki* Kohls, Sonenshine and Clifford. *Journal of Medical Entomology*, **7**, 46–74.

Stiller, D. and Ranchitham, S. T. (1974). Production of cement substance by larval argasid ticks at the site of attachment: a preliminary report. *Southeast Asian Journal of Tropical Medicine and Public Health*, **6**, 445–447.

Stone, B. F., Doube, B. M. and Binnington, K. C. (1979). Toxins of the Australian paralysis tick *Ixodes holocyclus*. *Proceedings of the 5th International Congress of Acarology*, 1978, Vol. 1, pp. 347–356.

Tatchell, R. J. (1967). Salivary secretion in the cattle tick as a means of water elimination. *Nature (London)*, **213**, 940–941.

Tatchell, R. J. (1969a). The ionic regulatory role of the salivary secretion of the cattle tick. *Boophilus microplus*. *Journal of Insect Physiology*, **15**, 1421–1430.

Tatchell, R. J. (1969b). Host–parasite interactions and the feeding of blood sucking arthropods. *Parasitology*, **59**, 93–104.

Tatchell, R. J. (1969c). The significance of host-parasite relationships in the feeding of the cattle tick *Boophilus microplus*. *Proceedings of the 2nd International Congress of Acarology*, 1967, pp. 341–345.

Tatchell, R. J. (1971). Electrophoretic studies on the proteins of the haemolymph, saliva, and eggs of the cattle tick, *Boophilus microplus*. *Insect Biochemistry*, **1**, 47–55.

Tatchell, R. J. and Binnington, K. C. (1973). An active constituent of the saliva of the cattle tick, *Boophilus microplus*. *Proceedings of the 3rd International Congress of Acarology*, 1971, 745–748.

Tatchell, R. J. and Moorhouse, D. E. (1968). The feeding processes of the cattle tick *Boophilus microplus* (Canestrini). II. The sequence of host-tissue changes. *Parasitology*, **58**, 441–459.

Tatchell, R. J. and Moorhouse, D. E. (1970). Neutrophils: their role in the formation of a tick feeding lesion. *Science*, **167**, 1002–1003.

Theis, J. H. and Budwiser, P. D. (1974). *Rhipicephalus sanguineus:* Sequential histopathology at the host–arthropod interface. *Experimental Parasitology*, **36**, 77–105.

Till, W. M. (1961). A contribution to the anatomy and histology of the brown ear tick *Rhipicephalus appendiculatus* Neumann. *Memoirs of the Entomological Society of South Africa*, **6**, 1–124.

Tracey-Patte, P. D. (1979). Effect of the bovine immune system on esterase deposited by *Boophilus microplus* larvae. *Australian Advances in Veterinary Science*, p. 78.

Utech, K. W. B., Wharton, R. H. and Kerr, J. D. (1978). Resistance to *Boophilus microplus* (Canestrini) in different breeds of cattle. *Australian Journal of Agricultural Research*, **29**, 885–895.

Vane, J. R. (1976). The mode of action of asprin and similar compounds. *Journal of Allergy and Clinical Immunology*, **58**, 691–712.

Wagland, B. M. (1978). Host resistance to cattle tick (*Boophilus microplus*) in Brahman (*Bos indicus*) cattle. II. The dynamics of resistance in previously unexposed and exposed cattle. *Australian Journal of Agricultural Research*, **29**, 395–400.

Weber, G. (1972). Glykolmethacrylat-einbettung und 1–2 μm-schnitt-technik fur zeckengewebe und ganze zecken. *Zeitschrift für Parasitenkunde*, **40**, 295–306.

Weber, G. and Friedhoff, K. (1979). Electron microscopic detection of initial and some subsequent developmental stages of *Babesia bigemina* in salivary glands of ticks. *Zeitschrift für Parasitenkunde*, **58**, 191–194.

Wikel, S. K. and Allen, J. R. (1977). Acquired resistance to ticks III. Cobra venom factor and the resistance response. *Immunology*, **32**, 457–465.

Wikel, S. K. and Allen, J. R. (1980). Immunological basis of host resistance to ticks. *In* "Physiology of Ticks" (F. D. Obenchain and R. Galun, eds.). Pergamon Press (in press).

Willadsen, P. W. (1980). Immunity to ticks. *In* "Advances in Parasitology" (W. H. R. Lumsden, R. Muller and J. R. Baker, eds.). Vol. **18**, pp. 293–313. Academic Press, London and New York.

Willadsen, P. and Riding, G. A. (1979). Characterization of a proteolytic enzyme inhibitor with allergenic activity. *Biochemical Journal*, **177**, 41–47.

Willadsen, P., Williams, P. G., Roberts, J. A. and Kerr, J. D. (1978). Responses of cattle to allergens from *Boophilus microplus*. *International Journal for Parasitology*, **8**, 89–95.

Young, A. S., Leitch, B. L. and Omwoyo, P. L. (1980). The induction of the infective stages of *Theileria parva* by exposure of host ticks to high temperatures. *Veterinary Record* (in press).

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