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HAEMATOPROTOZOAN PARASITES OF MARINE FISHES WITH SPECIAL REFERENCE TO MARICULTURE

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ABSTRACT

This research study is divided into 4 major sections.
Section A deals with the world-wide distribution of the haematoprotozoan parasites of marine fishes. These are tentatively divided into 3 major groups: the Haemogregarines, the Trypanosomes and the Trypanoplasms and one group of ill defined and controversal organisms including Haemohormidium sp. and Dactylosoma sp. The results of extensive surveys of the coastal waters of France, Scotland and Wales with added examples from the Mediterranean Sea substantiate the zoogeographical distribution of these parasites. Two species of haematoprotozoan parasites Haemogregarina simondi and Trypanosoma soleae are re-described, earlier accounts dating from the beginning of the century being considered incomplete. A new species of haemogregarine is described from the farmed turbot (Scophthalmus maximus) as Haemogregarina sachai n.sp. and an unidentified species of Haemohormidium found occasionally in turbot and Dover sole (Solea solea) is also described. In addition Haemogregarina sp. were encountered in certain wild marine fish from

the Atlantic coast of France e.g. Zeus faber, Sebastes sp., Trisopterus luscus, Pagellus bogaraveo and Raja sp. and from the coastal waters of Malta e.g. Peristedion cataphractum and Oblade melanura. However, they were not found in sufficient numbers to allow a definite description. The value of surveys of wild fish populations is discussed in the light of zoogeographical distribution, the apparent periodicity of these parasites and a seasonal variation of parasitism.

Section B attempts to summarize the knowledge of the mode of transmission of marine haematoprotozoan parasites by piscicolid leeches as intermediate hosts and vectors. The developmental stages of a trypanosome, probably Trypanosoma murmanensis from the Atlantic cod Gadus morhua are described in the marine leech Calliobdella nodulifera. Stages of a haemogregarine were observed in the same leech. The development of the turbot haemogregarine Haemogregarina sachai n.sp. in artificially infected leeches is also described up to the 20th day post infection. Transmission of this haemogregarine to apparently uninfected turbot via this leech was not successful. Various stages of development of Haemogregarina simondi are described in its apparently natural vector, the marine leech Hemibdella soleae, and transmission with infected leeches

to apparently healthy hatchery reared soles was achieved. Thus it was shown for the first time that marine leeches can serve also as vectors for haemogregarines. Stages of this haemogregarine are also described in the blood-sucking ectoparasitic copepod *Lernaeocera* sp. parasitizing the haemogregarine infected soles. These results are discussed in relation to the feeding behaviour and migration patterns of the fish hosts, the periodicity of the parasites and possible other vectors or other ways of acquiring an infection with these haematoprotozoan parasites.

In Section C the pathogenicity of the haematoprotozoan parasites of marine fishes is summarized from previous accounts among wild fish populations and compared with the situation in aquaculture. The pathogenicity of the two haemogregarines, *Haemogregarina simondi* and *Haemogregarina sachai* n.sp., accidentally introduced into several fish farming establishments connected with the effluent cooling waters of a nuclear power station is described with special reference to the possible source of the infection. The results of therapy trials and control programs are discussed in the light of the periodic reappearance of the parasites, the possibility of carrier fish existing, the immune status of the host and the possible role

of an intermediate host or vector in maintaining the infection. Possible means of controlling the pathogenicity of marine haemogregarines and perhaps other haematoprotozoan parasites when they occur in farmed marine fishes are also discussed.

In Section D are described for the first time, the ultrastructural characteristics of various stages of the haematoprotozoan parasites of marine fishes in fish hosts and vectors. The electronmicroscopical studies are limited to *Haemogregarina sachai* n.sp. and *Haemogregarina simondi*, for which the ultrastructure of schizonts from the spleen and blood, intracellular merozoites and free gametocytes were contrasted. In addition stages of *H.simondi* were demonstrated in *Hemibdella soleae* and *Lernaeocera* sp. The fine structure of the various organelles encountered was compared with that of related organisms from other cold-and warm-blooded vertebrates.

In conclusion attention is drawn to the need for more investigations in this field of host-parasite relationship of marine haematoprotozoan parasites and their vectors and their pathogenic action as seen in a confined and artificial environment such as the marine aquaculture.

GENERAL INTRODUCTION

The great majority of the organisms for which marine and freshwater fishes are hosts are referable either to the Protozoa or to the Helminthes (cestodes, trematodes, nematodes and leeches). Various crustaceans (belonging to the same broad group as the insects) often attach themselves to fishes too. Under natural conditions these all occur regularly, although seldom in heavy enough concentrations to bring about the death of the host: It is hardly in the best interests of a parasite to put an end to its source of nourishment.

Parasites in wild fishes are frequently only remarked upon when they are so obvious as to lead to rejection of fish by consumers. Serious parasitic damage to wild fish populations often goes unnoticed, particularly because under natural conditions the diseased, parasitized fishes are readily eliminated by predators such as other carnivorous fishes, seabirds or marine mammals. Such predation often guarantees the perpetuation of the parasite and is part of its life cycle, but heavy parasitic infestations of fishes associated with high mortality are exceptional under natural conditions.

The normal role played by parasites is that of one of the regulating mechanisms which keep the various components of the ecosystem in a state of biological balance with one another. Epizootics are usually associated, when they occur, with populations in which resistance to pathogens has been lowered through a general debility associated, for example, with spawning.

In aquaculture, however, parasites often cause serious outbreaks of disease. Fish are perhaps one of the most highly parasitized groups of animals and the unnatural crowded conditions usually encountered in aquaculture establishments such as hatcheries and aquaria, favour the greatly increased possibility of heavy infestation by some parasites. Such hazards have been recognized in freshwater fishes for some time and diseases of marine fishes are now beginning to receive increasing attention with the current trend towards marine fish farming under artificial conditions. In fish cultivation, environmental changes such as fluctuations in temperatures or particular concentrations of salts may favour the development of certain parasites so that their population increases to a very high level. Many parasite species are host specific to at least some degree and are capable of infecting one or only limited numbers of host species,

but some parasites may have adapted so well that they attain an unlimited range. At these high population densities they may seriously affect the health of the fish and cause high mortalities, particularly among stressed fish such as spawning brood stock or the young fish at the moment when they change from natural food to an artificial diet. The same parasite species may have also widely different effects on different host species. A fish farm situation also frequently provides an ideal microclimate for the vectors of these parasites, which can reproduce undisturbed by adverse environmental conditions and at the same time find an easy prey in the narrowly confined fish host. Thus the artificial conditions involving overcrowding, undernourishment, water pollution and other such factors as are often found in aquaculture may lead to an entirely different picture concerning the pathogenicity of the parasites. This will consequently lead to an economical impact on the farming of these highly valuable species of fish and the limitations which certain parasites might set for aquaculture cannot readily be forseen.

S E C T I O N A

HAEMATOPROTOZOAN PARASITES OF MARINE FISH

1. INTRODUCTION

The haematoprotozoan parasites of fish have been of longstanding interest to scientists and can be considered as pathfinders for research on haemato-protozoan parasites in general. Valentin (1841) described an "entozoon" in the blood of the trout, Salmo fario, which is considered by many authors to be the first description of a haemoflagellate parasite in any animal.

The genus Trypanosoma was created by Gruby (1843) for a haemoflagellate of frogs which he named Trypanosoma sanguinis. As it conformed to a similar parasite described earlier that year (Mayer, 1843) under Amoeba rotatoria the valid name for the type-species became Trypanosoma rotatorium. The new genus Trypanosoma is characterised briefly according to Laveran and Mesnil (1901): as flagellates of a fusiform body, which presents laterally an undulating membrane ending in a centrosome-like structure located in the posterior half of the body and prolonged anteriorly in form of a free flagellum. The division occurs by unequal longitudinal binary fission.

Knowledge of the trypanosomes of fishes developed

very early with the work of Gros (1845) and all future blood flagellates found in freshwater fishes were attributed to that genus. The fact that trypanosomes also occurred in marine fishes was briefly mentioned in a thesis by Labbé (1894). Sabrazès and Muratet (1901), described trypanosomes in the euryhaline fish species *Anguilla anguilla*. Laveran and Mesnil (1902) were the first to describe a marine fish trypanosome in detail, viz. *Trypanosoma scyllii* from *Scyllium stellare* and *Scyllium canicula*. Many more fish hosts for marine trypanosomes have been described since and numerous new species were created, though there appears today to be a wide range of synonymy. These trypanosomes seem to have a world-wide distribution but surveys have so far been limited to the activity of a few enthusiastic parasitologists. So far trypanosomes have been described from 10 orders, 7 suborders, 19 families and 43 species of marine fish, but it appears that certain families are more frequented than others (i.e. Rajidae; Pleuronectidae; Cottidae and Blenniidae) as seen from Table 1.

In 1901, Laveran and Mesnil discovered a trypanosome with 2 flagella in the blood of a freshwater fish for which they created the new genus *Trypanoplasma*. This parasite was described as *Trypanoplasma borreli* from *Leuciscus erythrophthalmus*. Thereafter many older

descriptions of trypanosomes in the blood of fishes appeared more likely to be descriptions of mixed infections of trypanosomes and trypanoplasms. The latter are occasionally also encountered in marine fishes. The validity of the genus Trypanoplasma is still under discussion.

The genus Haemogregarina was created by Danilewsky (1886) for the intracorpuscular parasite Haemogregarina stepanovi from lizards (Family Lacertidae). Labbé 1894 created the genus Karyolysus for a haemogregarine of Lacerta sp. because during the growth of the gametocytes within the nucleated red blood cells the nucleus tended to be fragmented. Miller (1909) created the genus Hepatozoon for a haemogregarine found in white rats. Schizogony of his Hepatozoon perniciosum occured in liver cells and merozoites were carried by leucocytes into the peripheral circulation.

The haemogregarines of fish belong to the genus Haemogregarina and it is in marine fish that this group of haematoprotozoan parasites has been studied most widely. They were first described by Laveran and Mesnil (1901) but there is still much confusion regarding the grouping of the haemogregarines of marine fish. Henry (1912) had coined the name Schizohaemogregarines because of their unique course of gametocyte formation. The earliest schizogony results

from the initial invasion of fish host cells by sporozoites which come from an invertebrate host. These divisions result in the production of considerable numbers of large merozoites which invade the circulating corpuscles and develop into gametocytes. Laird (1952) distinguished the haemogregarines according to differences in morphological features. He divided the schizo-haemogregarines into two groups. In group I a final schizogony terminating in the production of gametocytes takes place in the circulating blood, each parasitized erythrocyte thus coming to contain 2 or more mature gametocytes. In group II the micromerozoites which develop in the bloodstream or in capillaries of internal organs invade red cells and there develop into single gametocytes. This group is again subdivided into haemogregarines with the presence of polar masses of extranuclear material and called the "rovignensis-group" after the type-species Haemogregarina rovignensis from Trigla lineata (Minchin and Woodcock,1910) and into haemogregarines where the polar cap is absent as in Haemogregarina dasyatis from Dasyatis americana (Saunders, 1958). Haemogregarines are the most abundant group of all haematoprotozoan parasites of marine fish. A total of 11 orders, 6 suborders, 56 families and 158 species of

marine fishes have been found to harbour these parasites (Table 1). This is by no means a complete compilation because the majority of this information has been gathered by systematists and is scattered throughout the literature. There is also a great degree of synonymy among species of haemogregarines due to the very sketchy nature of the early descriptions and this contributes even more to the confusion. Numerous species were established merely on the basis of their occurrence in hosts from which haemogregarines had not been previously described. Indeed Minchin (1907) remarked that many of these names should not be taken as necessarily describing distinct natural species but were just a matter of labelling for matters of convenience so that reference could be made more briefly to them. Thus a re-examination of many of the older descriptions is indeed very much to be desired. Laird (1953) suggested a re-examination of material from four European species of flatfish from which Lebailly (1904, 1905) had briefly described four different species of haemogregarines. Laird and Bullock (1969) pointed out that various haematoprotozoan parasites reported from European marine fishes early this century were greatly in need of redescription, e.g. Haemogregarina cotti from European sculpins (Brumpt and Lebailly,1904). Laird and Morgan (1973) gave a short review on Haemogregarina platessae to stimulate the interest of European

parasitologists to either search for the original material of Lebailly or to try to find further material from Pleuronectes platessa which would furnish the original specimen of haemogregarine, because, as they contended " a detailed redescription of H. platessae is clearly needed and equally clearly a neo-type should be derived from the original host species and from the type locality".

Much confusion exists also with regard to the two "haemosporidian" or "piroplasmodian" parasites of fishes.

The genus Haemohormidium created by Henry (1910) comprises fish and amphibian babesioids having rather large, oval, ellipsoid or irregularly amoeboid trophozoites in which the well stained periphery contrasts with a refractile central zone. Such babesioids divide by schizogony, binary fission or budding into no more than four merozoites, the schizonts commonly being rosette-shaped or cruciform.

The genus Dactylosoma was created by Labbé (1894) for a similar amoeboid parasite from European frogs (Family Ranaidae), the only difference being the production of more than four merozoites at schizogony.

Dactylosoma also produces only one type of gametocyte while Haemohormidium produces both, micro- and macrogametocytes. These parasites are occasionally encountered also in fish and have been described only once so far from marine fish (Saunders, 1966). Their taxonomic position is still under discussion.

All of these haematoprotozoan parasites described from surveys of wild marine fish populations in various parts of the world raise a number of questions which still remain mostly unanswered. Many of the parasites have been most inadequately described and new species names have been given merely on the basis of their occurrence in new hosts or new locations. Unfortunately the incompleteness of the recorded information is such that it is only possible often to suspect the existence of synonymy. It is necessary therefore to obtain more information on how frequently these parasites are found in marine fish, and whether this depends on a seasonal fluctuation of parasites or a special preference of the parasites for certain hosts. It would also be of interest to know how they are transmitted and if they have any pathological effect under natural conditions or if they are purely commensals. The distribution of parasites and hosts might also indicate whether or not a certain habitat has an influence on parasitism so that a study

of the zoogeographical distribution and hostspecificity of these parasites would be of great value.

Since it has been shown recently that haematoprotozoan parasites of marine fishes can be of some importance in the closed and restricted environment of an aquaculture establishment (Kirmse, 1975) an attempt was made to answer some of these questions during the present study and also to re-describe some of the original European type-species of these parasites of marine fishes.

2. REVIEW OF THE LITERATURE

2.1. MASTIGOPHORA (Diesing, 1865): ZOOMASTIGOPHORA (Calkins,1909 PROTOMONADINA (Blochmann, 1895): TRYPANOSOMATIDAE:

2.1.1. *Trypanosoma* (Gruby, 1843)

Laveran and Mesnil (1901) had the idea of investigating the poorly studied marine fishes for the presence of trypanosomes. They examined two elasmobranchs, the dogfishes *Scyllium canicula* and *Scyllium stellare* and described *Trypanosoma scyllii*, the first trypanosome from the marine environment. This was a large parasite, the total length being 70-75 micron and the width 5-6 micron. The free flagellum measured 14 micron. The parasite was mostly found curled up and it was only rarely seen in the blood. Laird (1951) examining 45 related dogfish (*Squalus kirki*) in New Zealand was surprised to find them all free of trypanosomes. Since this first record of marine trypanosomes many more have been described from various locations (Table 2) and these have often shown such a marked polymorphism that it leads to the assumption that some of these trypanosomes, especially from fishes of the same family and described as distinct species, are in fact synonymous. It also also appears that certain families of fish are more commonly parasitized than others.

European and North American skates (Family Rajidae) are commonly infected with very large trypanosomes. *Trypanosoma rajae* was first described in *Raja mosaica*, *Raja macrorhynchus*, *Raja clavata* and *Raja punctata* (Laveran and Mesnil, 1902) and consequently in *Raja microcellata* (Robertson, 1906); *Raja batis* (Coles, 1914); *Raja ocellata* (Kudo, 1923); *Raja* sp. (Minchin and Woodcock, 1910); *Raja* sp. (Henry, 1913); *Raja erinacea* (Bullock, 1958); and *Raja radiata* (Laird and Bullock, 1969; So, 1972). The original description for *T. rajae* gives the measurements as 75-80 micron for the total length; 6 micron for the width and 20 micron for the free flagellum. However, Minchin and Woodcock (1910) found the total length to be 55-72 micron, the width 4.0-5.6 micron and the free flagellum 10-15 micron long and suggested that Laveran and Mesnil (1902) had only described fully grown specimens. Neumann (1909) described *Trypanosoma variabile* from *Raja punctata* which he distinguished from *T. rajae* only because of a greater polymorphism. But Minchin and Woodcock (1910) referred *T. variabile* to *T. rajae* and Robertson (1909) also believed in a synonymy of the two trypanosomes. *Trypanosoma giganteum* was described from *Raja oxyrhynchus* by Neumann (1909) as ranging from 125-130 micron in length and having a width of 8 micron. The free flagellum measured 25-30 micron. A *Trypanosoma* sp. was reported from *Raja capensis*

(Fantham, 1919) but no morphological details were given. Trypanosoma marplatensis was described from Psammotics microps, a member of the Rajidae from South America (Bacigalupo and De la Plaza, 1948). Trypanosoma gargantua was described from Raja nasuta by Laird (1951). This trypanosome is the largest recorded for its genus and showed marked polymorphism. The measurements were 66.7-131.1 micron in length and 4.6-13.7 micron in width. It was characterized by not having a free flagellum. All of these skate trypanosomes have very small sized kinetoplasts in proportion to the large size of their bodies.

Among the orders Pleuronectiformes, members of three families (Soleidae, Pleuronectidae and Bothidae) were commonly found infected with trypanosomes. The first record is Trypanosoma soleae from Solea solea (Family Soleidae) (Laveran and Mesnil, 1902) measuring 40-47 micron (length), 3-8 micron (width) and 5-8 micron (free flagellum). Trypanosoma dorhni from Solea monochir has a length of 41 micron (Yakimoff, cit. Laveran and Mesnil, 1912).

Many more species of the Pleuronectidae appear to be infected with trypanosomes. Here the first species described was Trypanosoma platessae from Pleuronectes platessa (Lebailly, 1904). It was followed by

Trypanosoma flesi from *Pleuronectes flesus* (Lebailly, 1904); *Trypanosoma limandae* from *Limanda limanda* (Brumpt and Lebailly, 1904); *Trypanosoma caulopsettae* from *Caulopsetta scapha* and *Rhombosolea plebeia* (Laird, 1951); *Trypanosoma* sp. from *Glyptocephalus cynoglossus* (So, 1972). There appears to be some synonymy concerning *T. platessae*. Robertson (1906) found this parasite in both, *Pleuronectes flesus* and *Pleuronectes platessa* and since the dimensions were also the same in both fishes it was considered that *T. platessae* and *T. flesi* were synonymous. Laird (1951) found that the morphology and the dimensions of *T. caulopsettae* closely resembled those of *T. platessae*, the only difference being that it is not known if *T. platessae* exhibits polymorphism. The author believed that there was a possibility that both flagellates may prove to be conspecific in which case *T. platessae* should be given priority.

Among the family Bothidae only one species, *Scophthalmus rhombus* was found with trypanosomes and described as *Trypanosoma bothi* (Lebailly, 1905). It measured 42 micron in length and 3 micron in width, the free flagellum being 13 micron long.

Blennies (families Blenniidae and Clinidae) from all over the world have been reported to contain trypanosomes.

Brumpt and Lebailly (1904) described Trypanosoma delagei from Blennius pholis in France. It measured 33 micron in length and 2.5 micron in width, the free flagellum was 12 micron long. Trypanosoma blenniclini was reported from Blennius cornutus and Clinus anguillaris in South Africa (Fantham, 1930). The total body length ranged from 50-77 micron, the width from 3-7 micron, a free flagellum was not present. Trypanosoma tripterygium was described from Tripterygium varium and Trypterygium medium by Laird (1951) in New Zealand. The overall length was 50.9-100.1 micron, the width 1.3-7.4 micron and the length of the free flagellum measured 3.3-16.8 micron.

Interesting also is the parasitism of the euryhaline family Anguillidae. Trypanosoma granulosum (Laveran and Mesnil, 1902) has been recorded from Anguilla anguilla in many parts of Europe. Johnston and Cleland (1910) described Trypanosoma anguillicola from Anguilla mauritanica, Anguilla bengalensis and Anguilla reinhardtii. However, Doré (1921) examined 400 Anguilla dieffenbachii without finding any infected with trypanosomes and Laird (1951) studying blood smears of 37 eels of the same species did not encounter any trypanosomes. Most of the other described trypanosomes of marine fishes were mainly single findings in isolated species (Table 1,2).

2.1.2. Trypanoplasma (Laveran and Mesnil,1901)

Laveran and Mesnil (1901) described Trypanoplasma borreli from the blood of the fresh-water fish Leuciscus erythrophthalmus and thus created the new genus Trypanoplasma. Soon thereafter some confusion about the taxonomic position of these haemoflagellates occurred when Léger (1905) extended the environment of the parasite to the alimentary tract of marine fishes, reporting the new species Trypanoplasma intestinalis from oesophagus and stomach of Box boops. Also Keysselitz (1906) found a flagellate in the stomach of the marine fish Cyclopterus lumpus and described it as Trypanoplasma ventriculi. In addition Trypanoplasma sp.was described as an ectoparasite on Carassius auratus by Swezy (1919). Since there appeared no morphological difference of these trypanoplasms from a parasite described by Leidy (1846) as Cryptobia helicis from the seminal vesicles of the molluscs Helix albolabris, Helix tridentata and Helix alternata, some authors using morphological criteria only as a basis for generic distinction have given Cryptobia the priority over Trypanoplasma (Crawley,1909; Swezy,1919; Katz,1951; Bullock,1953; Strout,1965; Laird and Bullock, 1969).This confusion has unfortunately been perpetuated and the term Cryptobia as well as Trypanoplasma has been

used to describe certain biflagellate haematoprotozoan parasites from the blood of freshwater and marine fishes. Laveran and Mesnil (1912) agreed that there was a great morphological resemblance between the parasite from the snail and that from the blood of the fish. They justified the genus Trypanoplasma, however, claiming that a parasite from the seminal vesicles of the snail with no intermediate host could not belong to the same genus as the trypanoplasm which lives in the blood of fishes and carries out one phase of its development in leeches serving as intermediate hosts and vectors. Wenyon (1926) agreed that the correct name of the genus should be Cryptobia Leidy (1846), found in the blood of fishes, the intestinal tract of fishes and in the seminal vesicles of molluscs. However, he suggested that the blood inhabiting forms have so long been known under the name Trypanoplasma Laveran and Mesnil (1901) that it was better to retain them as a distinct genus. Also Mackerras and Mackerras (1961) did not agree with a synonymy between Cryptobia and Trypanoplasma. They suggested the use of Trypanoplasma for the blood dwelling parasites of fish, leaving Cryptobia for intestinal parasites. Although most of the North American authors favour the generic name Cryptobia and have given it priority over Trypanoplasma , all bi-flagellate haematoprotozoan parasites of fish have been classified in the present study as Trypanoplasma

since it was felt that these haemoflagellates of fish belong into their own genus distinct from the parasite of molluscs.

Among the trypanoplasms the majority of species have been described from freshwater fishes and only very rarely were they found in the marine environment (Table 3). Keysselitz (1906) came to the conclusion that they all belonged to the same type species, Trypanoplasma borreli. Other observers, however, have frequently given specific names to the forms occurring in different fishes so that a certain amount of synonymy cannot be excluded. The first description of a trypanoplasm from the marine habitat is by Mackerras and Mackerras (1925) who found Trypanoplasma parmae in Parma microlepis. This species was monomorphic and rather broad and normal specimens measured 12.5-14.7 by 3.8-5.0 micron. Many abnormally rounded and probably disintegrating specimens, measuring 9.5-11.0 by 7.0-8.5 micron were also encountered. The cytoplasm stained faintly blue-pale, was vacuolated anteriorly and contained numerous round or irregular chromatoid bodies of 0.25-1.25 micron or more in diameter. The nucleus was reniform or ovoid and measured 3.0-3.7 by 1.5-2.3 micron and the large, elongate or oval kinetoplast measured 1.7-2.0 by 1.0-1.3 micron. The anterior free flagellum was 18-25 micron long, the free

part of the posterior flagellum measured 18.0-26.8 micron. No description is given of the morphology of Trypanoplasma flesi from Pleuronectes flesus (Nowitzky,1940). Trypanoplasma newingtoniensis was suggested by Bullock (1953) for a bi-flagellate parasite of 18 micron length from the blood of Pseudopleuronectes americanus but since no detailed description was given, Strout (1965) declared it a nomen nudum. He gave the new name Trypanoplasma bullocki to a similar parasite in the blood of the same fish host. He also encountered this parasite in Liopsetta putnami, Fundulus heteroclitus and Fundulus majalis. This parasite measured 10.9-23.1 by 1.2-6.0 micron. The cytoplasm was alveolar with few scattered vacuoles and often larger, darkly staining chromatin granules were seen. The nucleus measured 3.4-3.6 by 1.4-1.8 micron, the kinetoplast was usually rod-shaped or elongate and measured 3.3-3.6 by 1.1-1.6 micron. The length of the anterior flagellum was 13.1 micron, the free part of the posterior flagellum measured 8.5-8.7 micron in length. Laird and Bullock (1969) described Trypanoplasma bullocki from the same fish hosts in Canada. Their specimens measured 12.5-23.1 by 1.2-4.5 micron. The anterior flagellum was 8.3-19.1 micron long and the posterior one 4.4-15.7 micron. The nucleus measured 3.4 by 1.4 micron, the kinetoplast 3.3 by 1.1 micron. Kirmse (1975) described a Trypanoplasma sp.

from Scophthalmus maximus . The parasite was broad and measured from 13.5-17 micron in length and from 10.5-15 micron in width. The cytoplasm was vacuolated and contained numerous chromatoid bodies of 0.5-1.5 micron in diameter. The nucleus measured 3.0-4.0 by 2.5-3.0 micron, the kinetoplast 1.5-2.5 by 1.5 micron. Most of the parasites appeared bloated and in a state of disintegration and no measurements of the flagella were taken for that reason.

2.2. SPOROZOA (Leuckart,1879) : TELEOSPOREA (Schaudinn,1900) COCCIDIA (Leuckart,1879):SCHIZOCOCCIDIA:ADELEIDEA:

Haemogregarina (Danilewsky,1886)

Haemogregarines of fish were first discovered from the marine environment by Laveran and Mesnil (1901) who described Haemogregarina simondi from Solea solea and Haemogregarina bigemina from Blennius pholis and Blennius gattorugine. Since then numerous other forms have been described from marine fish by many other authors in Europe (Laveran and Mesnil,1902 ; Brumpt and Lebailly,1904; Lebailly,1904,1906; França,1908; Neumann,1909;Minchin and Woodcock, ,1910; Minchin, 1909;Henry,1910,1912,1913; Kohl-Yakimoff and Yakimoff,1915;

Yakimoff,1917; Bentham,1917;and Noble,1957); North America (Mavor,1915; Fantham,Porter and Richardson, 1942; Saunders,1954,1955,1958,1959,1964 and 1966; Bullock,1958; Laird,1961; Laird and Bullock,1969; So,1972; Laird and Morgan,1973; and Bridges,Pedro and Laird,1975); Bahamas (Saunders,1958); South America (Laveran,1906; Carini,1932); Pacific Islands (Laird, 1951,1958); New Zealand (Laird,1952,1953); Australia (Mackerras and Mackerras,1925,1961); India (De Mello and Vales,1936); and Africa (Fantham,1919; and Saunders,1960). Haemogregarines occur much less frequently in fresh-water fishes and even then it is mostly in fish that spend part of their lives also in sea water,e.g. Anguillidae, Salmonidae, Esocidae.

As an example of the life cycle of a typical haemogregarine reference is made to the description of Haemogregarina stepanowi by Reichenow (1910). Asexual multiplication takes place in the bone marrow or the internal organs of the vertebrate host. Repeated schizogonies culminate in the production of merozoites larger than those concerned with the continuation of the asexual cycle. Each of these large merozoites invades an erythrocyte in the circulating blood and there develops into a micro-or macro-gametocyte.

The marine fish haemogregarines resemble those of other animals. Reproducing schizogony occurs in the erythrocytes

of the peripheral blood and division takes place in *H.bigemina* of *Blennius pholis* into two (Laveran and Mesnil,1901); *H.quadrigemina* of *Callionymus lyra* into four (Brumpt and Lebailly,1904); *H.simondi* of *Solea solea* into eight (Laveran and Mesnil,1901); and *H.polypartita* of *Gobius paganellus* into sixteen gametocytes (Neumann,1909).

Occasionally haemogregarines are also seen in white blood cells and have been considered by several authors as a separate group of parasites (*Leucocytozoon* by Henry,1913; Fantham *et al*.1942; *Leucocytogregarina* by Yakimoff,1917). However, Laird (1953) who for the first time described the life cycle of a piscine haemogregarine in the vertebrate host, was able to show that intraleucocytic stages of the parasite are part of its life history. He described *H.bigemina* in *Ericentrus rubrus* and since this haemogregarine is the most frequently encountered species (Table 5),its development appears to be a good example for fish haemogregarines and a short account will be given here.

The initial invasion of various cells of the vertebrate host by sporozoites derived from a possible invertebrate vector has not been observed to date. The earliest stages observed are schizogonies leading to the formation

of a large number of small merozoites. Once they have invaded various leucocytes, such as small and large lymphocytes and monocytes, these small merozoites develop into schizonts. Binary fission takes place and secondary schizonts are produced. Two such schizonts are usually present in a parasitized cell, each of them giving rise to two merozoites, the dominant number of merozoites per cell thus being four. Binary fission of the schizonts of this second series may lead to the production of six or eight merozoites. Eight merozoites are seldom formed in a small lymphocyte but are quite commonly seen in large lymphocytes and monocytes. More than five schizonts or 10 merozoites per host cell are not observed. These merozoites, resulting from the intermediate schizogony in leucocytes, are vermicular bodies that are finally released from the leucocyte and generally invade erythroblasts,or,occasionally, erythrocytes. Here they develop into schizonts and after division two young gametocytes are formed which mature and become long,slender and somewhat crescentic in shape. Free gametocytes are often narrower and longer than the intra erythrocytic forms.

This type of development was confirmed by Saunders(1958) who found and described the same stages of H.bigemina. The earliest stage of development was seen by this author in the fish host Thalassoma bifasciatum where a small

lymphocyte had recently been invaded by a small and oval shaped merozoite.

No descriptions of a life cycle similar to the one in H.bigemina have been given for the numerous other marine fish haemogregarines.

In addition there is the description of a haematoprotozoan parasite of Acanthoclinus quadridactylus which appears quite distinct from any of the haemogregarines previously described from fish (Laird,1953). The author suggested that it might belong to the genus Hepatozoon because of the presence of myonemes and the staining properties of the cytoplasm. Only mature gametocytes were encountered with deeply blue staining cytoplasm,a few small vacuoles and a few bright red granules of chromatic material. This parasite was designated Haemogregarina (Hepatozoon?) acanthoclini but has never been encountered since.

2.3. SPOROZOA : PIROPLASMEA : DACTYLOSOMATIDAE (Jakowska & Nigrelli,1955;Levine,1971)

2.3.1. Haemohormidium (Henry,1910) (Babesiosoma, Jakowska & Nigrelli,1956)

This parasite was first described by Henry(1910) as an irregular,round or oval body,lying embedded in the cytoplasm of the erythrocyte and measuring 2.0-4.5 micron

in length and 1.0-3.0 micron in width. He proposed the name *Haemohormidium cotti* for this parasite of *Cottus bubalis* and *Cottus scorpius*. In 1913 the same author made no mention of the name he had proposed three years previously when he described this intracorpuscular parasite. He now apparently took the amoeboid bodies to be developmental stages of *Haemogregarina cotti*. Later (Henry,1913 b) he found another babesioid in British *Solea solea* but did not recognize it as such. He now considered this parasite as a stage or "granule shedding phase" of *Haemogregarina simondi*, although his figures quite clearly substantiate his earlier description of a *Haemohormidium* sp. Mackerras and Mackerras (1925) reported *Haemohormidium aulopi* from *Aulopus purpurissatus* and from *Parma microlepis* in Australia but described it as a haemogregarine under the name *Haemogregarina aulopi*. Their figures and illustrations show a strong resemblance to *Haemohormidium cotti* and this was recognized by them later (Mackerras and Mackerras,1961). They also suggested that bodies seen by Johnston and Cleland (1909) in red cells of another Australian marine fish (*Monocanthus* sp.) might have been related to this parasite.

Wenyon (1926) validated *Haemohormidium* by publishing a recognizable account of the parasite. Jakowska and Nigrelli (1956) established the genus *Babesiosoma* for certain babesioid parasites of freshwater teleosts and

Trichuridae and included Henry's parasite. However, Laird and Bullock (1969) relegated Babesiosoma to synonymy with Haemohormidium and thus gave the name of Henry priority. Following their lead, Levine(1971) amended the description of the family Dactylosomatidae (Jakowska and Nigrelli,1955) as follows: " in the erythrocytes of cold-blooded vertebrates, schizogony generally present, with formation of four to sixteen merozoites, vector unknown". He recognized three genera in this family: Dactylosoma Labbé,1894; Haemohormidium Henry,1910 and Sauroplasma Du Toit,1938.

Most of the more recent descriptions of Haemohormidium sp. are from marine fish (Table 6a). Haemohormidium (=Babesiosoma) rubrimarensis was found in erythrocytes and erythroblasts of Lethrinus xanthochilus, L.variegatus, Cephalopholis miniatus, C.hemistictus, Mugil troscheli and Scarus harid from the Red Sea (Saunders,1960). The earliest stage was a small ovoid body, but also binucleate and tetranucleate schizonts were seen. Two types of gametocytes i.e.slender,club shaped micro-gametocytes and shorter, broader macro-gametocytes with both ends rounded,were also observed. The author found Haemohormidium sp. also in Epinephelus summana and in Cheilinus diagrammus.

Laird and Bullock (1969) gave the first report of a Haemohormidium sp.from North American marine fish.

Two Hemitripterus americanus and one Hippoglossoides platessoides from Canada were found to be lightly infected. Affected erythrocytes contained from one to four amoeboid bodies with a central zone paler than the surrounding host cell cytoplasm and a faintly blue staining periphery. The overall measurements were 2.3-4.7 micron by 0.8-3.2 micron. No other stages were present and haemogregarines were not known from either host. So (1972) described two new species of this parasite. Haemohormidium terraenovae was reported from the following marine fish hosts: Ammodytes americanus, Hippoglossoides platessoides, Limanda ferruginea, Urophycis tenuis, Melanogrammus (syn.Gadus)aeglefinus, Glyptocephalus cynoglossus. The last species was also infected with Haemogregarina platessae. The second species described, Haemohormidium beckeri, was found in Myoxocephalus octodecemspinosus and showed cruciform division stages. So (1972) suggested that because of the total absence of rosette-shaped or cruciform schizonts from Haemohormidium terraenovae in contrast to Haemohormidium beckeri there might be at least two different groups of babesioids in marine fish. One group characterized by conspicuous oval,ellipsoid or amoeboid trophozoites but lacking division stages appears closer related to the genus Sauroplasma from lizards. The other group is somewhat larger and exhibits intraerythrocytic rosette-shaped or cruciform schizonts.

2.3.2. Dactylosoma (Labbé, 1894)

Dactylosoma has been reported from the blood of reptiles,amphibians and fish. It was first found in a *Rana* sp. by Lankester (1882). Labbé (1894) created the genus with the description of *Laverania* (= *Dactylosoma*) *splendens* in the same host. It is an unpigmented parasite commonly found within the erythrocytes of the circulating blood of the family *Ranidae*. Reproduction occurs asexually by means of schizogonies which form four to sixteen merozoites. These develop into gametocytes similar to those found in haemogregarines except that they stain less intensively and are usually smaller.

In addition two new species were described from *Urodeles*. Fantham (1905) described *Lankesterella tritonis* (later amended to *Dactylosoma*) from *Triton cristatus* and Nigrelli (1929) reported *Dactylosoma jahni* from *Triturus viridescens*.

Only three species of *Dactylosoma* are known from fish and two of these are from freshwater fish. *Dactylosoma mariae* was described by Hoare (1930) from four species of *Haplochromis* taken from Lake Victoria, Uganda. It was characterized by producing four merozoites from

a schizont being arranged in a rosette or cruciform manner without any residual body. Baker (1960) described the same species from *Tilapia* sp. of Lake Victoria and Lake George, East Africa.However, he considered the differences between *Dactylosoma* and *Haemohormidium* as insufficient to allow generic separation and believed that his *D.mariae* should be transferred to *Haemohormidium*.

Dactylosoma salvelini was observed by Fantham *et al.* (1942) in *Salvelinus fontinalis*. Blood and organs showed the parasites as binucleate schizonts,but tetra- to octonucleate schizonts were also seen.

The only *Dactylosoma* from marine fishes is *Dactylosoma lethrinorum* described by Saunders (1960) from *Lethrinus nebulosus* and *L.mahsenoides* and found in the cytoplasm of erythroblasts and erythrocytes of the circulating blood. Several stages were observed from small individual parasites with a round nucleus to binucleate,tetranucleate, sexinucleate and octonucleate wedge shaped schizonts of 10.5 by 8.0 micron producing eight merozoites. Free merozoites measured 1.18 by 2.35 micron. No gametocytes were observed. A *Dactylosoma* sp. with either four or six free merozoites in the cytoplasm of an erythroblast were seen also in *Chrysophrys haffara*.

Jakowska and Nigrelli (1956) considered D.mariae as a representative of their genus Babesiosoma which was later amended by Laird and Bullock (1969) to the genus Haemohormidium. They also showed that the type species should be D.ranarum (Lankester,1882) Labbé,1894. Laird and Bullock (1969) also included D.jahni (Nigrelli, 1929) in the genus Haemohormidium because both these Dactylosoma produced never more than four merozoites in schizogony which were always arranged in a rosette or cross like formation. In addition their nucleus showed no definite nucleolus, the cytoplasm was rich in vacuolization and two distinct types of gametocytes were produced.

3. MATERIAL AND METHODS

3.1. Surveys of wild fish populations

3.1.1. France (Biarritz, Atlantic Coast)

A total of 184 marine fishes of 19 different species were sampled during the winter 1975/76 from fish landed at the nearby port or at the port of St. Jean de Luz (Table 7 a.) by commercial fishermen. During the months of June to August 1976 a total of 1,288 marine fishes belonging to 39 different species were examined for haematoprotozoan parasites (Table 7 b.). The majority of the fish were either pleuronectids (374) or anguillids (215). The flatfishes had been gutted already at sea and were thus dead several hours before reaching the port. Most of the other marine fishes were still alive when samples of blood were taken or had just been killed.

3.1.2. Scotland (Oban, Argyll)

In a search for other haematoprotozoan parasites

of marine fishes, especially marine flatfishes, a large number of several different species were collected from the Dunstaffnage Marine Laboratories at Oban. A short beam trawl was either pulled by two persons along the beach in front of the marine laboratory through waters of 3-4 feet of depth or it was dragged behind a fibreglass boat powered by a 25 hp motor through approximately 6-10 feet deep waters. In addition a seine net was employed from the boat on one occasion (September 1976). On each field trip several hauls were made along the inner bay mainly at low or incoming tide.

In early September 1976 a total of 276 fishes of 7 different species were thus collected. Most of these fishes were small herring (Clupea harengus) of about 3-5 cm in length (a total of 252 specimens). In addition with the short beam trawl 3 saithe (Gadus virens); 6 poor cod (Trisopterus minutus); 5 fifteen-spine stickleback (Spinachia spinachia); 3 plaice (Pleuronectes platessa); 6 sea trout (Salmo trutta) and 1 gobi (Pomatoshistus minutus) were collected. Eleven eels (Anguilla anguilla) from the holding tanks at the marine laboratories, previously collected from the wild, were also made available for examination of the blood.

At the end of June 1977 only the short beam trawl was employed, both for beach fishing and boat trawling. Approximately 12 hauls were made and a total of 174 marine fishes belonging to 9 different species were collected. At this time of the year most of the fishes caught were marine flatfishes, e.g. plaice of a size range of 1.2-3.0 cm in length. These are found in this area from May until the end of August. The age of these plaice was considered to be approximately 3-4 months. A total of 124 plaice were caught. In addition the following species were captured: 7 juvenile cod (Gadus morhua) of 2-3 cm length; 1 eel pout (Zoarces viviparus) of 5-6 cm length; 9 butterfishes Pholis gunellus) of 3-5 cm length; 2 three-spined stickleback (Gasterosteus aculeatus) of 5 cm length; 2 fifteen-spined stickleback (Spinachia spinachia) of 10 and 23 cm length, respectively, 1 gobi (Pomatoschistus minutus) of 3 cm length; 13 sculpins (Cottus scorpius) of 1.8-3.0 cm length; and 5 soles (Solea solea) of 1.2-1.5 cm length.

3.1.3. Scotland (Millport, Clyde)

Bloodsamples were collected from a small number of fishes at the Universities Marine Station Millport. These fishes were collected by the staff of that

laboratory by otter trawl from the main channel of the Clyde at approximately 25-30 fathoms depth. A total of 7 cod (*Gadus morhua*), one juvenile and 6 adults, measuring from 20-47.5 cm in length were examined. In addition blood was collected from two adult plaice (*Pleuronectes platessa*) of 38 cm and 40 cm in length, respectively.

3.1.4. Scotland (Aberdeen, North Sea)

During July 1977 fishermen caught 50 adult plaice (*Pleuronectes platessa*) from approximately 50 fathoms depth off the Aberdeen coast with trawlers for the Department of Biology, University of Aberdeen. These were kept in a holding tank for 24 hours until their blood could be sampled for blood smears. These plaice had an average size of 29.8 cm (range 19.5-38.0).

3.1.5. Malta (Mediterranean Sea)

During August 1977 blood samples were also collected from marine fishes of the Mediterranean Sea by Mr.C.Agius, University of Malta. The following species of fish were examined for haematoprotozoan parasites: 3 *Boops boops*; 3 *Trachurus* sp.; 2 *Merluccius merluccius*; 2 *Gadus minutus*; 2 *Mullus barbatus*; 2 *Peristedion cataphractum*; 2 *Zeus faber*; 2 *Oblade melanura* and

and 2 Coris julis. They were all adult fish collected at 100 fathoms depth by trawl at approximately 10 miles off shore of the Isle of Malta.

3.2. Surveys of marine fish populations in aquaculture

3.2.1. White Fish Authority at Hunterston, Ayrshire, Scotland

This fish farming establishment had been sampled already earlier in 1974/75 at different times of the year (Kirmse,1975) and this survey was continued through 1975, in 1976 and 1977. Altogether 135 turbot (Scophthalmus maximus) of age group 1+, having derived originally from North Wales; 165 sole (Solea solea) of age group 0+ and hatchery reared; and 5 lemon sole (Microstomus kitt) were examined for haematoprotozoan parasites. In addition blood smears were taken from 20 adult sole derived from the English Channel and introduced for breeding purposes in 1977. These had a size range of 32-45 cm length. Also examined were 4 adult hatchery sole of age group 1+ and measuring more than 20 cm in length.

3.2.2. British Oxygen Company at Hunterston

During February 1977, 200 turbot from a total of 2,500 fish held in one circular tank were investigated.

All of these fish had been obtained as very small fish of age group 0+ from beaches in North Wales and belonged to age group 1+ when tested for haematoprotozoan parasites. They measured approximately 25-40 cm in length and their weight was in the range of 280-650 grams.

3.2.3. Fitch Lovell Company at Hunterston

Here in February 1977 a survey was performed on farmed fish obtained either from North Wales or hatched from the egg at Hunterston. A total of 138 wild turbot were examined, 28 of which were from a tank with runts having a history of inhibited growth and rather unexpected mortality. All of the other wild turbot examined were of age group 1+ and their weight and size were considerably higher. Consequently on several field trips in March, June and July 1977 a total of 35 additional turbot from the same lot were tested. The blood of 48 hatchery reared turbot of age group 1+ was also examined. In addition 125 small sole approximately 2-3 months of age and 25 small turbot of approximately 4-5 months of age and hatched in 1976 were sampled during 1976/77.

3.2.4. White Fish Authority at Ardtoe, Argyll

Fish obtained from this station differed from the above

examined ones in that they were reared in tanks of circulated natural seawater at normal temperature independent of any power station effluents. They were transferred to sea cages at the age of 1+. Only turbot were tested for haematoprotozoan parasites at this site. Blood was sampled from 25 specimens of age group 1+ and 10 specimens of age group 2+, all wild turbot originally derived from Wales. In addition 12 turbot of age group 1+ derived from the hatchery at Ardtoe were also examined. None of these fish had a previous history of the proliferative condition associated with a haematoprotozoan parasite as observed and described in turbot from the White Fish Authority at Hunterston (Kirmse,1975).

3.2.5. British Oxygen Company at Wylfa, Anglesey, North Wales

A total of 50 turbot were examined from the tanks of this aquacultural operation which had commenced operations in April 1977 and had received its wild fish, for stocking purposes, from the beaches of North Wales. They belonged to age group 0+ when examined in July 1977.

3.3. Techniques of sampling blood from wild and farmed marine fishes in the surveys

In marine flatfish, blood samples were mainly taken from a major artery or vein, usually the renal caudal artery or vein or the heart according to the methods described by Kirmse (1975). In all other juvenile or adult fish blood samples were taken directly from the hearts of living fish. These were anaesthesized in a solution of tricaine methane sulphonate (MS 222; Sandoz, Basle) at a dose rate of 50-65 ppm. Anaesthesized fish were placed on the bench between two blocks of wood covered with a wet cloth to avoid damage of the skin. Using a No.23 or 25 gauge sterile, disposable needle and a 1 ml or 2 ml hypodermic syringe blood was withdrawn from the heart inserting the needle at the cross point of an imaginary line between the anterior ends of the pectoral fin base and the linea alba. In fish which were already dead blood was collected from the heart or by making wet impressions of the spleen on a microscopic slide. In some instances where there was no need to preserve the fish alive it was sacrificed and the heart blood sample taken at this time. In the very small young fishes of age group 0+ the peduncle was severed with a sharp pair of surgical scissors and a small drop of blood collected.

A thin smear was made of each sample of blood on a microscopic slide which was immediately air dried on collection. It was fixed at the laboratory in absolute methyl alcohol and stained with Giemsa at a dilution of 1:10 (pH 6.8-7.2) for 20 minutes. Often smears were somewhat overstained because some haematoprotozoan parasites are thus easier detected, e.g. the intra-erythrocytic stages of marine fish haemogregarines. In some cases thin blood smears from individual fish were made by blotting the gills with a paper towel and then jabbing the gill filaments with a capillary pipette and quickly sucking up a few drops of blood. This method was used especially in some larger fish and proved to be quite useful for reading the packed-cell-volume (PCV) at the same time. Fish blood is remarkable for the extreme rapidity with which it clots, either in the body after death or on being drawn from the living fish. Blood smears must therefore be made as soon as the fish is caught and it is necessary to work very quickly once the blood is drawn or the heart is opened in order to obtain even 2 or 3 satisfactory preparations. In freshly killed fish, the opportunity was also taken to collect wet impression smears of the organs in order to discover possible intracellular stages of development of the parasites. These were dried, fixed and stained as above.

Once the microscopic slides had been stained, the whole area of each preparation was scanned microscopically for the presence of parasites using a x 10 ocular and a x 40 oil immersion objective or a x 100 oil immersion objective.

Observations on living stages of haematoprotozoan parasites to test for different degrees of motility, especially in fish trypanosomes, were readily made by examining a drop of whole blood from heavily parasitized fish or dilutions of blood with drops of saline or distilled water sealed under a cover slip. For more extended observations the hanging drop technique was employed in which the drop of blood is sealed in with vaseline under a coverslip using a special microscopic slide. The vital stains used for the wet preparations included methyl green, neutral red, Lugol's iodine and methylene blue.

The haematocrit centrifugation technique described by Woo (1969) was used in some live flatfish species as turbot, sole and plaice. This technique was used for the rapid detection of trypanosomes because these parasites accumulate in the buffy coat between blood plasma and serum after centrifugation and can thus be more readily found in pooled blood of several fish from the same species. Centrifugation at 1,800 rpm for

8 minutes was performed either in heparinized capillary tubes or in small centrifuge tubes.

3.4. Measurements of haematoprotozoan parasites

When intra- and extracellular organisms such as haemogregarines or piroplasmoid organisms were measured a photographic micrometer was used. This was done only via the oil immersion lens at x 100 (i.e. a total magnification of x 1,000) Measurement of the dimensions of trypanosomes was more difficult. Generally they were obtained by projecting coloured slides of known magnification through a Rollei P 2.8/85 slide projector onto a piece of white paper and then tracing out the course of the mid line with a piece of thread along the middle of the parasite.

3.5. Photography of parasites

The parasites and their dividing and developing stages, when observed in wet preparations, were photographed with phase contrast using various coloured filters. Photographs of parasites found in Giemsa stained smears were either taken with Black & White Film (Kodak Panatomic-X) at high power (x 100 oil immersion) or for better contrast a Kodak colour film 2483 daylight PCF 135-36 was employed and pictures taken at ASA 16 - 13 DIN

through a photomicroscope. In most instances the area of observation was enlarged in addition with the aid of a zoom lens.

3.6. Taxonomy of fish

Although the taxonomy of fish appeared very confused with many synonomies an attempt was made to sort out scientific names and authorities according to the most recent publications on this subject (Wheeler,1970; Shino,1972; American Fisheries Society,1970; Grassé,1959; Berg,1958;UNESCO,1973; De Haas and Knorr,1966).

4. RESULTS

4.1. Surveys of wild fish populations

4.1.1. France

During winter 1975/76 a total of 84 marine fish of 19 different species (Table 7 a) were examined for haematoprotozoan parasites. *Haemogregarina simondi* was demonstrated in 6/12 *Solea solea* tested, both in blood smears and wet impressions of spleens. The ocurrence of these parasites was limited and no trypanosomes or any other blood protozoans were detected.

In summer 1976 a total of 1,288 marine fish of 35 different species (Table 7 b) were investigated for haematoprotozoan parasites. *Haemogregarina simondi* was detected in 67/241 *Solea solea* and *Trypanosoma soleae* was found in only 1/241 soles. The impression smears of 6/10 spleens of *Solea solea* revealed haemogregarines in different stages of development.

Impression smears of the kidneys of Solea solea and of spleens and kidneys of Scophthalmus rhombus did not show any haematoprotozoan parasites. Likewise no parasites were detected in histological sections.

In addition single unidentified haemogregarines or their developmental stages were seen in 1/46 Pagellus centrodontus; 1/8 Trisopterus luscus; 1/50 Sebastes sp. 1/18 Zeus faber; and 1/12 Raja sp. Except for the haemogregarine of Raja sp. which was found singly inside a large lymphocyte, all other haemogregarines were seen free in the blood plasma. Their morphology was too ill-defined to allow a detailed description.

4.1.2. Scotland

All blood smears examined from marine fishes at Oban, Millport and Aberdeen, regardless of the age of the fish, failed to reveal any infection of these marine species with haematoprotozoan parasites.

4.1.3. Malta

Blood samples examined from 20 mediterranean fishes of 9 different species showed a single haematoprotozoan parasite only in 1/2 Peristedion cataphractum. The parasite was found free in the blood plasma and

resembled the gametocyte of a *Haemogregarina* sp. because of its shape of a reversed "S". Its length was 13.6 micron and the width 0.9 micron. The intensely purple staining nucleus of 0.3 by 0.25 micron was in central location. A few dark staining granules were located in the anterior portion of the parasite.

4.2. Surveys of farmed fish populations

4.2.1. Scotland

No haematoprotozoan parasites were detected in the blood samples from the White Fish Authority at Ardtoe. Also no parasites were found in turbot and sole from the White Fish Authority at Hunterston examined in 1975-1977 except for the occurrence of *Haemogregarina simondi* in 20 of 72 adult *Solea solea* serving as brood stock collected April 1977 off Portsmouth in the English Channel. Other adult soles collected earlier or later from the same location were apparently free of these parasites. However, brood stock soles from the North Sea and captured in October 1977 harboured this haemogregarine. No trypanosomes were found in any of these infected soles.

The blood of 200 turbot examined from the British Oxygen Company at Hunterston did not reveal any

haematoprotozoan parasites despite the fact that this particular tank had a previous history of the proliferative condition associated with haematoprotozoan parasites (Kirmse,1975). However, 7/185 wild turbot from Fitch Lovell examined in spring 1977 showed moderate to high parasitemias with intracorpuscular organisms parasitizing the leucocytes of these fish. None of the hatchery reared turbot or any other species of marine fish revealed an infection with these haematoprotozoan parasites. A detailed description of this parasite is given later.

4.2.2. Wales

All of the blood smears examined from farmed turbot at this locality failed to show any infection with haematoprotozoan parasites.

4.3. Description of a haematoprotozoan parasite of farmed turbot (*Scophthalmus maximus*)

The earliest stage of this parasite observed was a vermicular intracorpuscular organism parasitizing a *small lymphocyte*. Its average length was 5.5 micron (range 4.5-6.6) and its width was 1.4 micron (range 1.3-1.5). The nucleus stained a pale pink with Giemsa

FIGURES 1 - 19. HAEMOGREGARINA SACHAI N.SP.

in blood smears

1. Sporozoite entering a neutrophil (N). Also present are erythrocytes (E), one erythroblast (Eb) and a "lone nucleus" type thrombocyte (Th). x 1,600

2. Merozoite entering or leaving monocyte (M) which already contains two merozoites. Also a neutrophil parasitized by two merozoites. x 1,600

3. Large lymphocyte (LL) with one merozoite; two monocytes and two neutrophils with two merozoites each and a free micro-gametocyte. x 1,250

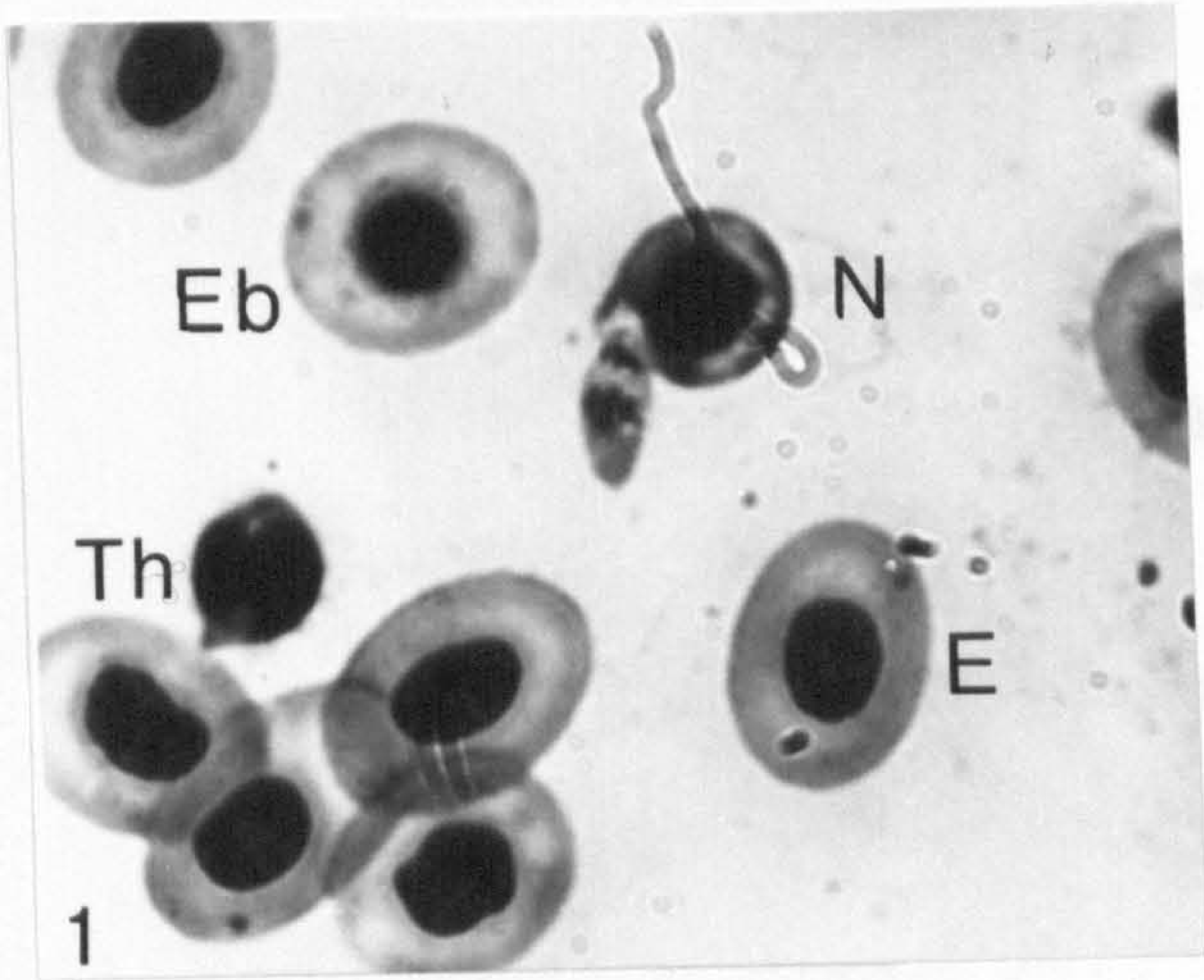
4. Schizont with 10 nuclei in monocyte. x 1,500

5. Schizont with 36 nuclei, some of these undergoing further division. Host cell nucleus (HCN) laterally compressed. x 2,500

6. Two schizonts, one with 30 nuclei, the other with 32 nuclei. x 1,600

7. Two merozoites,each penetrating into an erythrocyte. Also intracellular merozoite in small lymphocyte (SL). x 1,600

8. Merozoite entering an erythroblast. Intracellular merozoites in a neutrophil and an erythrocyte. Note "oval" type thrombocyte (Th). x 1,600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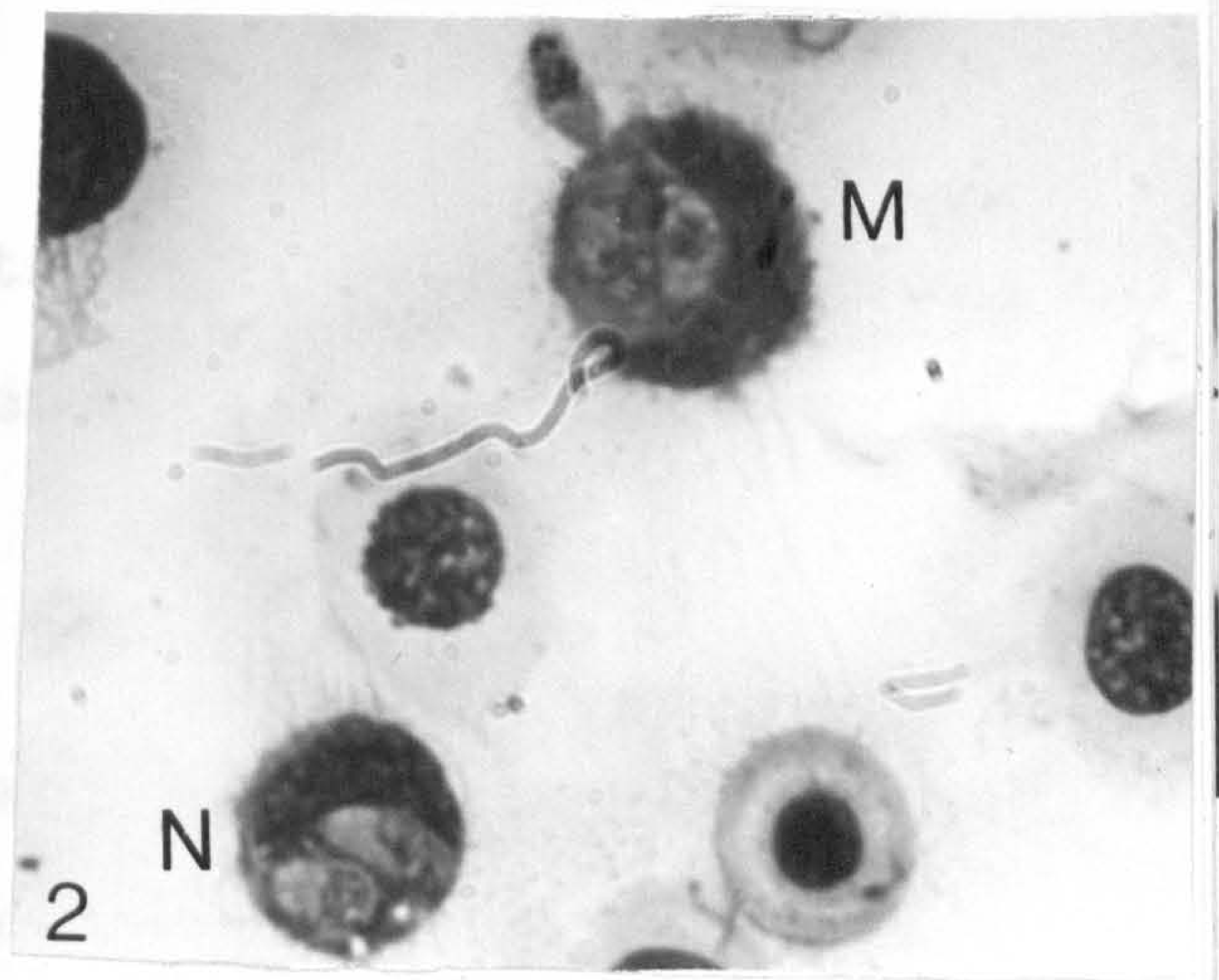
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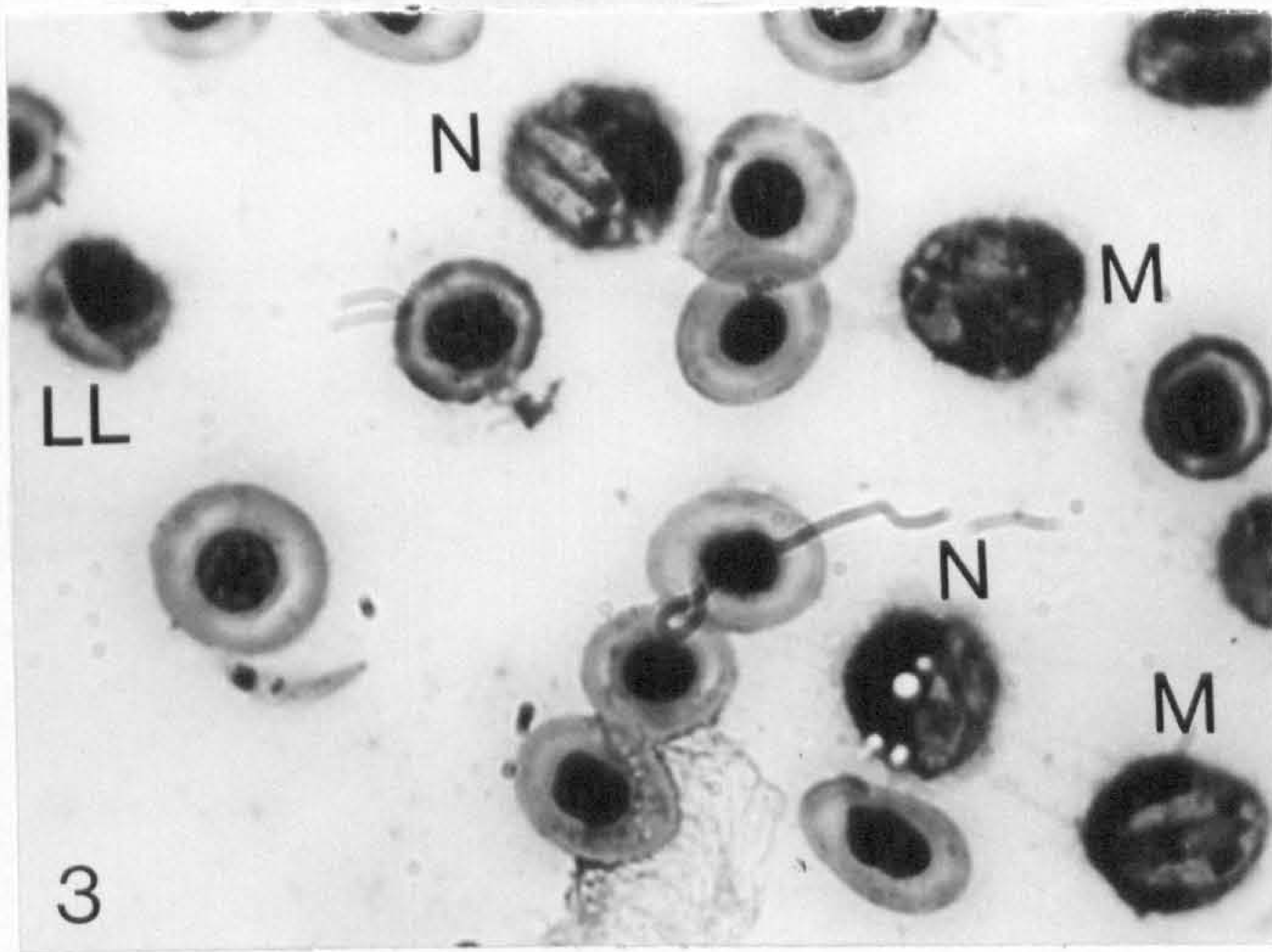
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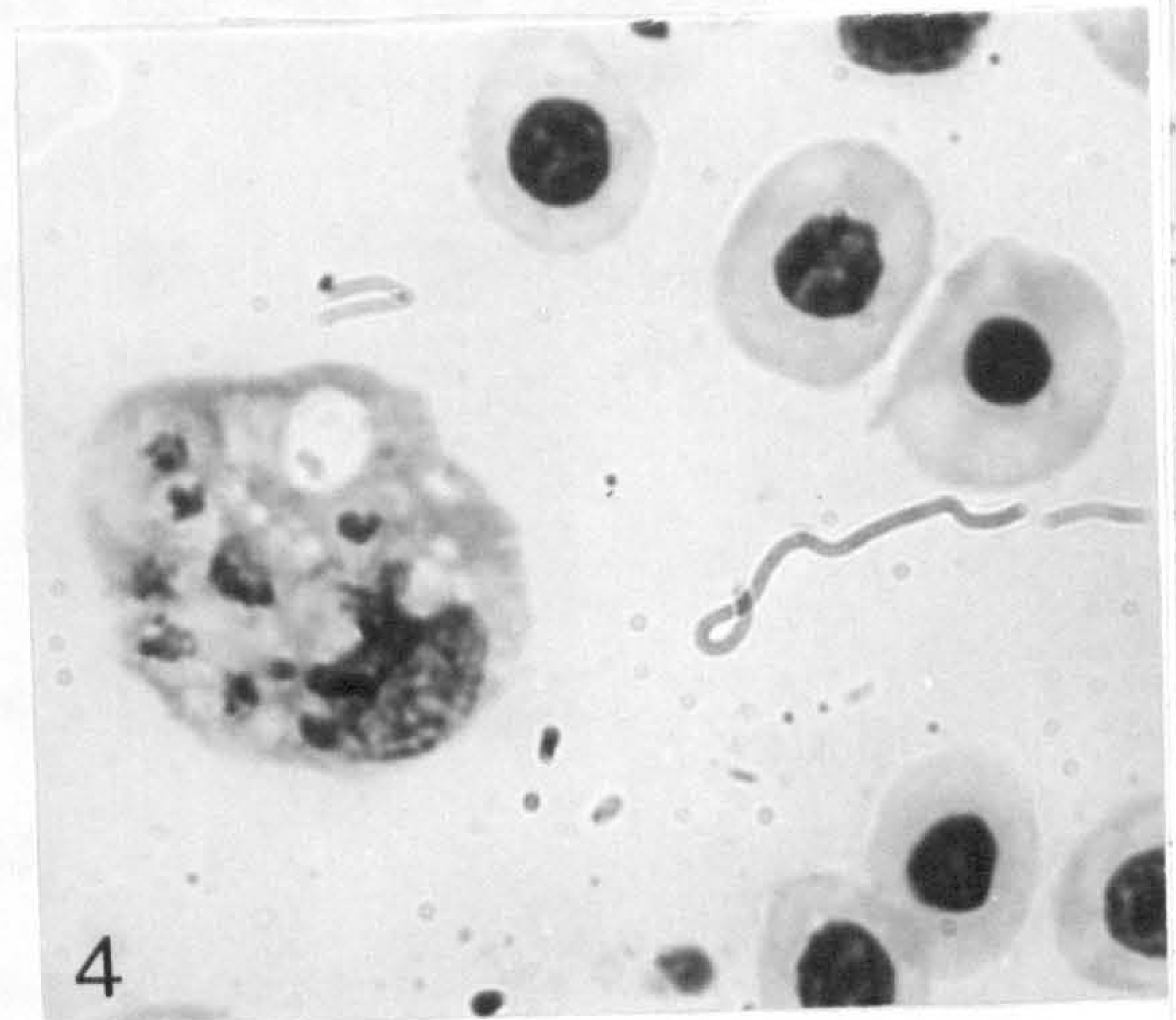
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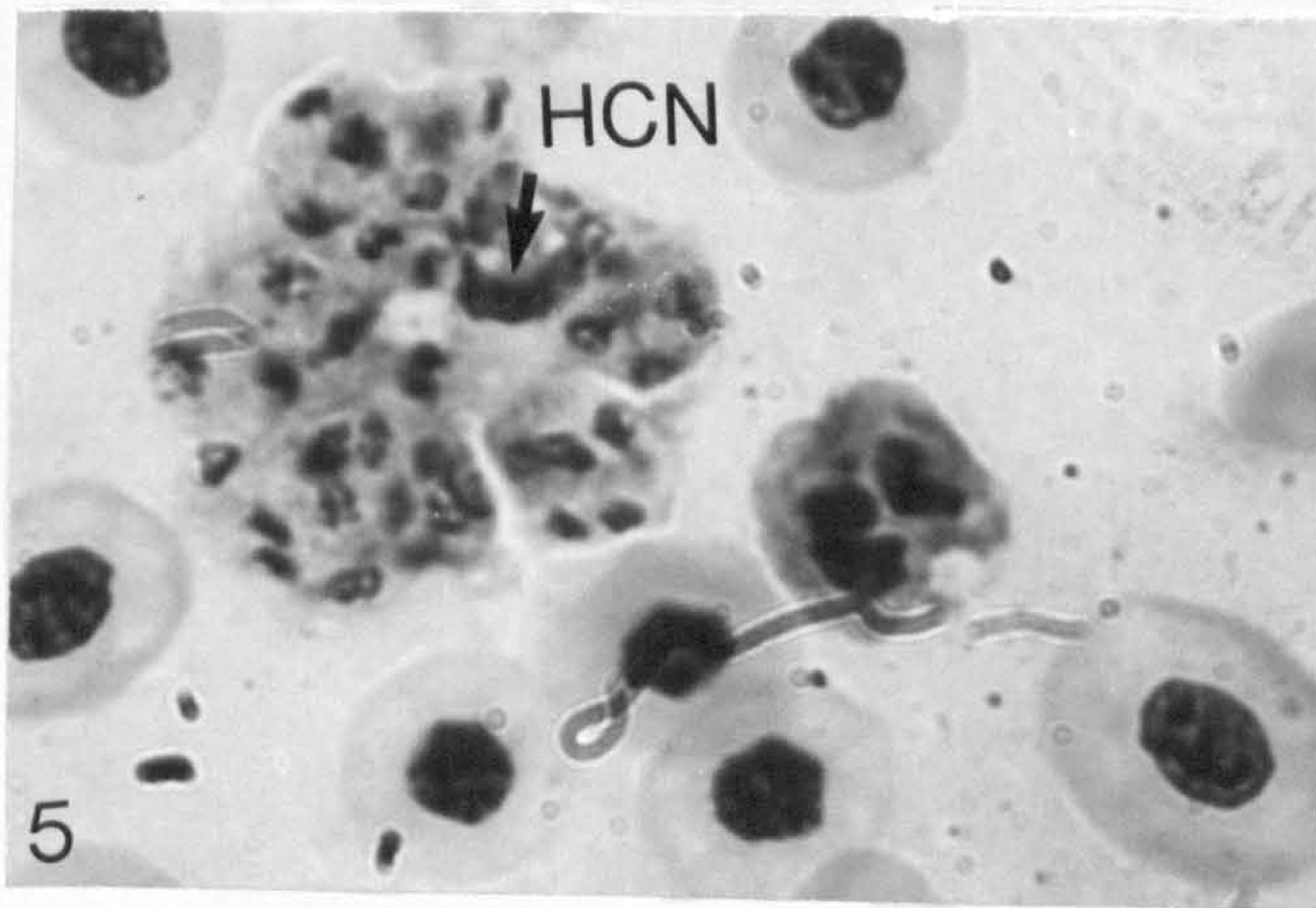
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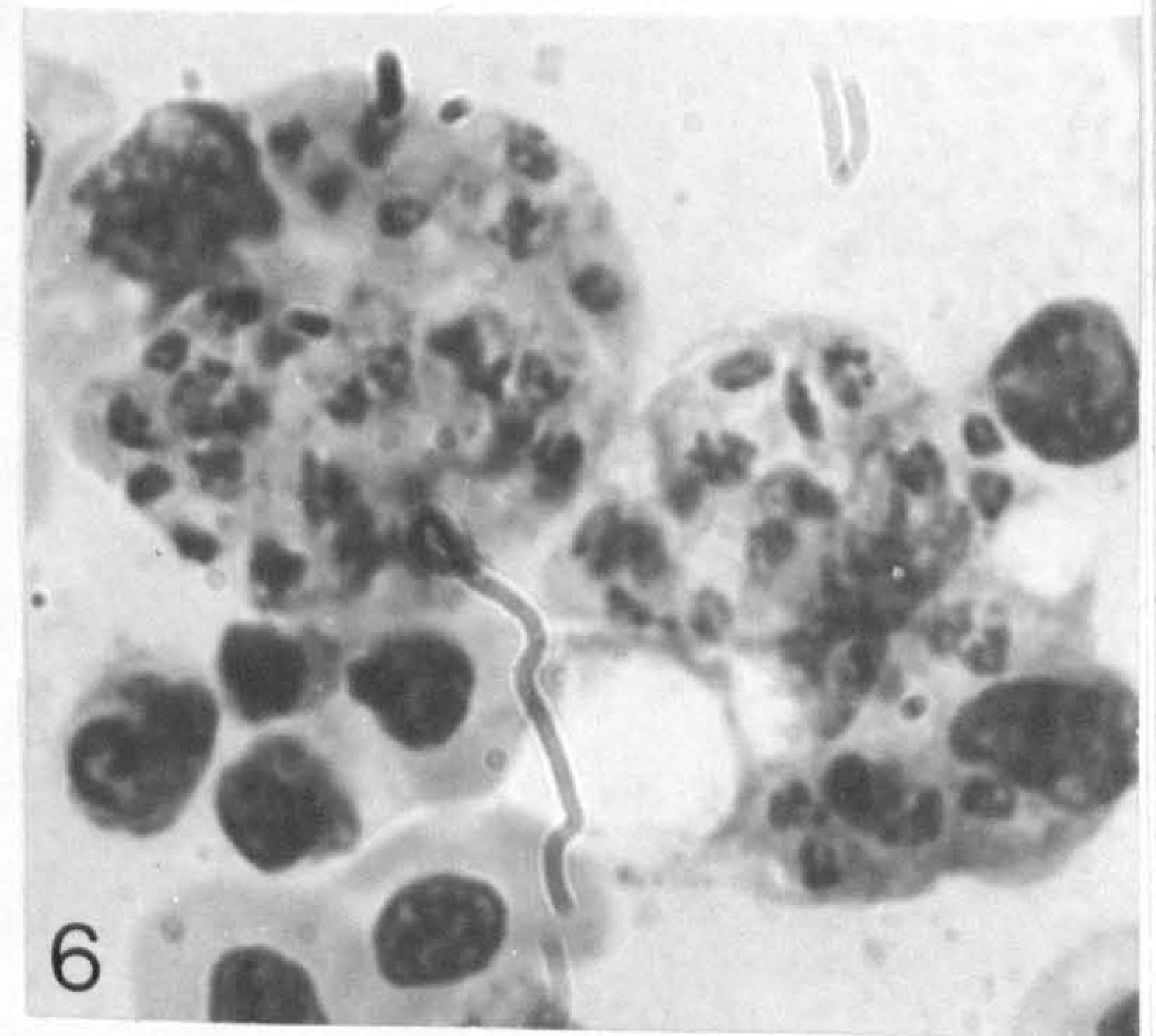
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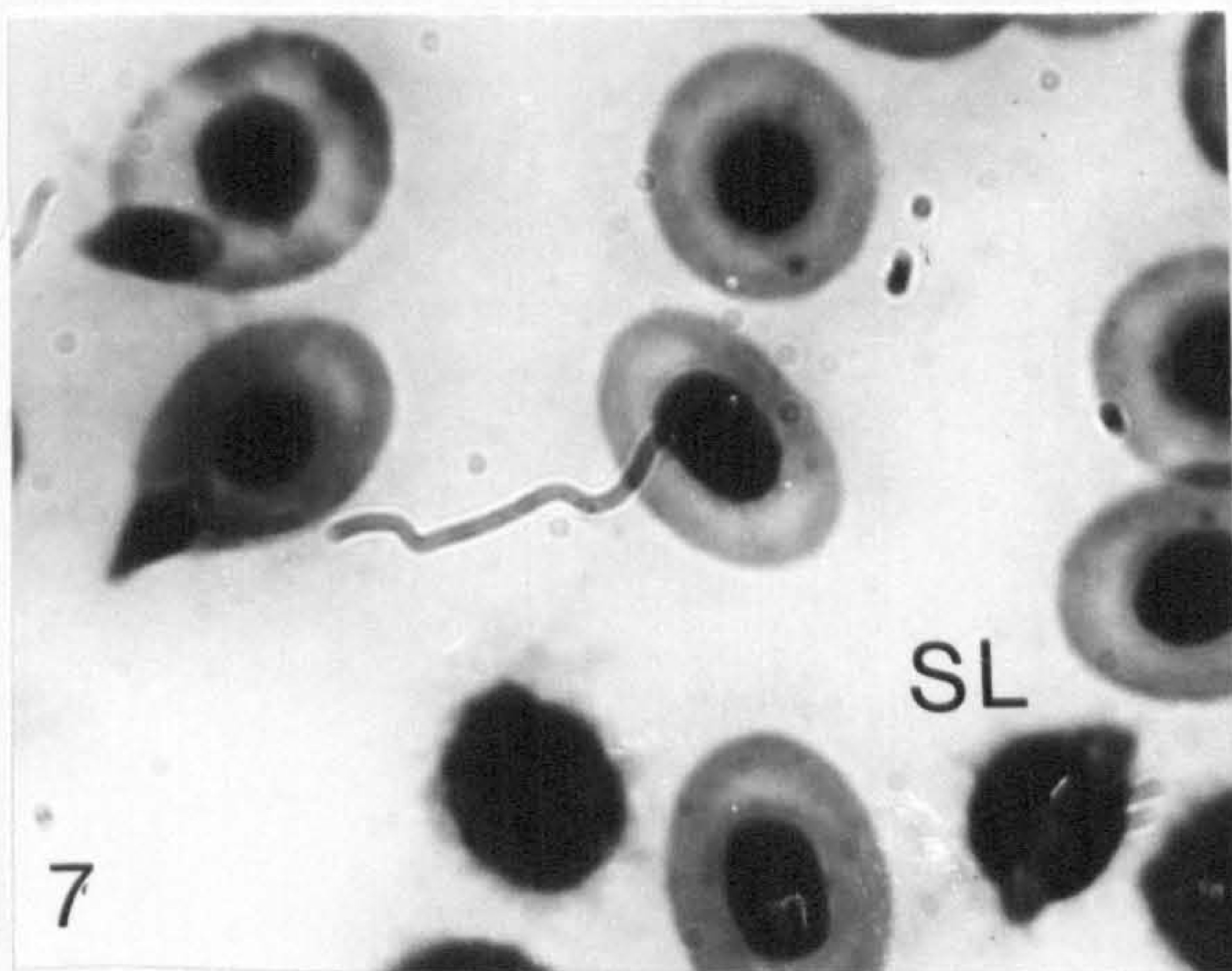
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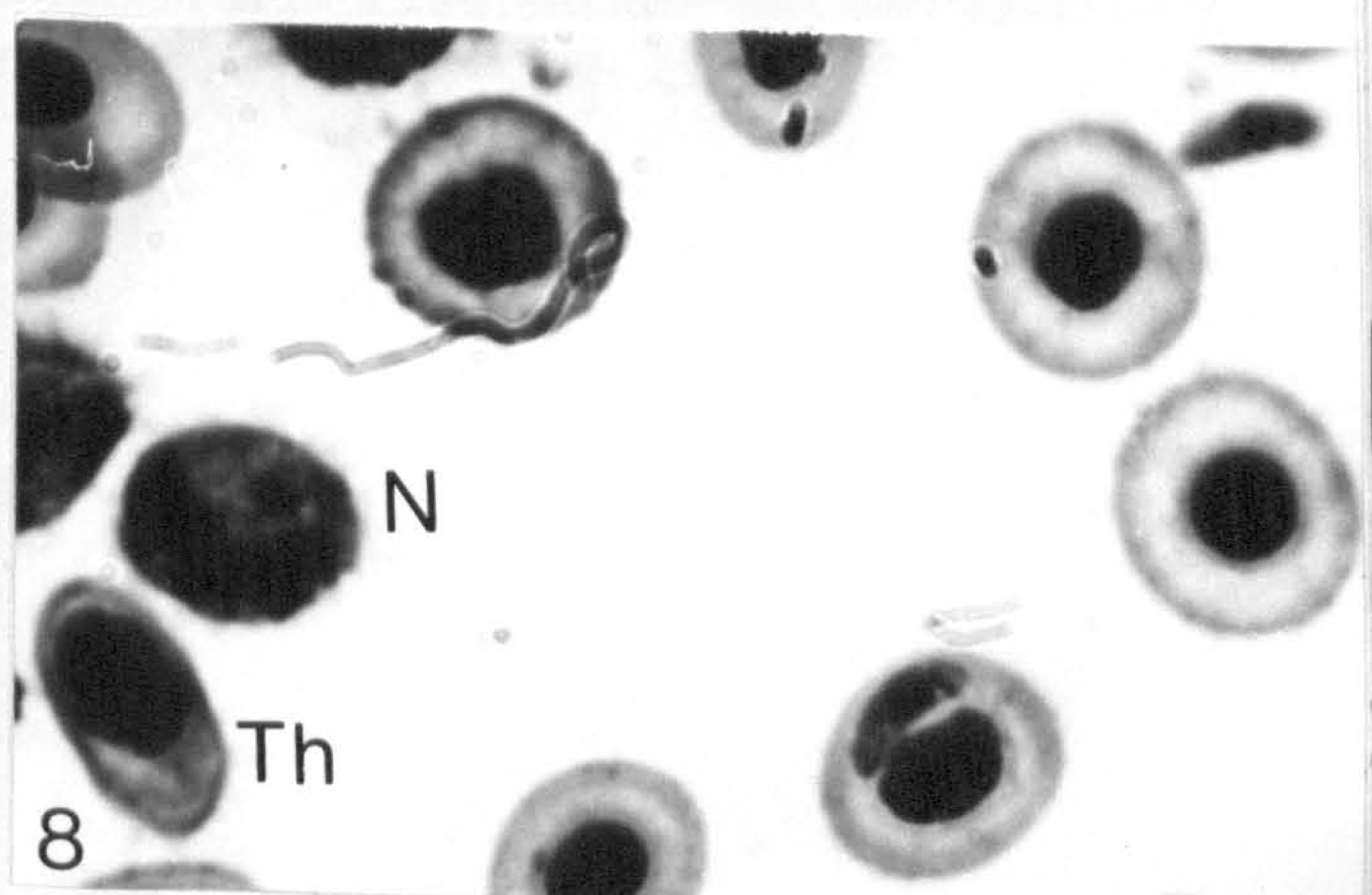
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and was hardly visible in its central location. More prominent was a dark purple staining mass of 1.3 by 1.5 micron at one of the poles. In most of the cases a single parasite had penetrated the small lymphocyte in such a way that the host cell nucleus was indented (Fig.1,7). Also since the normal small lymphocyte measures from 3.6-3.9 micron in length and 3.3-3.9 micron in width it was apparent that the intruding parasite was too long. It therefore expanded the cytoplasmic membrane at both poles so that it appeared in the smear that the imbricate parasite was overlapping the host cell nucleus at both ends (Fig.7). These infected small lymphocytes harbouring a single organism measured on average 5.2 by 5.3 micron. Up to 4 parasites were found on occasion and such host cells measured 5.8 by 5.2 micron in the peripheral blood and 5.8 by 5.5 micron in the lymph. These first developmental stages of the parasite were considered to be early merozoites (the parasite.in Fig.1 might have been even a sporozoite) beginning a first schizogonic cycle in leucocytes. After intracellular multiplication and rupture of the host cell these merozoites apparently invade other leucocytes as well (Fig.2). The organisms measured from 5.2-6.5 micron in length (average 5.7) and from 2.6-2.8 micron in width (average 2.7). The centrally located nucleus stained quite distinctly and

9. Merozoite penetrating into erythrocyte. "Banded" neutrophil containing two merozoites. x 2,750

10. Merozoite inside an erythrocyte. x 2,750

11. Two merozoites penetrating two erythrocytes. One intracellular merozoite in large lymphocyte. x 1,600

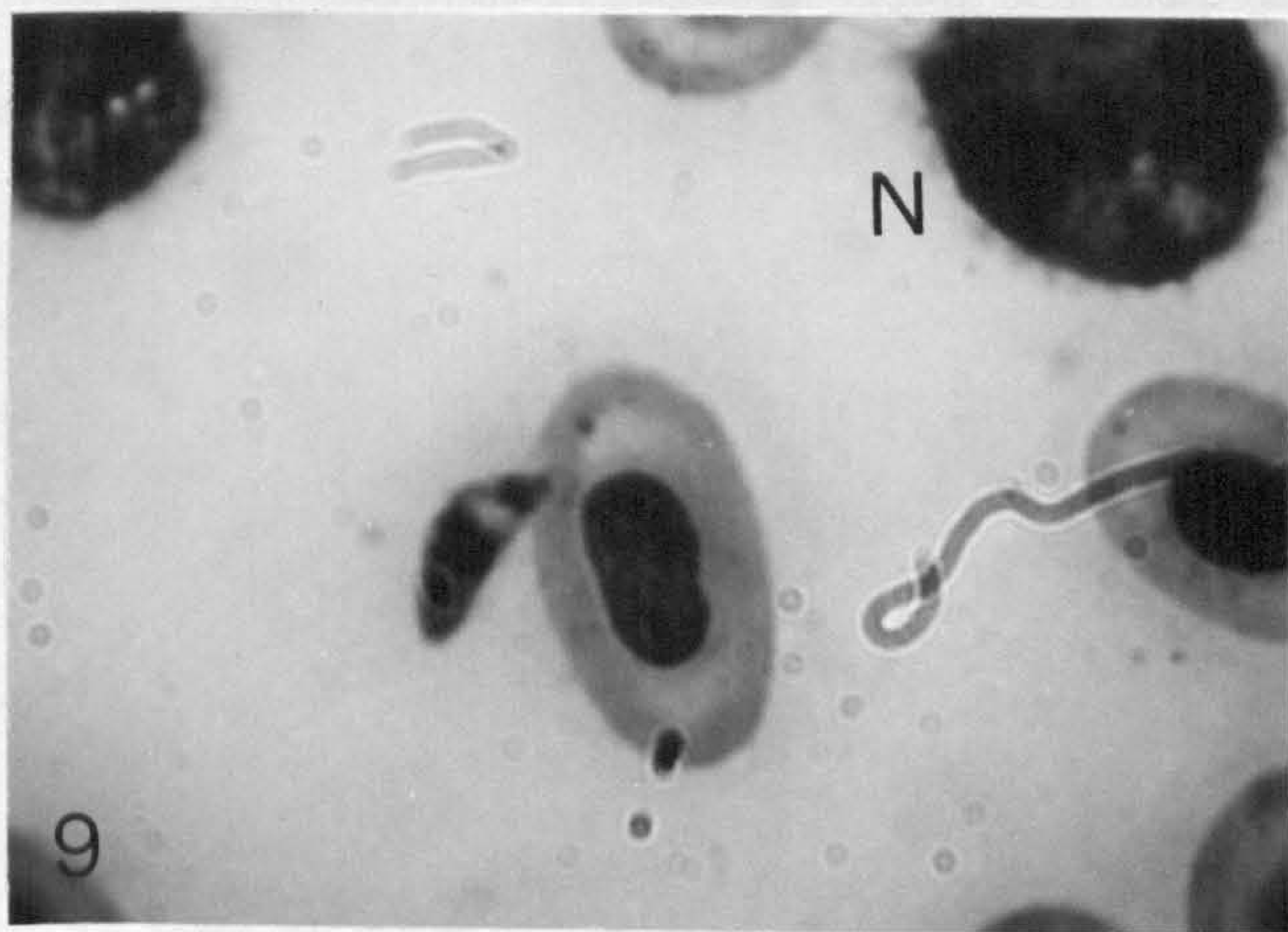
12. Merozoite penetrating an erythrocyte. Also present a large lymphocyte. x 2,000

13. Merozoite entering erythroblast. One intra-erythrocytic merozoite. x 2,500

14. Intraeryhtrocytic merozoite. x 3,200

15. Pair of micro-gametocytes leaving an erythrocyte. Also two merozoites inside a monocyte. x 1,600

16. Pair of free micro-gametocytes and pair of free macro-gametocytes (ma). Also small lymphocyte. x 2,000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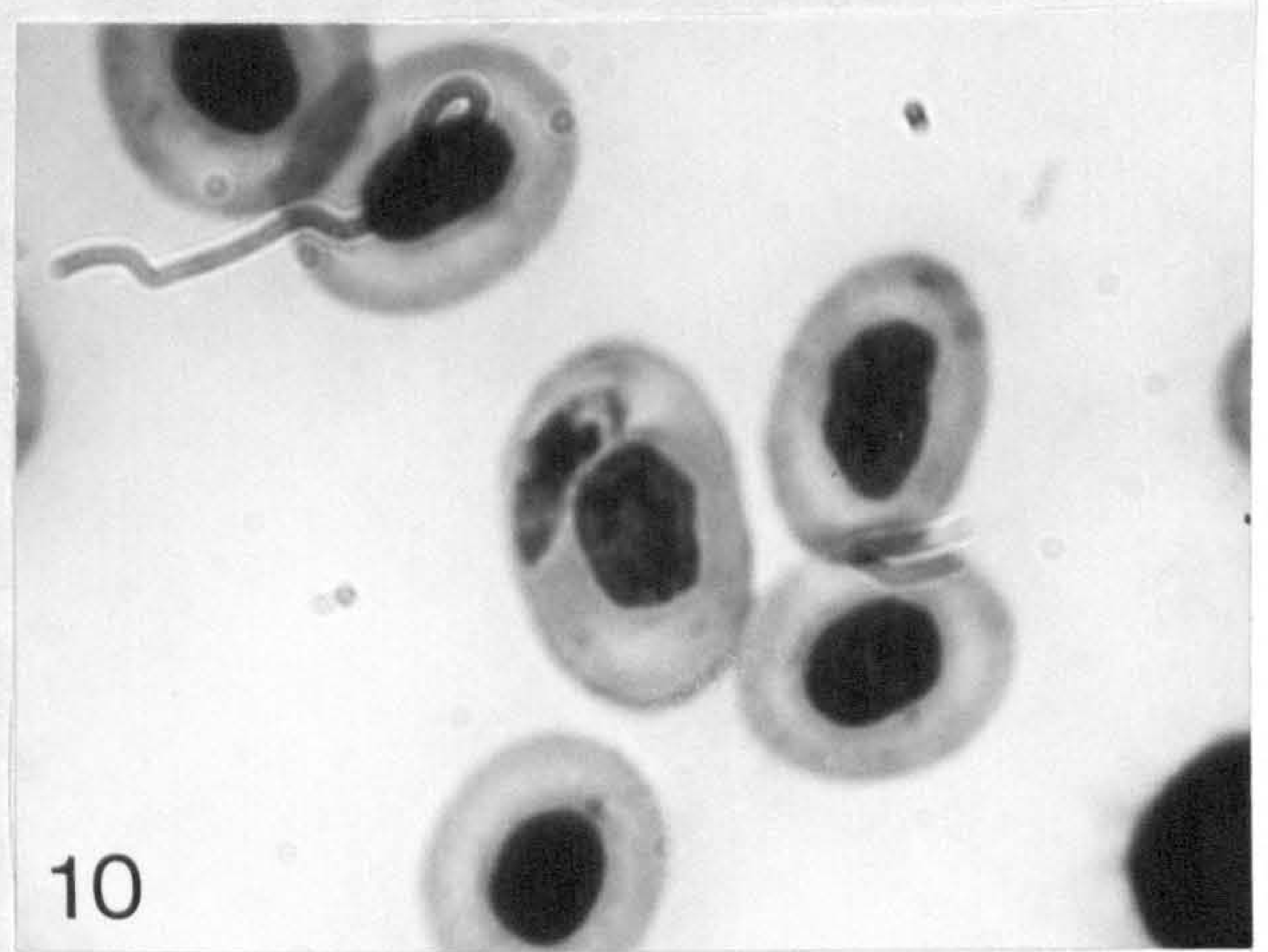
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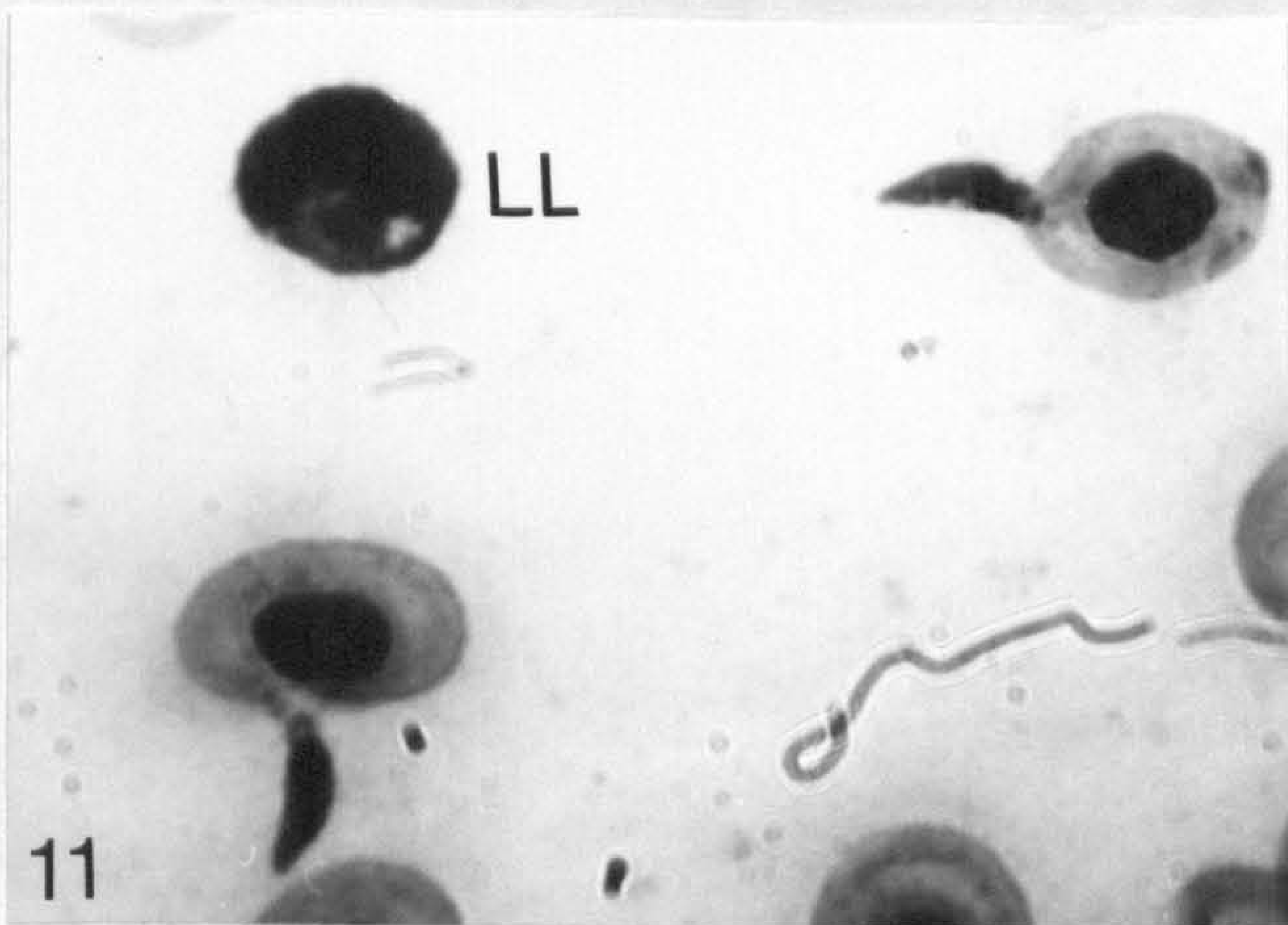
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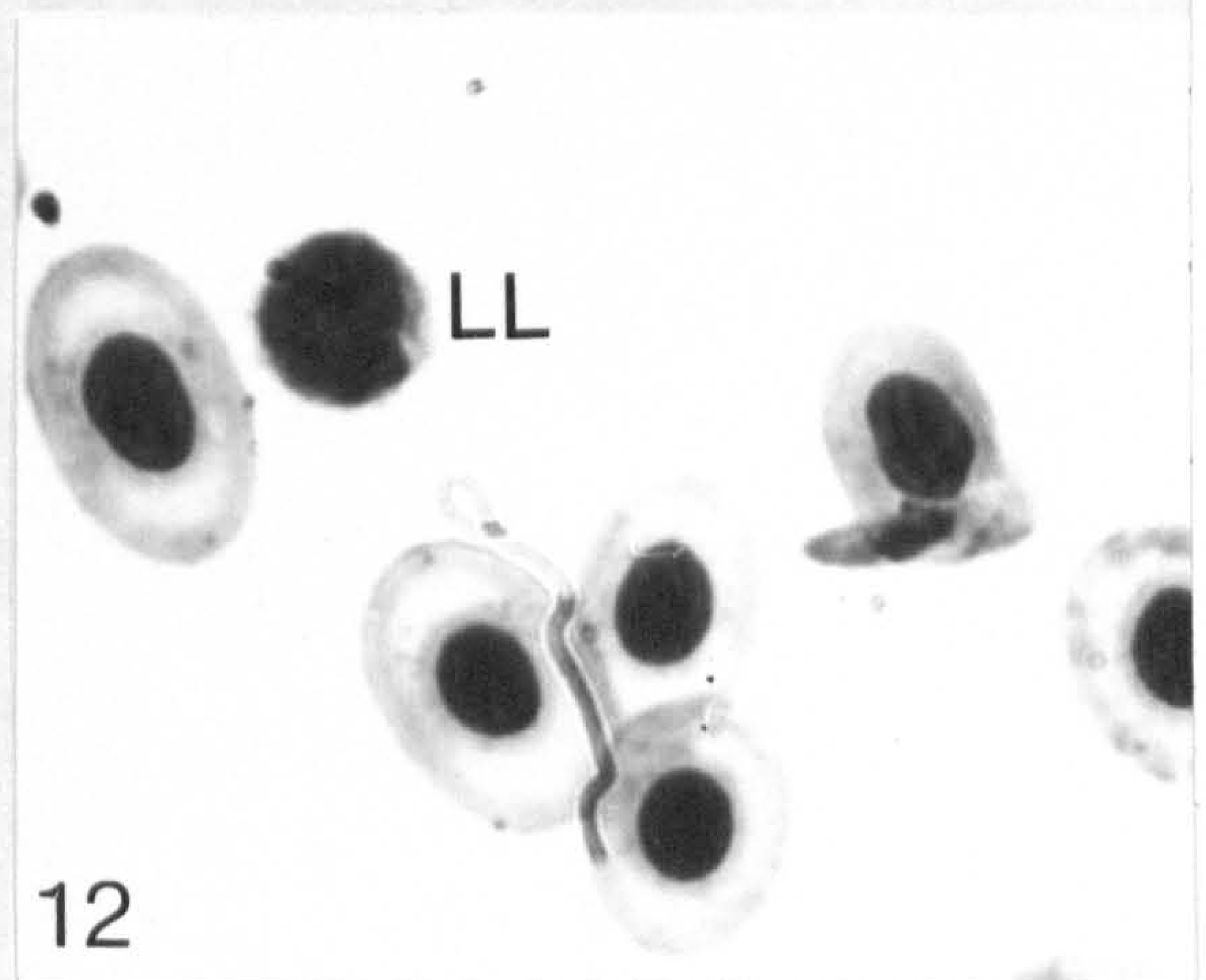
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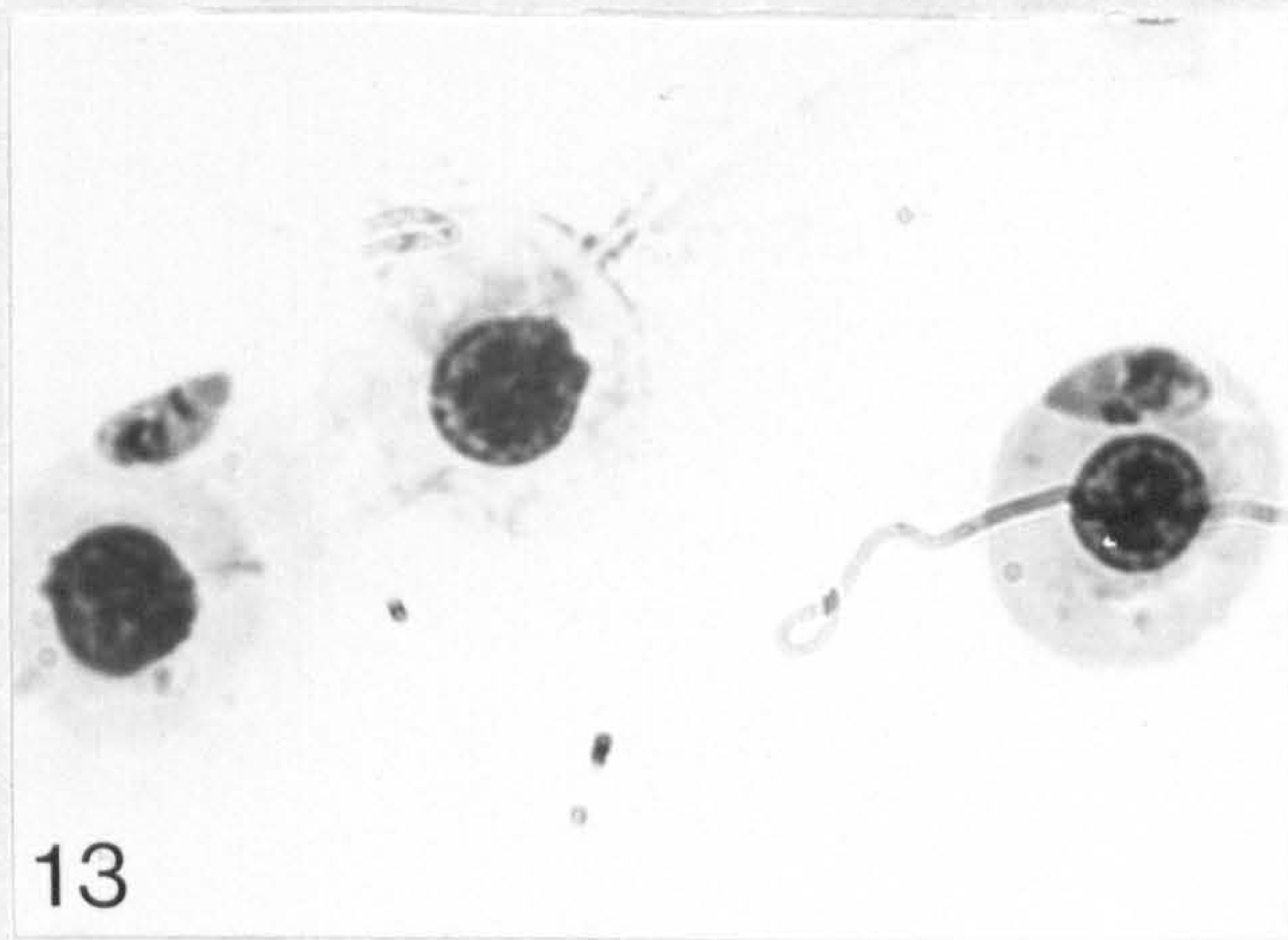
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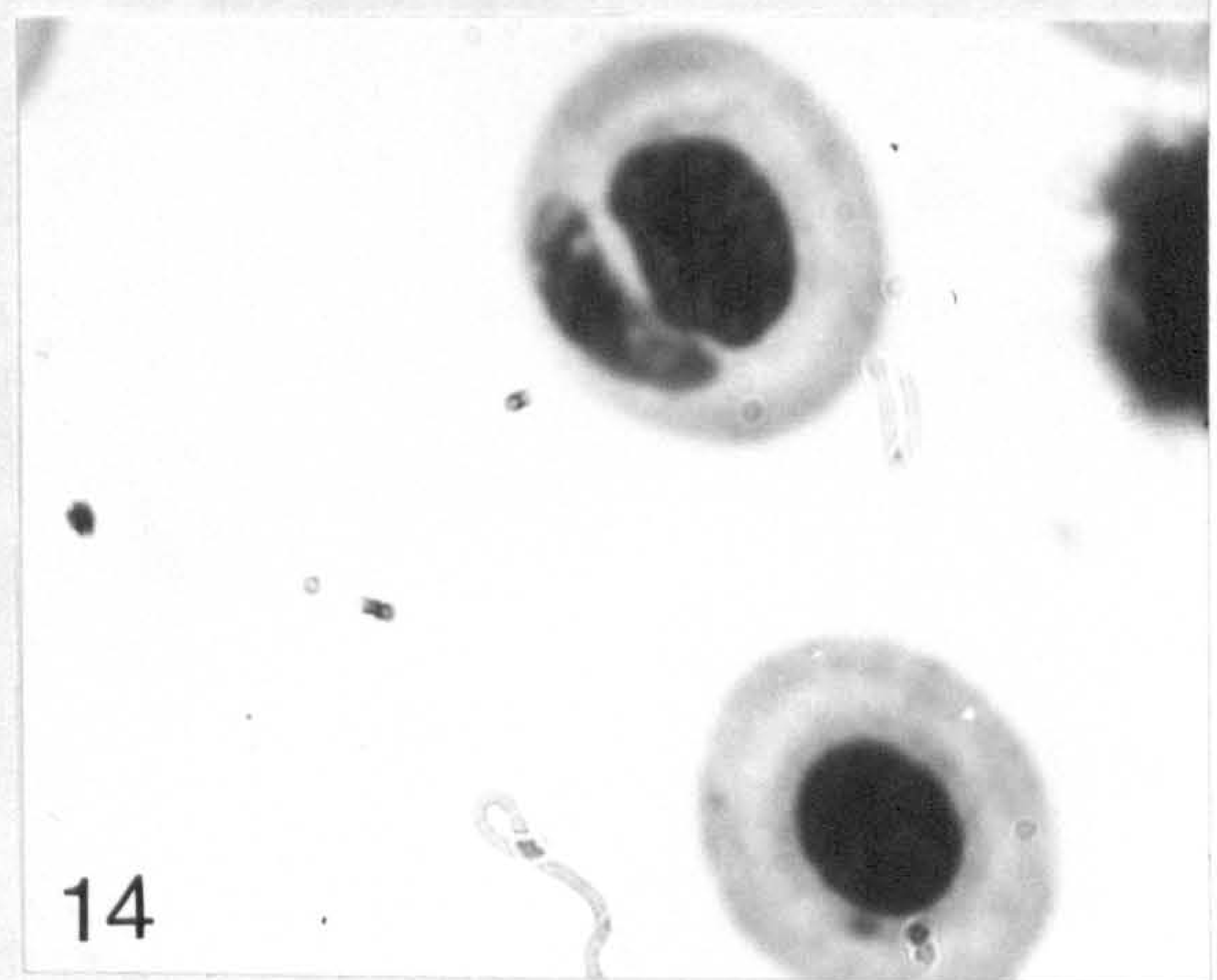
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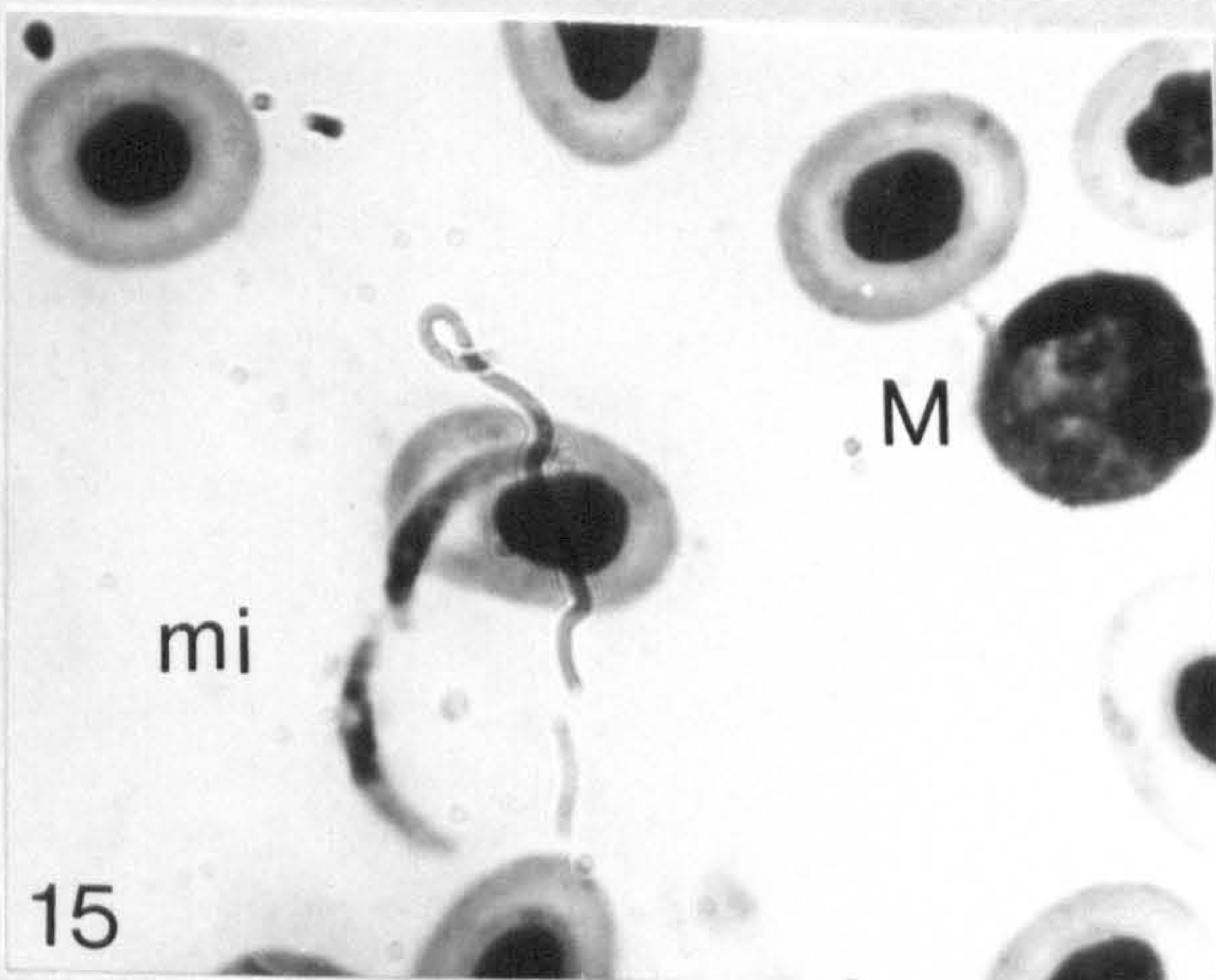
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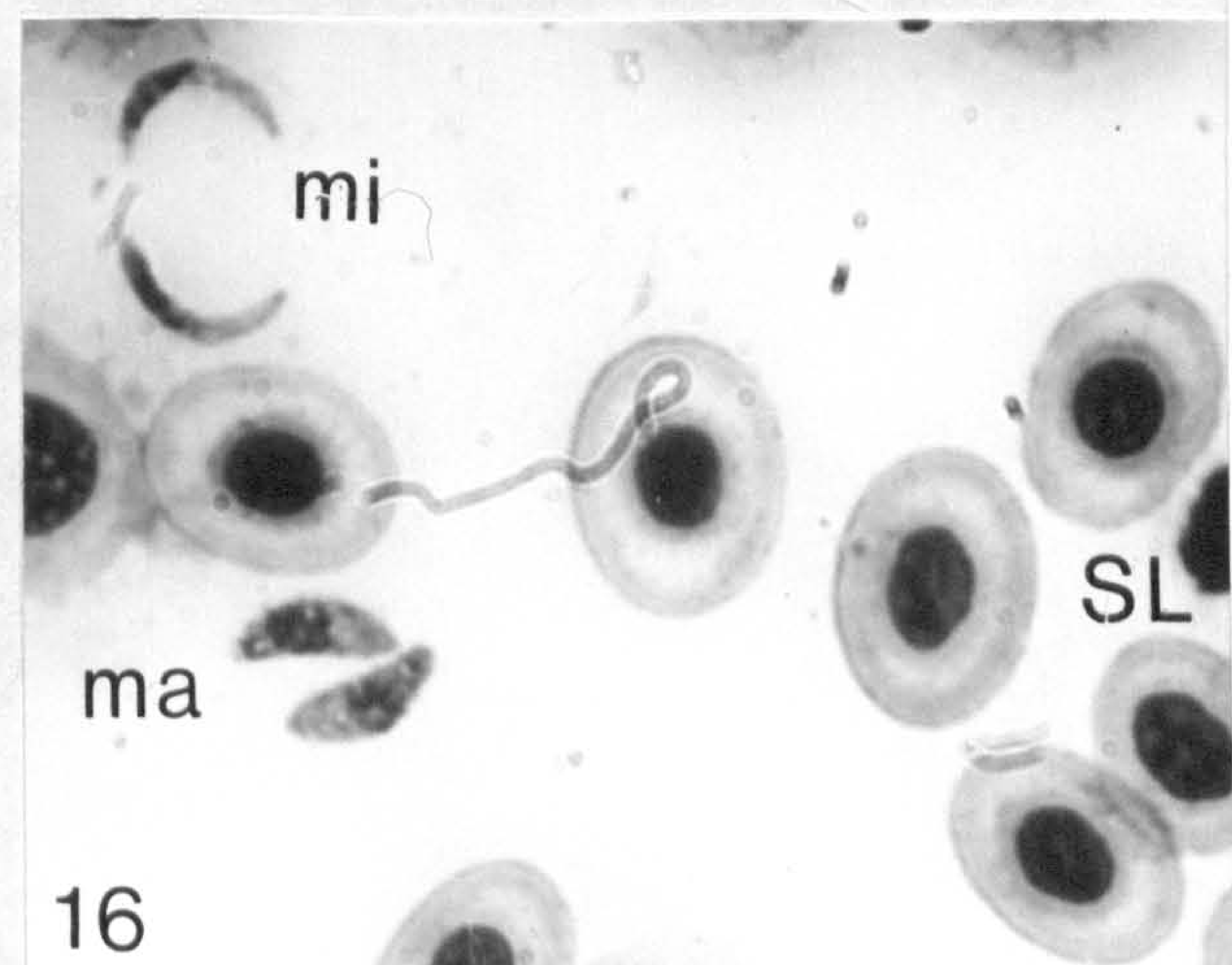
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occupied the whole width of the parasite. It measured an average of 2.6 by 1.7 micron. In large lymphocytes up to four parasites were encountered, the infected cells measuring from 6.5-8.4 micron in length (average 7.2) and 6.5-7.8 micron in width (average 7.0) when harbouring a single organism of 6.5 micron length, 2.0 micron width and a centrally located nucleus of 1.9 by 1.5 micron. Large lymphocytes with multiple infections measured an average of 7.9 by 6.6 micron in contrast to their normal cell size of 5.9-6.5 micron in length (average 6.1) and 5.2-6.2 micron in width (average 5.5). Large lymphocytes with one merozoite are seen in Fig.3 and 11. In neutrophils a differentiation of parasites was seen. Cells were found either parasitized by ovoid or rounded forms of 5.4 by 3.2 micron and a nucleus in central location of 1.9 by 1.5 micron (Fig.2). or they had been invaded by the more elongate form such as was found in small lymphocytes. This stage had a length of 7.8 micron and a width of 2.3 micron, the nucleus occupying the anterior part of the parasite (Fig.3) and measuring 1.4 by 1.3 micron. Neutrophils infected with a single merozoite measured from 9.1-10.4 micron (average 10.0) in length and 9.1-9.7 micron in width (average 9.2). Two or more parasites were seen less frequently but when these did ocurr the host cells had increased even further measuring now from 10.4-11.7 in

length (average 11.5) and 9.1-11.0 micron in width (average 10.1). In comparison normal neutrophils measured from 8.6-9.5 micron in length (average 9.1) and 7.8-8.5 micron in width (average 8.2). Their nucleus was spherical and compact and measured 6.5 by 4.6 micron. In infected cells there was no apparent difference in nuclear size. The round type nucleus measured 6.5-by 5.2 micron and the banded type nucleus 9.75 by 3.9 micron. In the monocytes the first stage observed was a single ovoid parasite in the cytoplasm of the host cell. It measured 5.2 by 3.2 micron. The nucleus covered almost the total width of the parasite and division appeared to take place quite commonly. This nucleus measured 2.8 by 2.4 micron. The host cell dimensions were 14.3 by 13 micron. Also more slender forms of the parasite were found at initial invasion of the monocytes. Such a single organism measured 6.2 by 4.5 micron, the compact and centrally located nucleus 1.5 by 1.3 micron (Fig.3). In the monocyte a further multiplication by binary fission seemed to take place, whereupon the size of the intra-leucocytic merozoites increased. Slender and banana-shaped organisms measured 8.1 micron in length (range 7.5-8.8) and 1.3 micron in width. Oval-shaped and broad organisms measured 6.0 micron in length (range 5.0-7.5) and 2.6 micron in width (range 2.5-3.2). Their rounded nucleus measured 2.3 micron in diameter. From 2 to 5 merozoites were

usually encountered but intracellular schizonts with up to 36 nuclei were also observed (Fig.4,5,6,19). Infected monocytes harbouring up to 5 merozoites measured an average of 12.4 micron in length (range 11.0-14.3) and 11.5 micron in width (range 9.8-13.1). In contrast the normal monocytes had dimensions of 9.1-10.4 micron in length (average 9.9) and 9.1-9.75 micron in width (average 9.3). The large granular and lobulated nucleus measured from 7.8-9.1 micron in length (average 8.4) and from 6.2-6.5 micron in width (average 6.3). The exact type of host cell for the larger schizonts could not be determined because the nucleus was either greatly hypertrophied and distorted or pushed aside and flattened and often had completely disintegrated. Multinucleated schizonts in the peripheral blood measured from 16.9-20.8 micron in length (average 18.8) and 13.0-14.3 micron in width (average 13.6). Schizonts found in lymph smears measured 13.0-26.0 micron in length (average 18.1) and 12.4-23.4 micron in width (average 17.3). It is assumed that most of the larger schizonts are derived from enlarged monocytes. Large schizonts had a tendancy to rupture readily and free vermicular stages were released into the peripheral circulation which differentiated again into more slender and slightly curved organisms of 6.5 by 1.3 micron and into smaller and broad organisms of 4.8 by 1.9 micron.

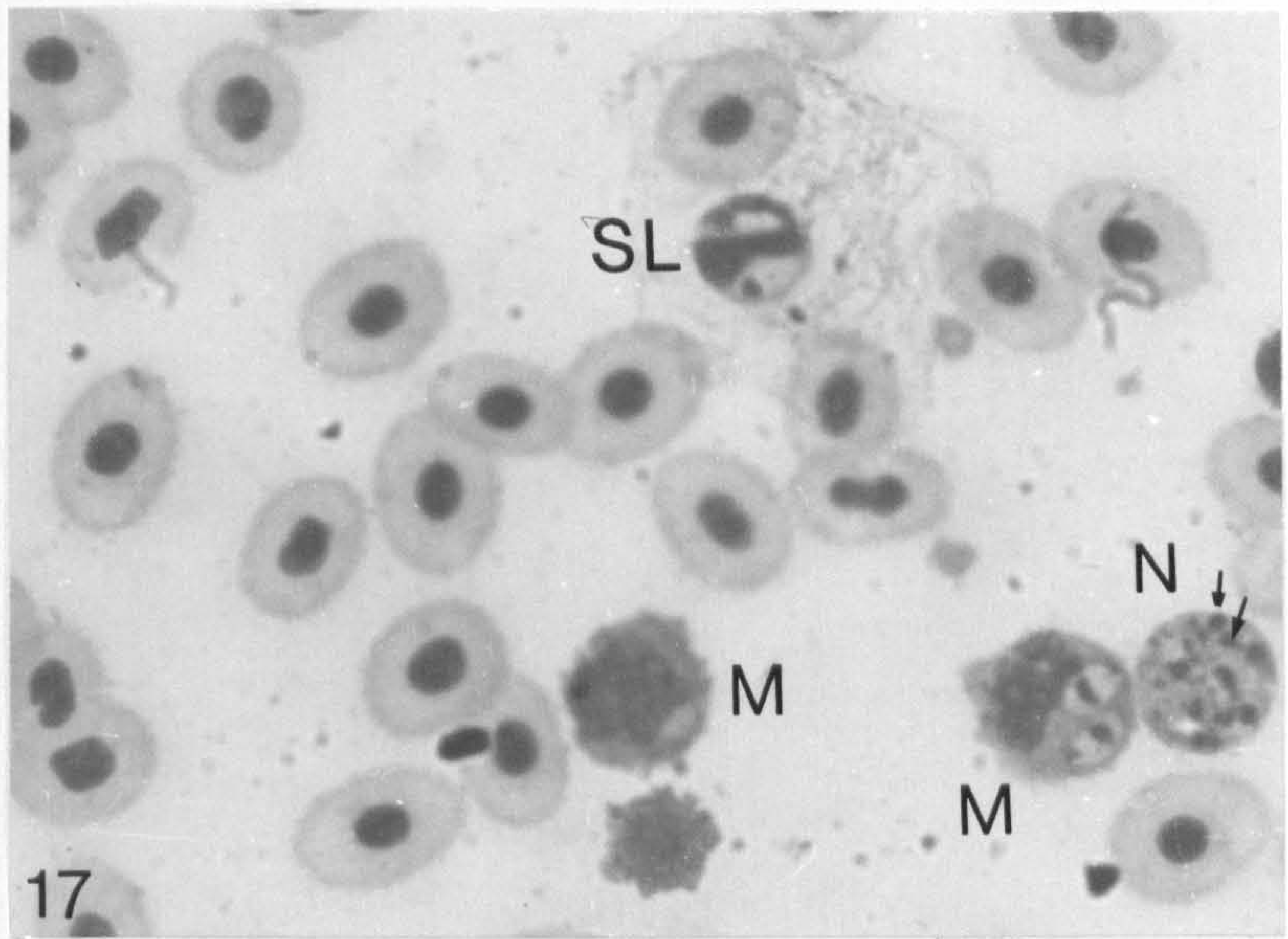
Located at the anterior pole of the slender type parasite was a distinct organelle, staining a deep purple with Giemsa and taking the shape of an arrow-head with a distinct apical spike. This was considered a polar cap modified to aid penetration of the cytoplasmic membrane of blood cells. This polar cap measured 1.9 by 1.3 micron (Fig.9). In the broader type parasite a similar structure in the form of a dark staining granule was observed anterior to the centrally located dark red nucleus of 1.6-2.6 micron in length (average 2.1) and 1.4-1.9 micron in width (average 1.6).

These merozoites apparently started a second schizogonic cycle in erythroblasts and erythrocytes. In moribund fishes with high parasitaemias this penetration of erythroblasts (Fig.8,13) and erythrocytes (Fig.7,9, 11,12) in addition to leucocytes was more frequently observed. Once the parasite was inside such an erythroblast it increased slightly in size and measured now 5.4-6.7 micron in length (average 6.2) and 1.4-2.0 micron in width (average 1.6). Its compact nucleus was centrally located, dark red staining and measured 1.9 by 1.4 micron. At this stage the erythroblast seemed slightly enlarged measuring 8.4-10.4 micron in length (average 9.0) and 7.1-9.7 micron in width (average 8.7) as compared with the size of the normal erythroblast (6.5-7.8 micron

17. Two merozoites inside small lymphocyte compressing host cell nucleus bi-laterally. Monocyte with two merozoites. Degenerating neutrophil with intracellular merozoites and nuclear fragments (arrow). x 1,200

18. Neutrophil with four merozoites. Neutrophil with 3-4 merozoites intracellular and fragmentation of host cell nucleus (arrow). Large lymphocyte with merozoites. Two free merozoites. x 1,200

19. Neutrophil with four merozoites. Three schizonts with up to 28 nuclei. Monocyte with two merozoites. Neutrophil with two merozoites. x 400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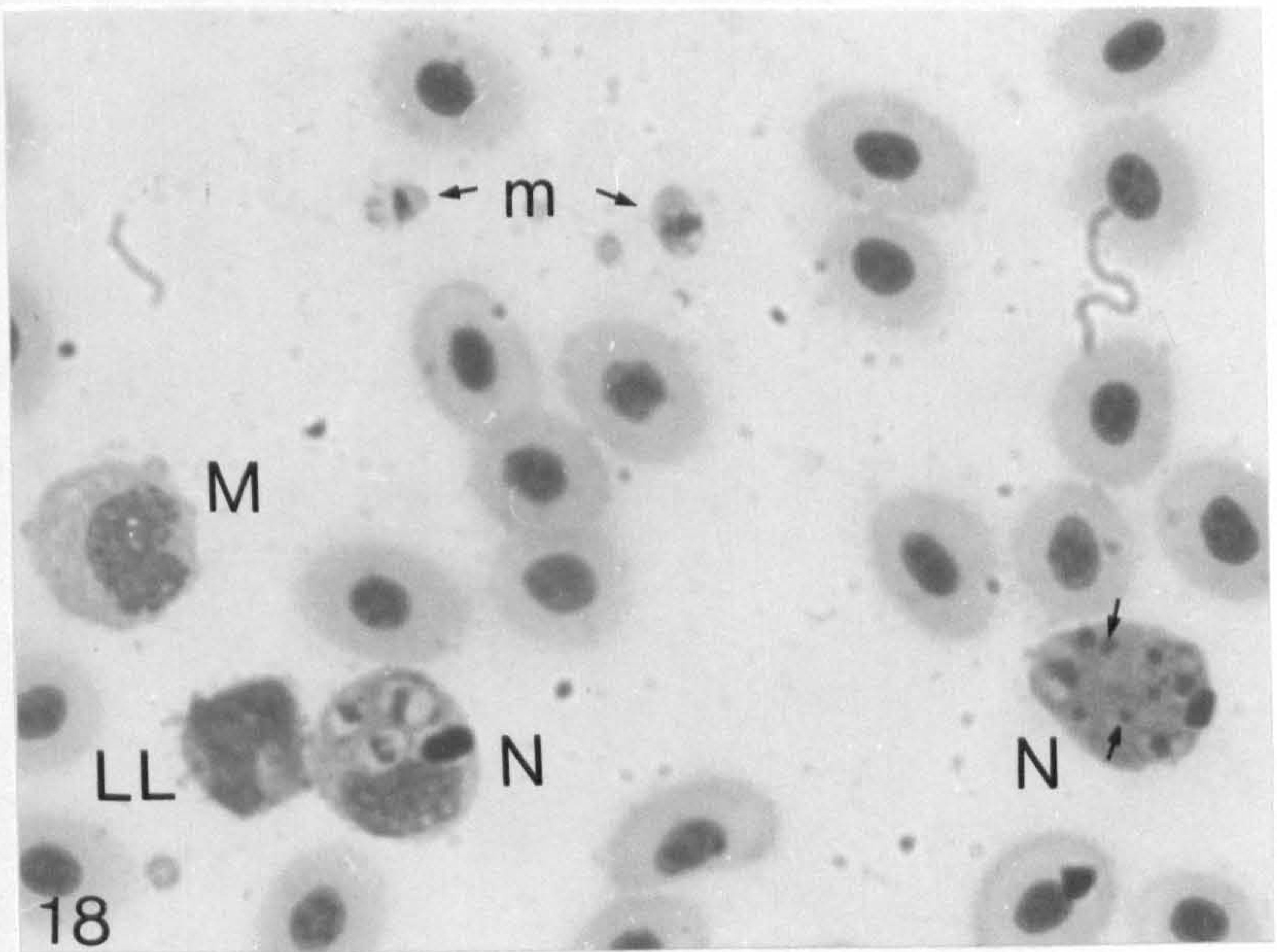
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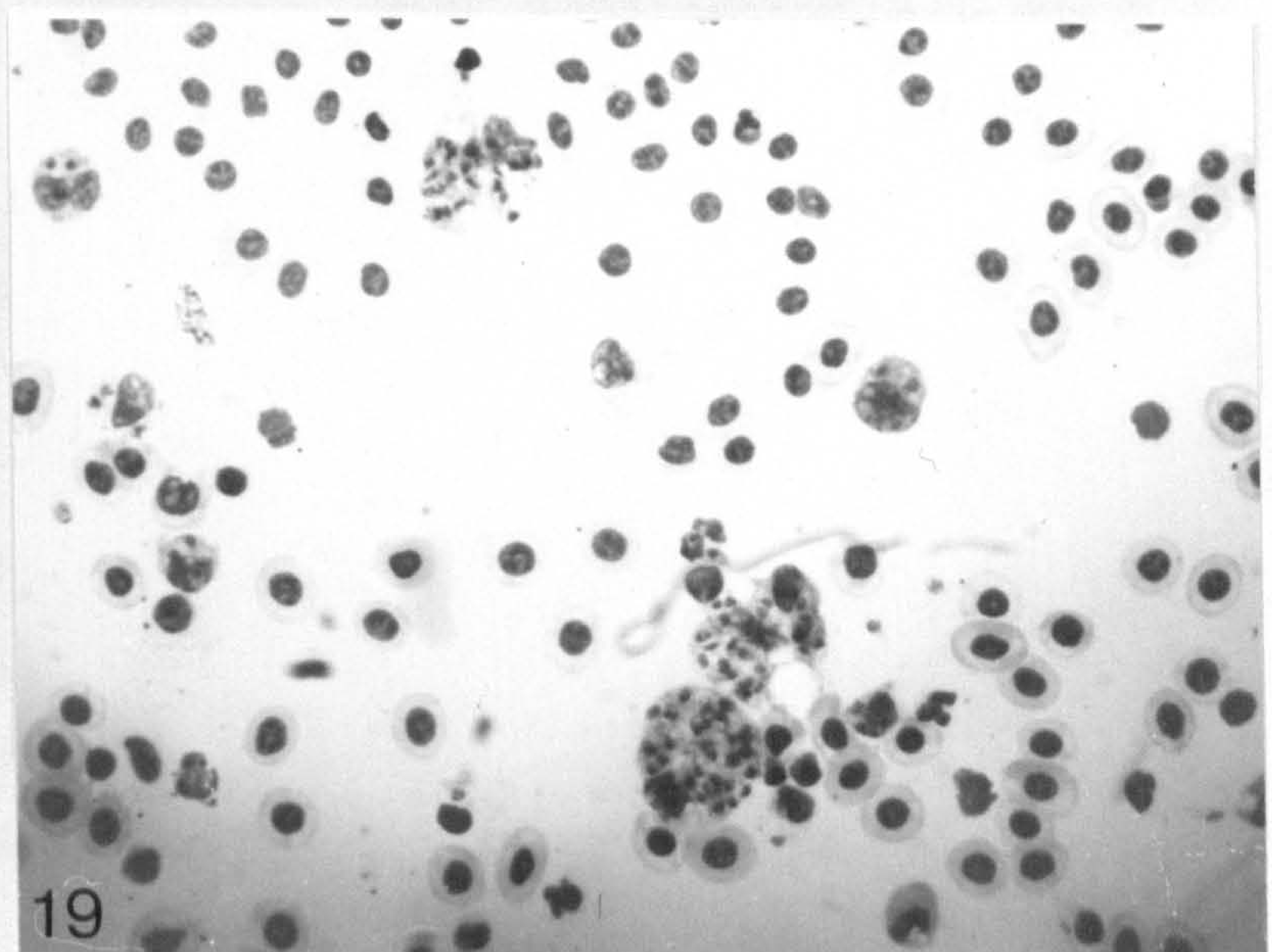
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length (average 7.3) and 6.5-7.8 micron width (average 7.3)). The host cell nucleus appeared unaffected by the parasite and measured 3.9 by 3.5 micron. Infected erythrocytes (Fig.10,14) appeared not to be enlarged by the parasite and retained their normal size of 11.7 by 6.5 micron average. No further divisions of the intraerythrocytic merozoites were observed and it is possible that the development into a single gametocyte takes place without further division. However, it is more likely that gametocytes develop in pairs from the one intraerythrocytic merozoite after a single division has taken place. This can be seen from Fig.15, where a pair of slender gametocytes appears in the process of leaving an erythrocyte. In this final phase of development a large number of free parasites were found in pairs in the blood plasma. Because of their morphological differences they were considered to be micro- and macro-gametocytes.

The micro-gametocytes were extremely slender and characterized by a dark staining granule approximately 0.6 micron posteriorly to the nucleus. Parasites which had just liberated themselves from the host cell (Fig.15) measured 6.8-9.3 micron in length (average 8.3) and 1.1-1.4 micron in width (average 1.2). Mature extracellular micro-gametocytes measured from 9.8-13.0 micron in length

(average 10.9) and from 1.0-1.2 micron in width (average 1.1). Their nucleus measured 1.2 by 1.5 micron.

The shorter and more crescent shaped macro-gametocytes (Fig.16) showed a wide range of sizes. They were considerable smaller than the micro-gametocytes and their length ranged from 4.4-6.2 micron (average 5.5) while their width was from 1.5-2.6 micron (average 2.0).

Since the parasite just described has a similar life cycle and morphology to that of Haemogregarina bigemina it was felt justified to designate this haematoprotozoan parasite as belonging to the same genus Haemogregarina.

It is proposed to name this parasite Haemogregarina sachai n.sp., the type host being Scophthalmus maximus and the type location Hunterston, Scotland.

4.4. Haematoprotozoan parasites from Solea solea

4.4.1. Haemogregarina simondi (Laveran and Mesnil,1901)

The earliest phase of development of this haematoprotozoan parasite encountered in the blood of Solea solea was the initial penetration of leucocytes of the peripheral blood by small crescentic organisms. These measured

an average of 12.5 micron in length and 2.25 micron in width with a diffuse granular nucleus of 2.5 by 2.0 micron. Mainly large lymphocytes (Fig.20) or neutrophils (Fig.21) appeared to be invaded by these early merozoites or perhaps sporozoites. An indication that these stages were most likely sporozoites was the presence of a distinct refractile body located in the anterior half of the parasite. Large lymphocytes thus parasitized had a size of 8.1 by 7.2 micron in average and did not differ from the size of normal large lymphocytes (average length 7.8 micron (range 6.5-9.1); average width 7.3 micron (range 6.5-8.5)). However, with the ensueing multiplication of these intraleucocytic parasites,also the host cells enlarged. Infected large lymphocytes or neutrophils (?) with two parasites measured 12.5 by 8.75 micron (Fig.25). Those with four organisms had a size average of 12.1 (range 11.8-12.5) by 9.4(range 8.75-10.0) micron (Fig.29). In contrast the normal neutrophils measured an average of 10.4 (9.1-11.7) micron in length and 9.9 (7.8-11.7) micron in width. Their nucleus had an average size of 6.5 by 5.7 micron. Enlarged leucocytes, probably neutrophils or monocytes, with up to eight nuclei in their cytoplasm were also detected. These intraleucocytic schizonts measured from 13.0-19.5 micron in length (average 15.9) and from 5.2-10.4 micron in width (average 7.5) (Fig.36). The intracellular parasites or merozoites did not appear to increase their length.

FIGURES 20 - 50. HAEMOGREGARINA SIMONDI

in blood smears

20. Sporozoite entering a large lymphocyte.
x 2,500

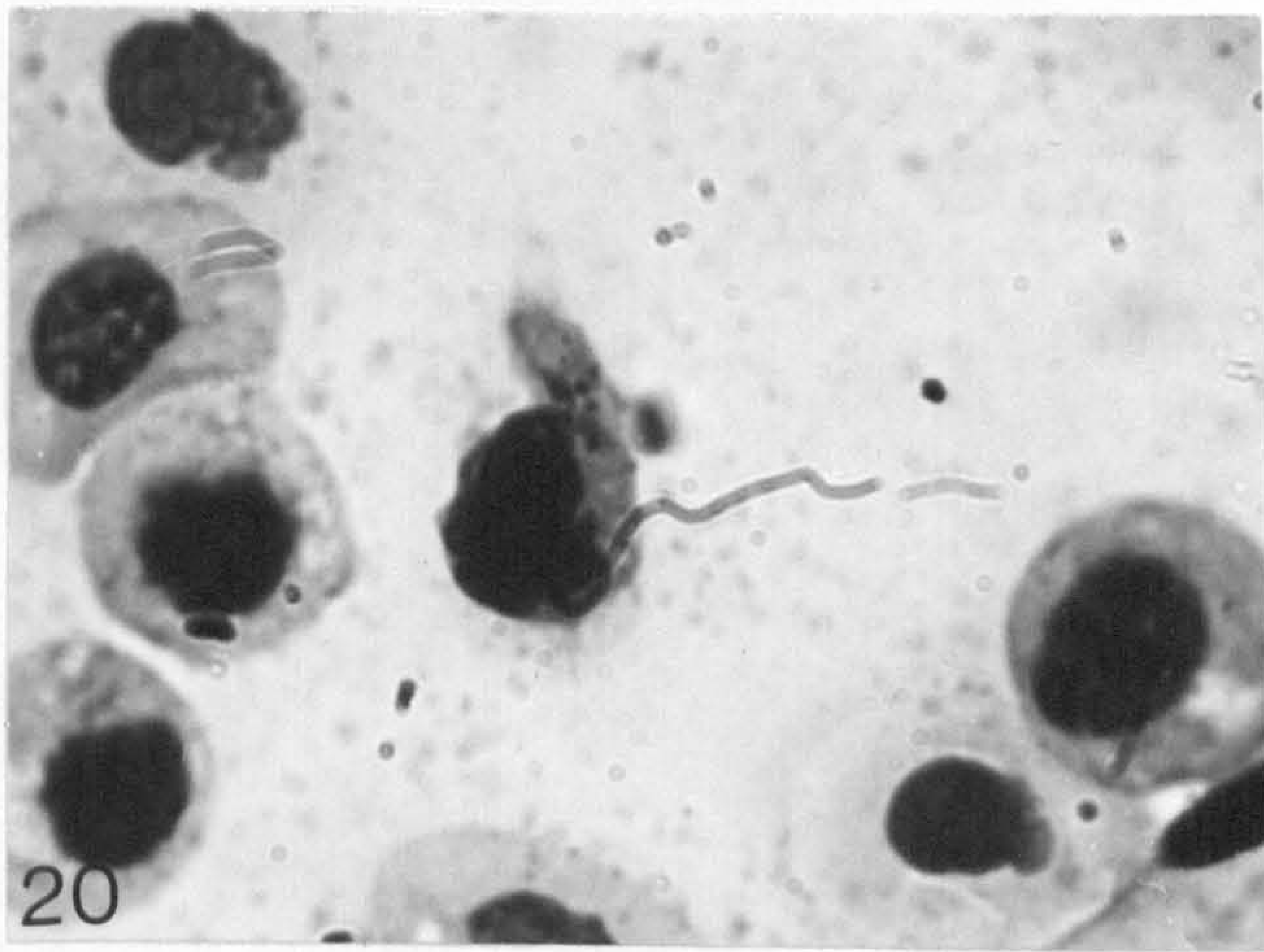
21. Sporozoite penetrating into a neutrophil.
x 3,200

22. Intracellular merozoite in large lymphocyte(?).
Immature neutrophil (progranulocyte)(arrow).
x 2,7500

23. Intracellular merozoite in large lymphocyte(?).
Small and large lymphocytes, degenerating lymphocytes and one neutrophil. x 1,200

24. Merozoite inside large lymphocyte dividing into two parasites. Small lymphocyte, "oval" type thrombocyte and parasitized neutrophil.
x 1,500

25. Two merozoites inside large lymphocyte(?).
Also present a neutrophil. x 2,500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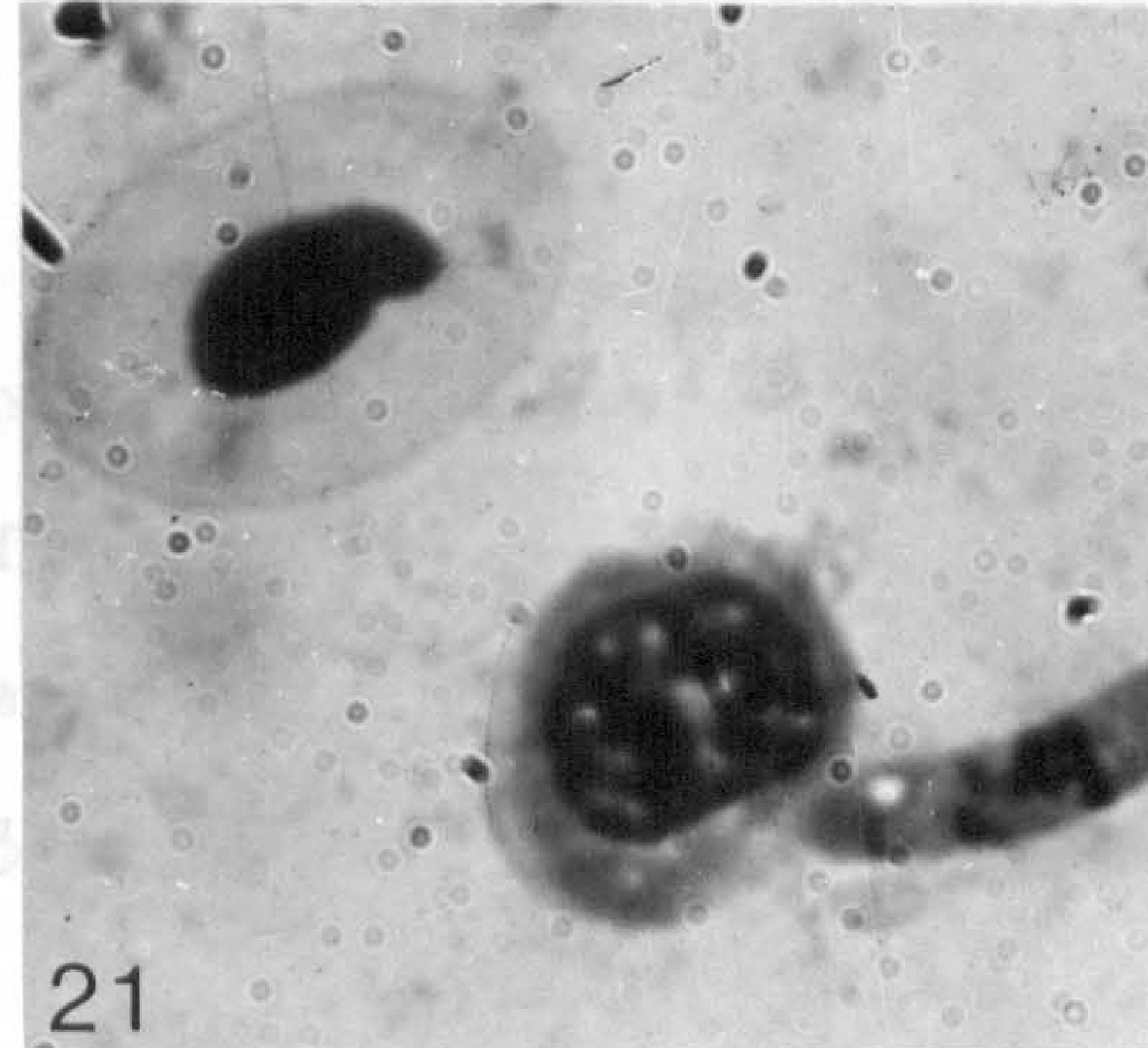
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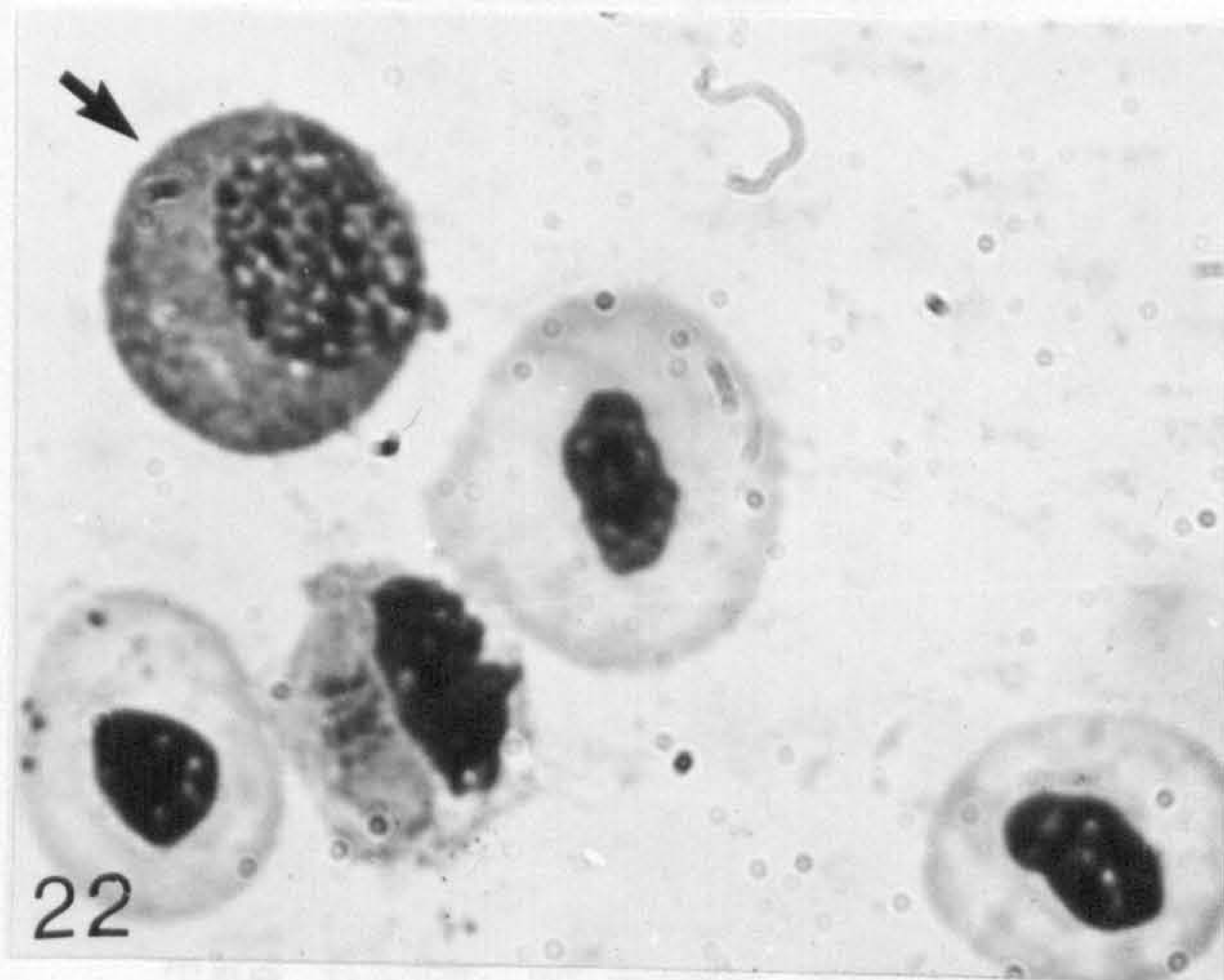
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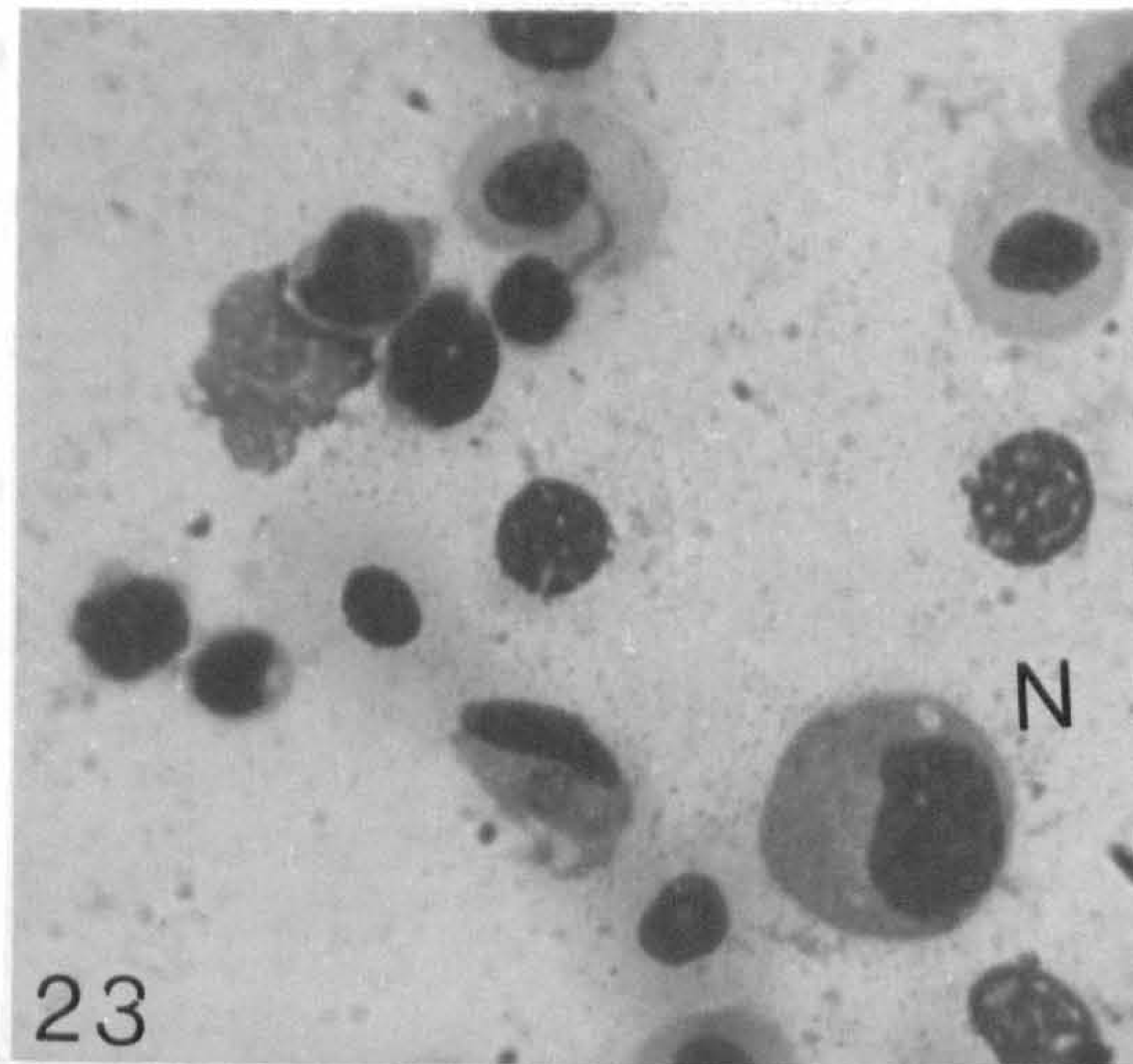
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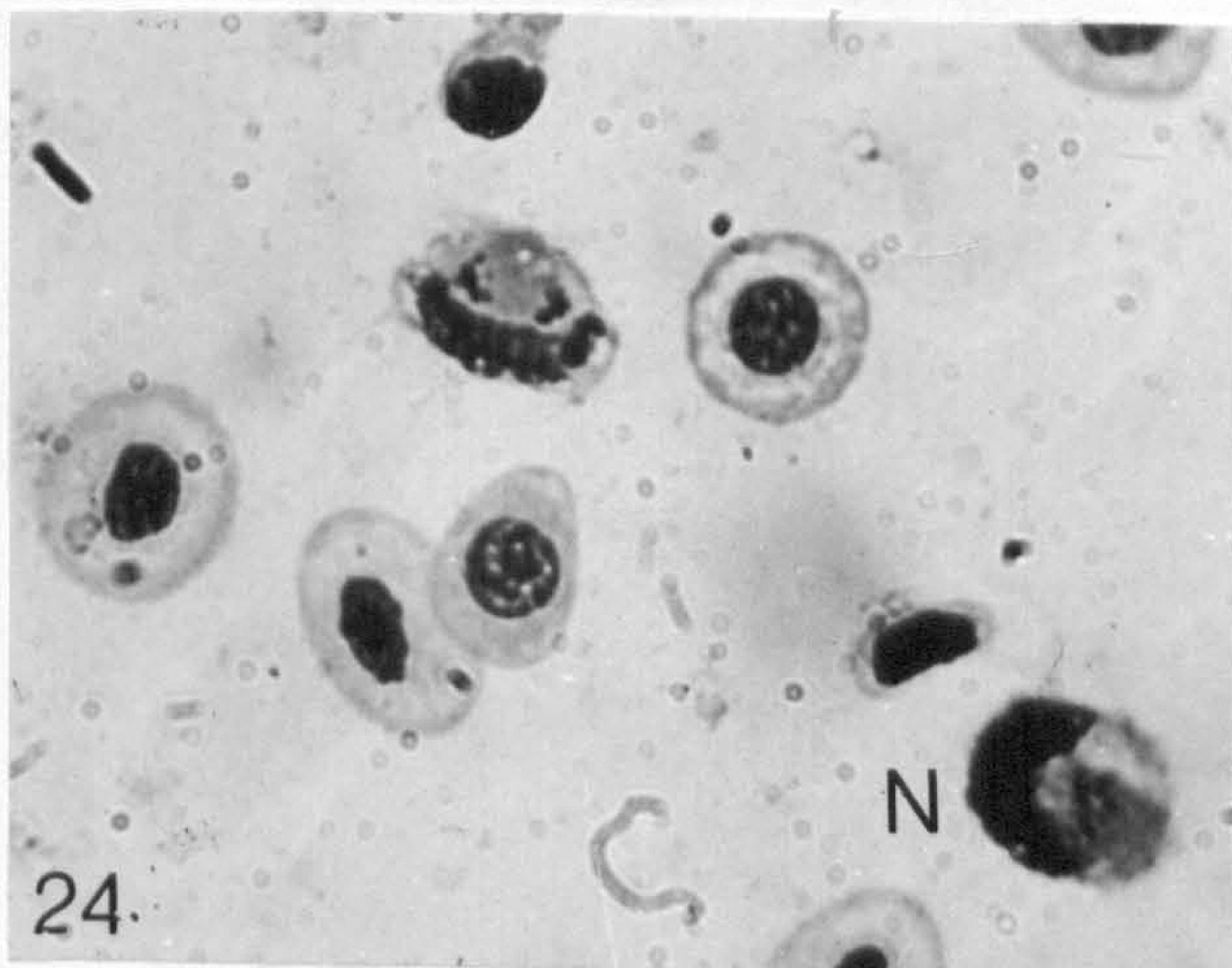
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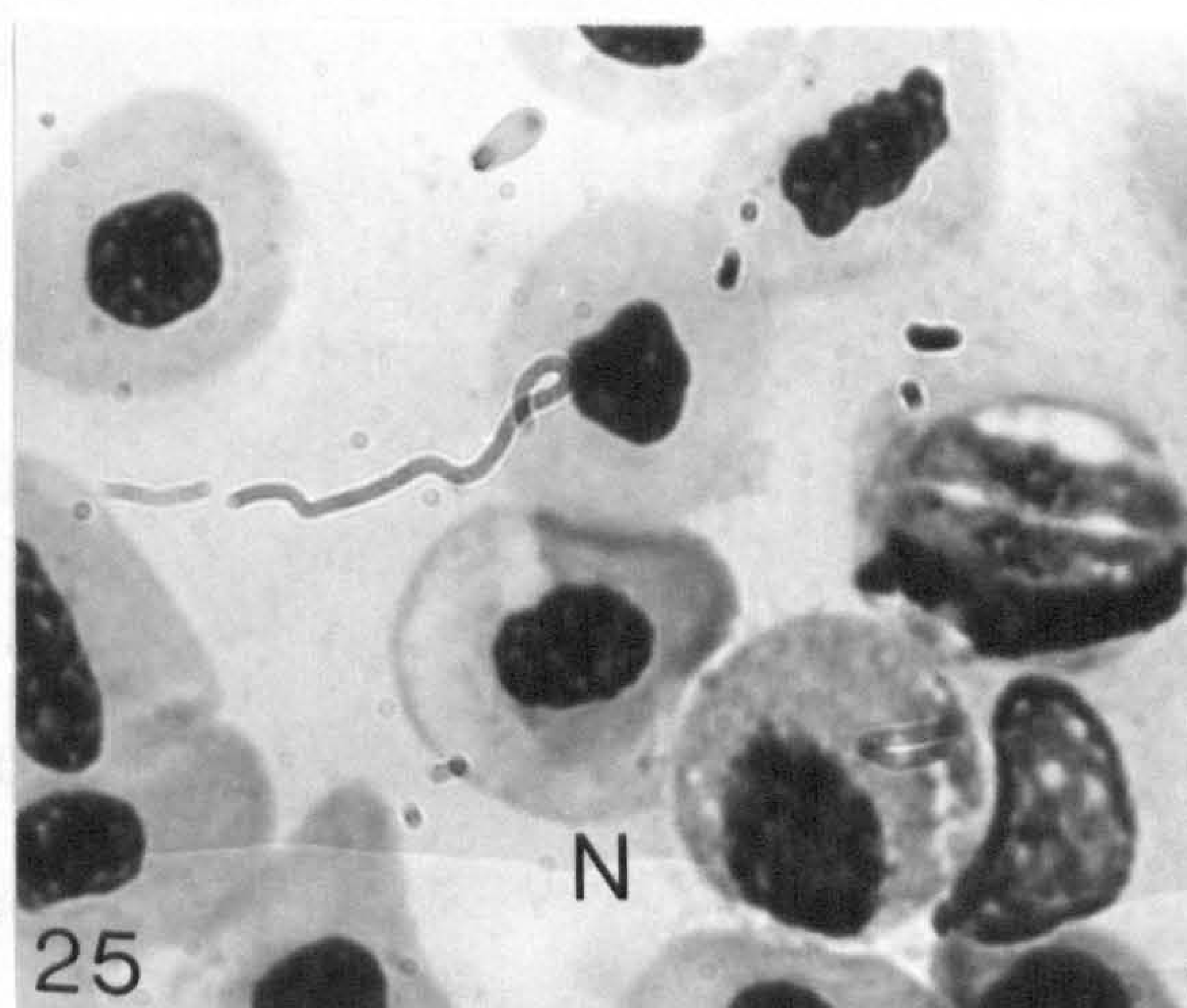
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Normal monocytes measured from 13.0-19.5 micron in length (average 14.9) and from 9.1-14.3 micron in width (average 11.1) with a nucleus of 9.5 by 6.5 average. Often free merozoites derived from leucocytes and sometimes connected with the remainder of a host cell nucleus were observed (Fig.27,31,32,33,35,37).They were seen in groups of two,four,six and eight still connected at one pole so that they appeared in the blood plasma in the shape of a fan. These stages measured 10.0-12.5 micron in length (average 11.0) and 2.25-2.5 micron in width (average 2.4) with a centrally located nucleus of 3.75 by 2.5 micron average and one or two distinct vacuoles at the posterior pole. A small but distinctly dark staining polar cap crowning the anterior pole was also observed (Fig.32,33).

The next stages seen were intraerythrocytic parasites. These were slender, elongate stages and right after the invasion of an erythrocyte they were found singly and curled up because of their length (Fig.38,39). The host cell appeared of normal size and erythrocytes measured 11.7-13.0 in length (average 12.6) and 6.5-7.8 micron in width (average 6.9). These intraerythrocytic merozoites measured an average of 16.3 by 1.7 micron. It appeared that division by binary fission took place consequently (Fig.40) and that the intracorpuscular multiplication

26. Two merozoites inside large lymphocyte (arrow). Also two large schizonts and a "spiked" type thrombocyte (Th). x 1,250

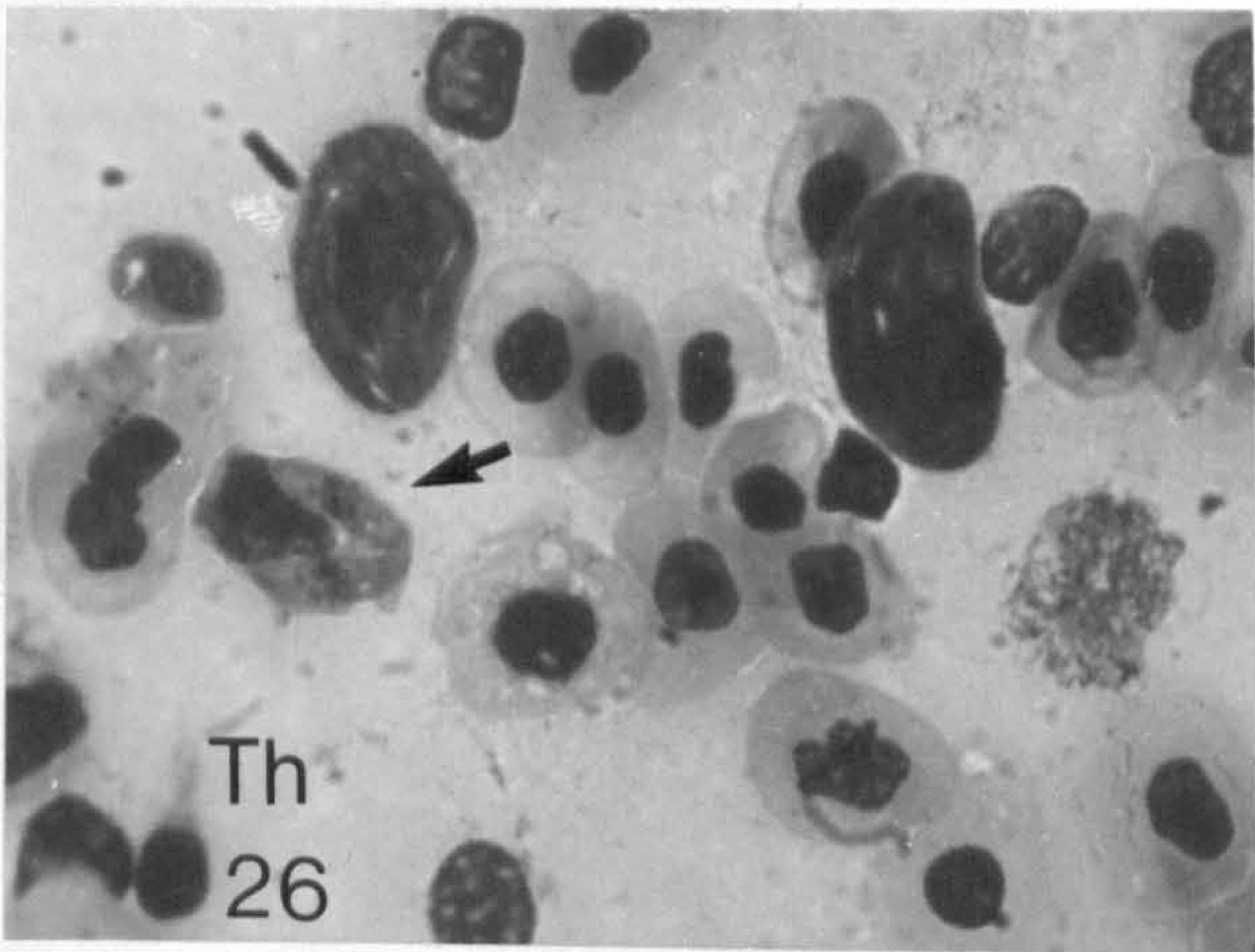
27. Two merozoites apparently leaving host cell with hypertrophied host cell nucleus, presumably monocyte(?). x 2,500

28. Dividing merozoites, four nuclei, compressing host cell nucleus bi-laterally. Host cell large lymphocyte or neutrophil (?). Also "lone nucleus" type thrombocyte.(Th).x 2,200

29. Four merozoites inside a neutrophil. x 2,500

30. Four merozoites with "tails" bend forwards in the process of breaking the cell wall of the host cell. x 1,600

31. Four merozoites free in the blood plasma but still attached to eachother at one end. Note absence of host cell nucleus. x 2,750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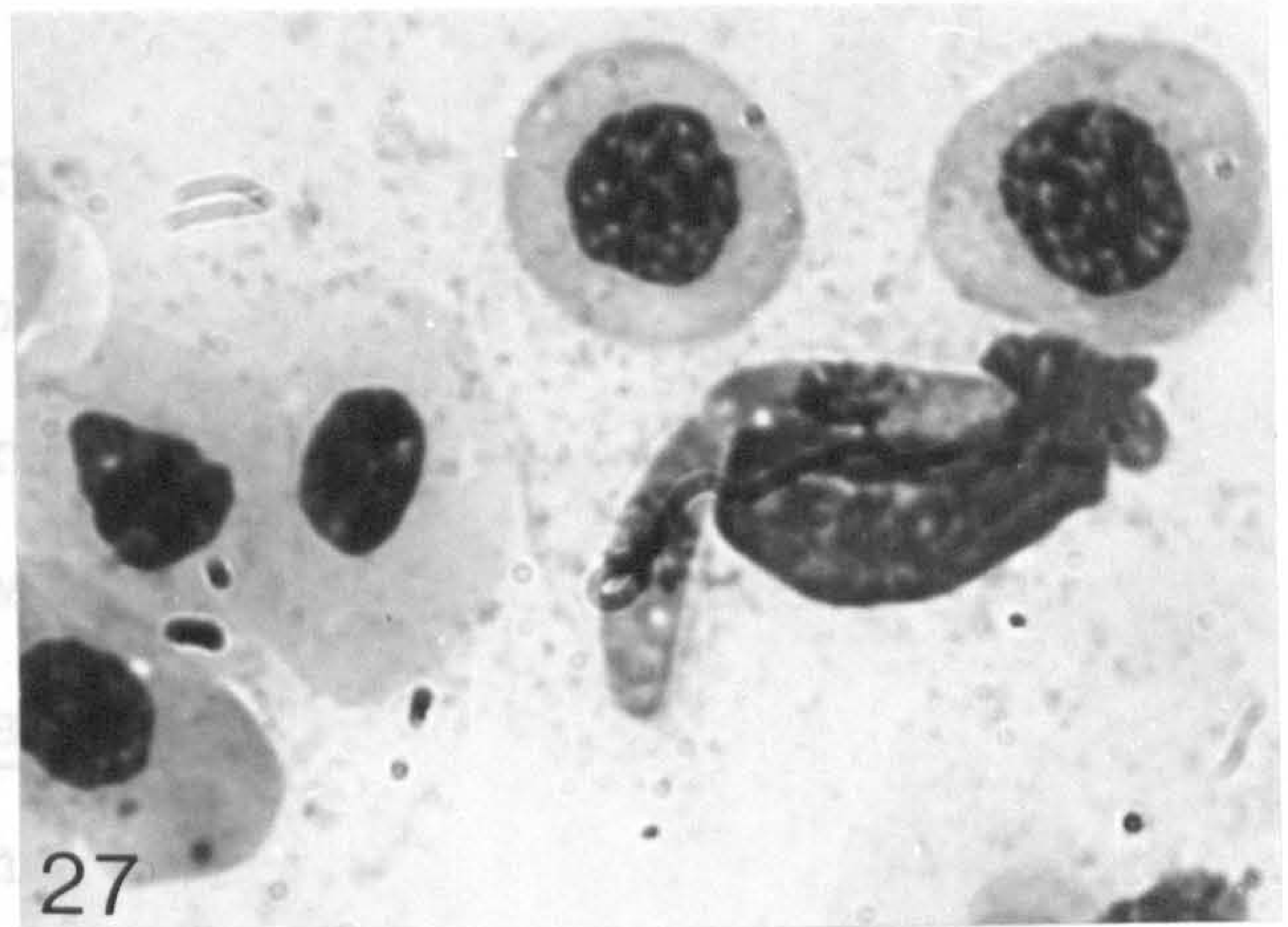
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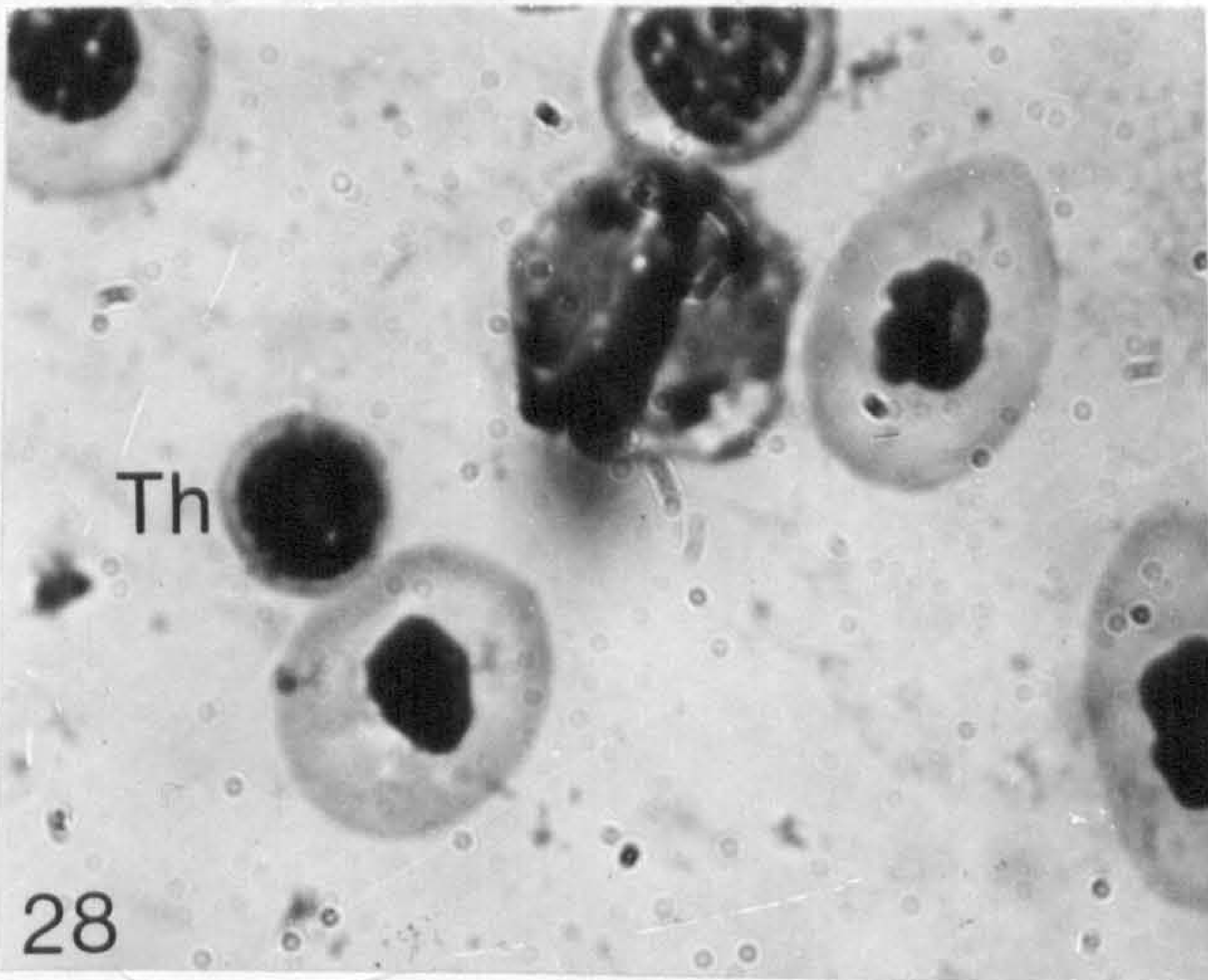
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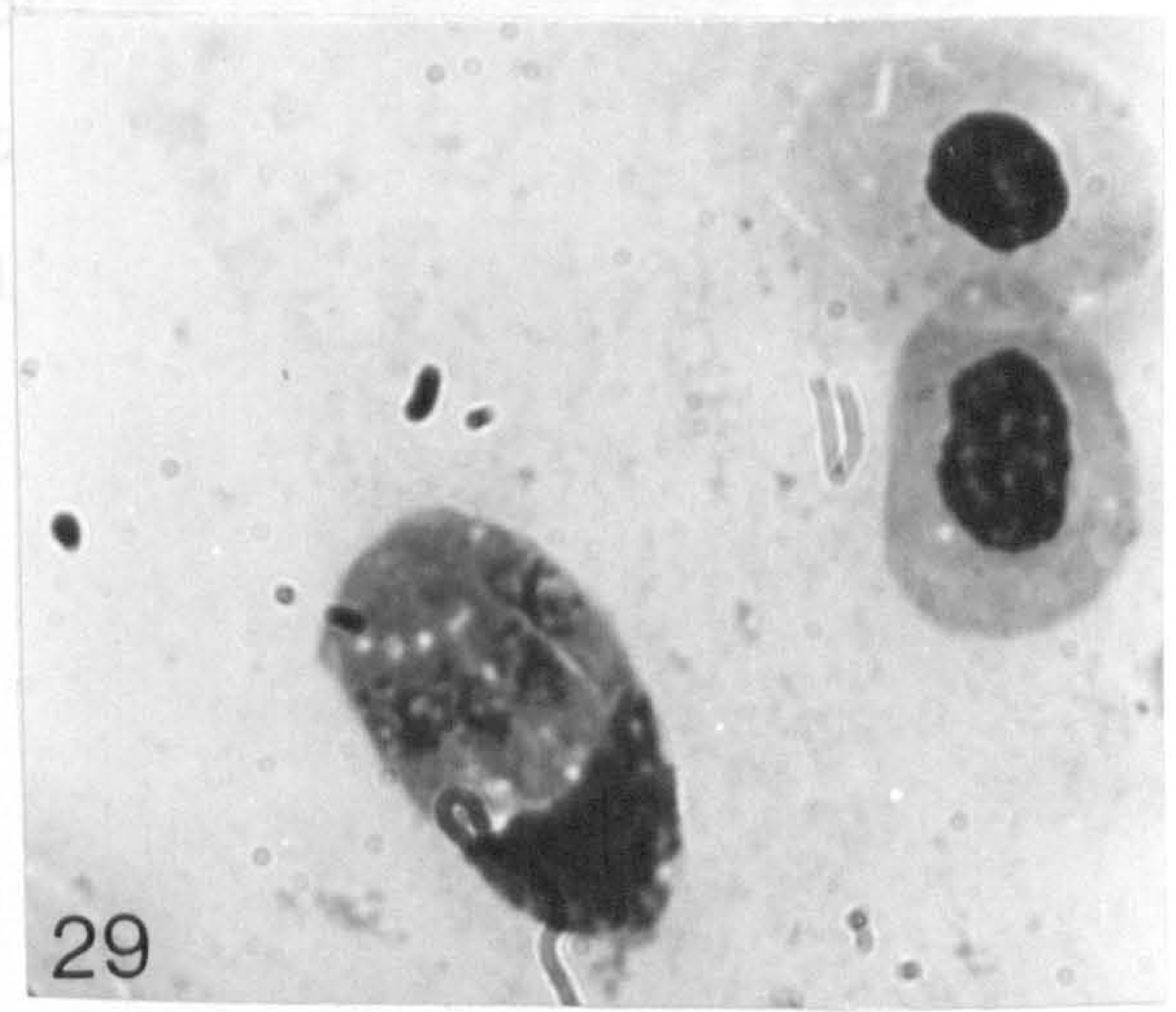
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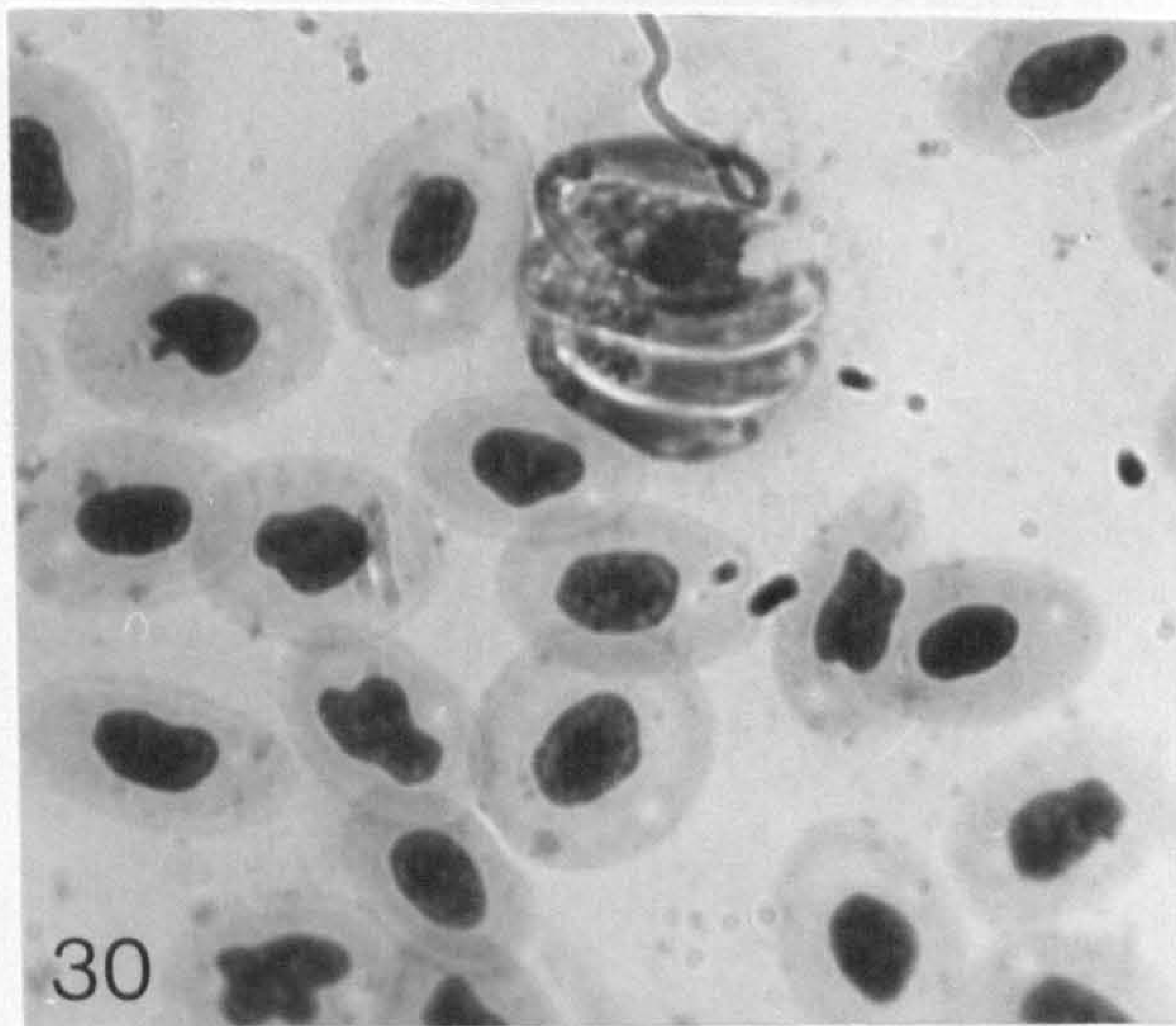
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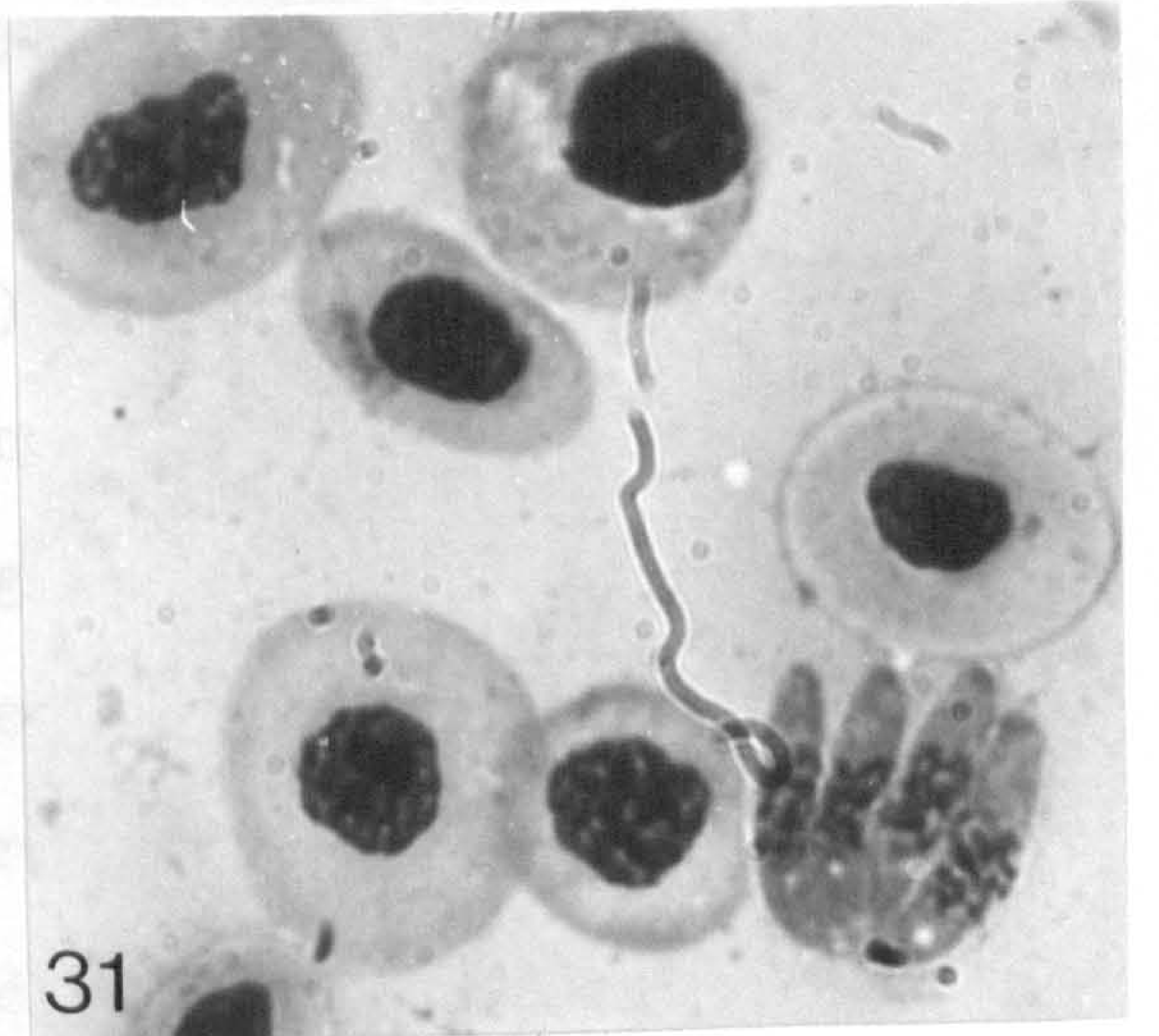
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of these merozoites had enlarged the erythrocyte host cell considerably, so that the host cell cytoplasm and its limiting membrane were hardly discernable (Fig.40, 41). Up to eight merozoites were observed per host cell and these intraerythrocytic schizonts had an average size of 19.1 micron in length (range 16.9-22.1) and of 9.3 micron in width (range 5.8-11.7). At times these schizonts were quite numerous in a blood smear (Fig.49). The nucleus of the host erythrocyte in mature schizonts was often pushed aside or flattened to the periphery and later appeared to have completely disintegrated (Fig.42). Such large schizonts measured from 20.0-21.2 micron in length (average 20.6) and from 6.8-7.5 micron in width (average 7.1). These would eventually break up and release the eight merozoites (Fig.43,50).

Finally, free stages were seen in the plasma showing a distinct differentiation into what appeared to be male and female gametocytes. Elongate, slender stages from *Solea solea* at Hunterston measured from 20.0-22.5 micron in length (average 21.2) and from 1.37-1.5 micron in width (average 1.4) with a centrally located nucleus of 3.75 by 1.5 micron on average situated at a distance of 8.1-8.75 micron from the posterior and slightly tapering end. The anterior third of the parasite was characterized by a dark stained polar cap,an occasional vacuole and few darker staining graules. The polar cap

32. Four merozoites free in the blood plasma but still posteriorly attached to each other. x 1,600

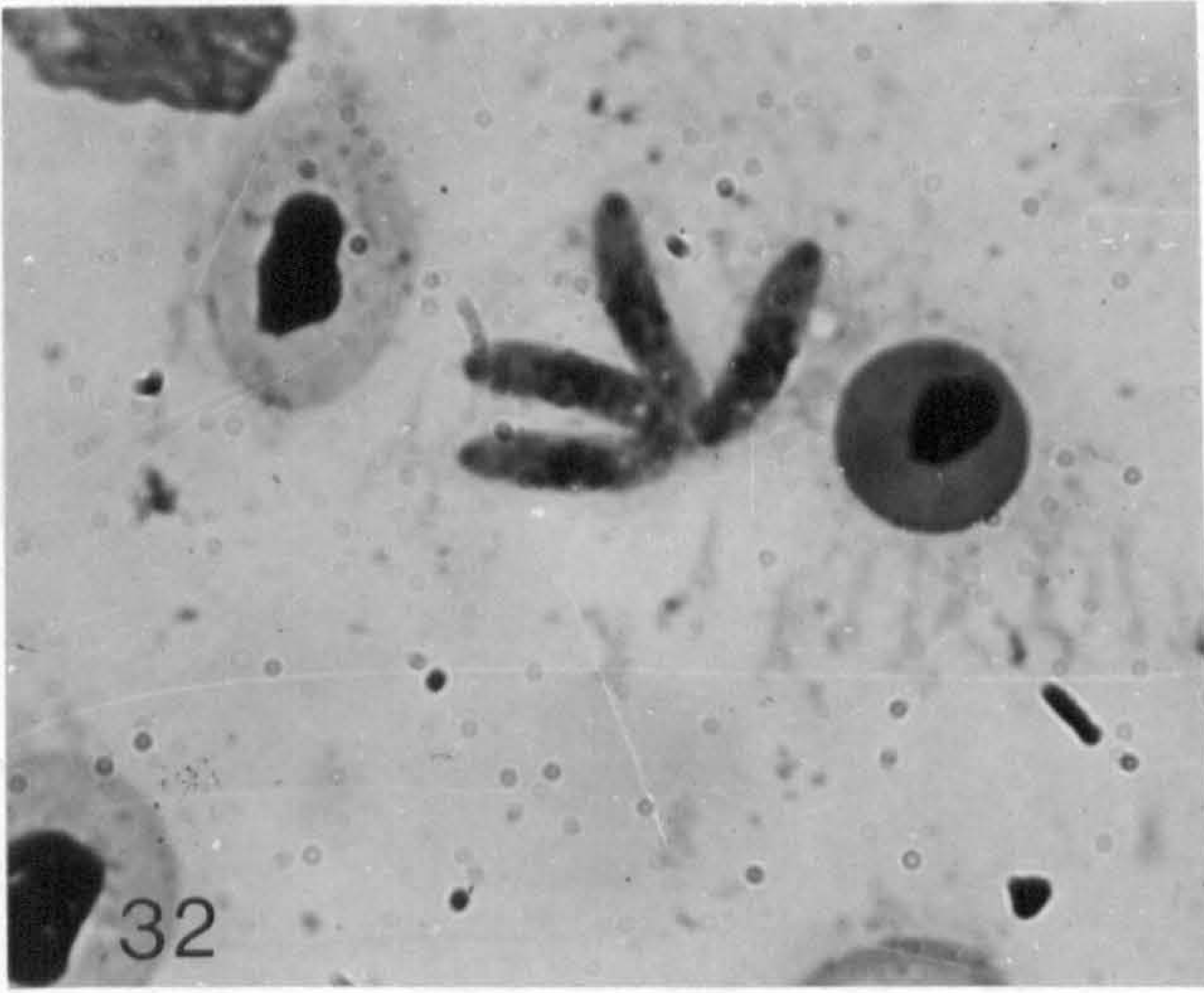
33. Four merozoites connected "finger-like" at their posterior ends in the process of breaking apart. x 2,200

34. Neutrophil with 6 nuclei (arrow). Large schizont (sch) and three neutrophils. x 1,250

35. Six merozoites breaking free from a neutrophil. Host cell nucleus appears unaltered. x 2,500

36. Enlarged monocyte with partly fragmented host cell nucleus holding 8 merozoites. Also two monocytes which are not parasitized. x 2,750

37. Eight merozoites,partly attached to each other, partly attached to enlarged host cell nucleus of monocyte (?) but free of host cell cytoplasm which has apparently disintegrated. Also large lymphocyte. x 2,500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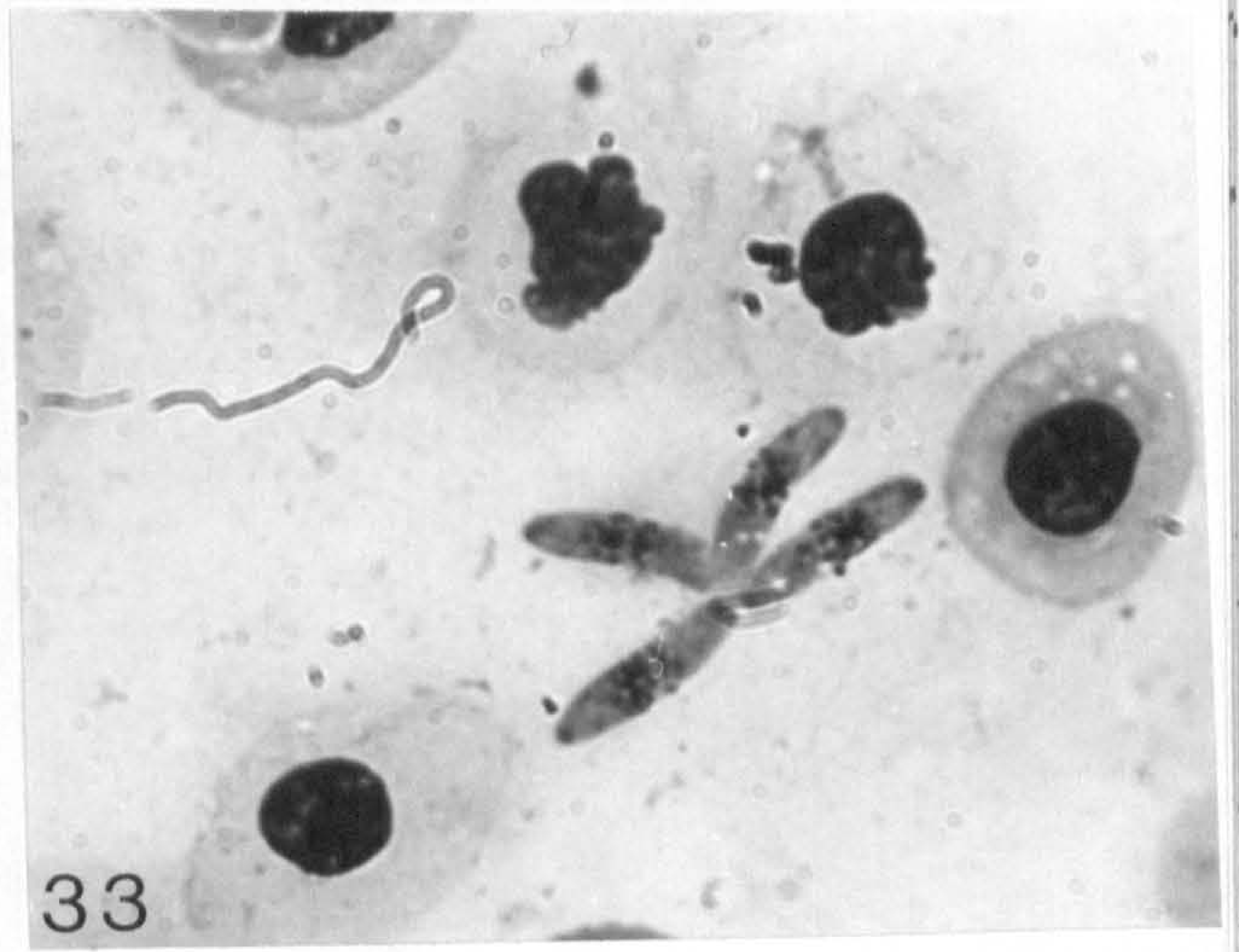
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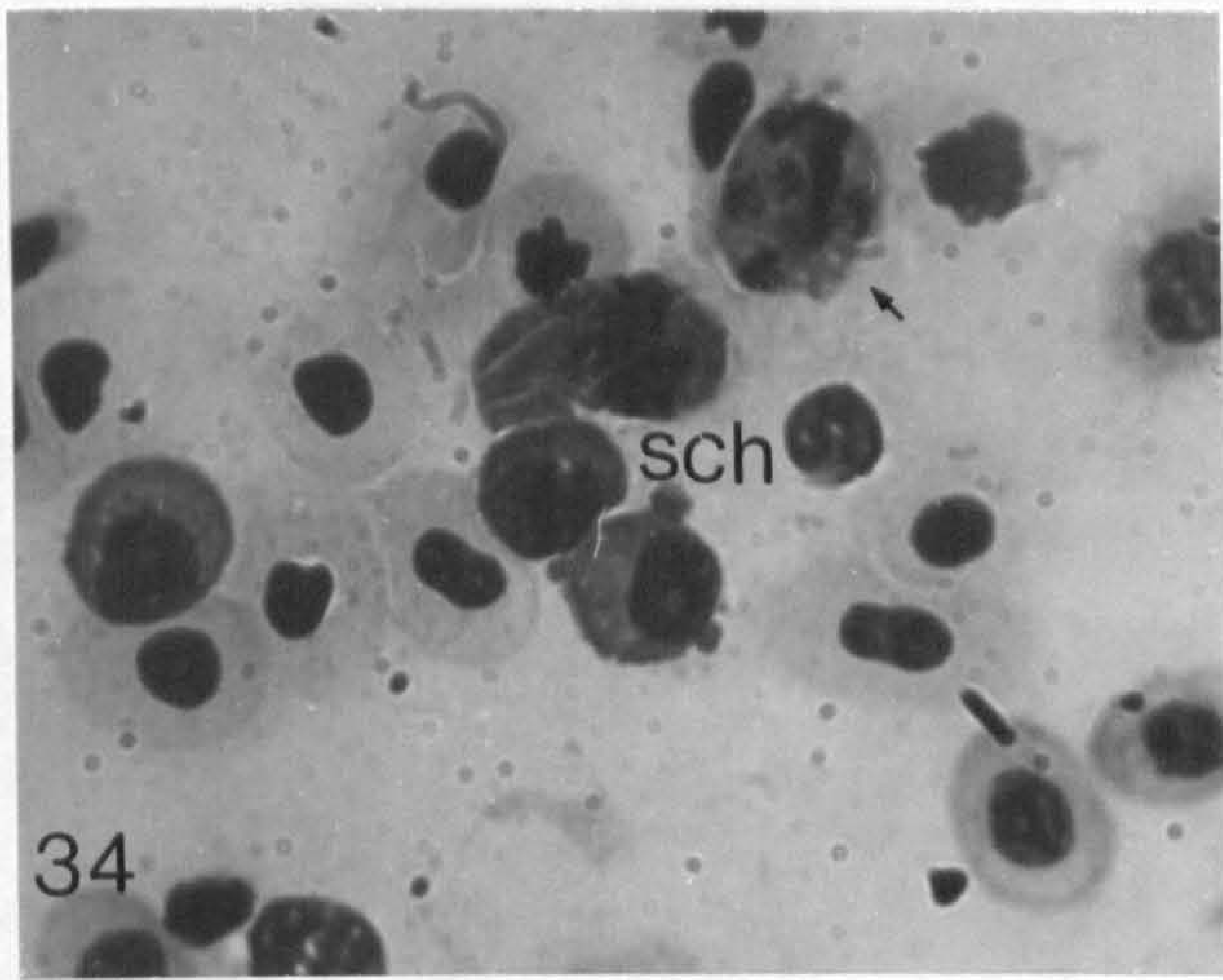
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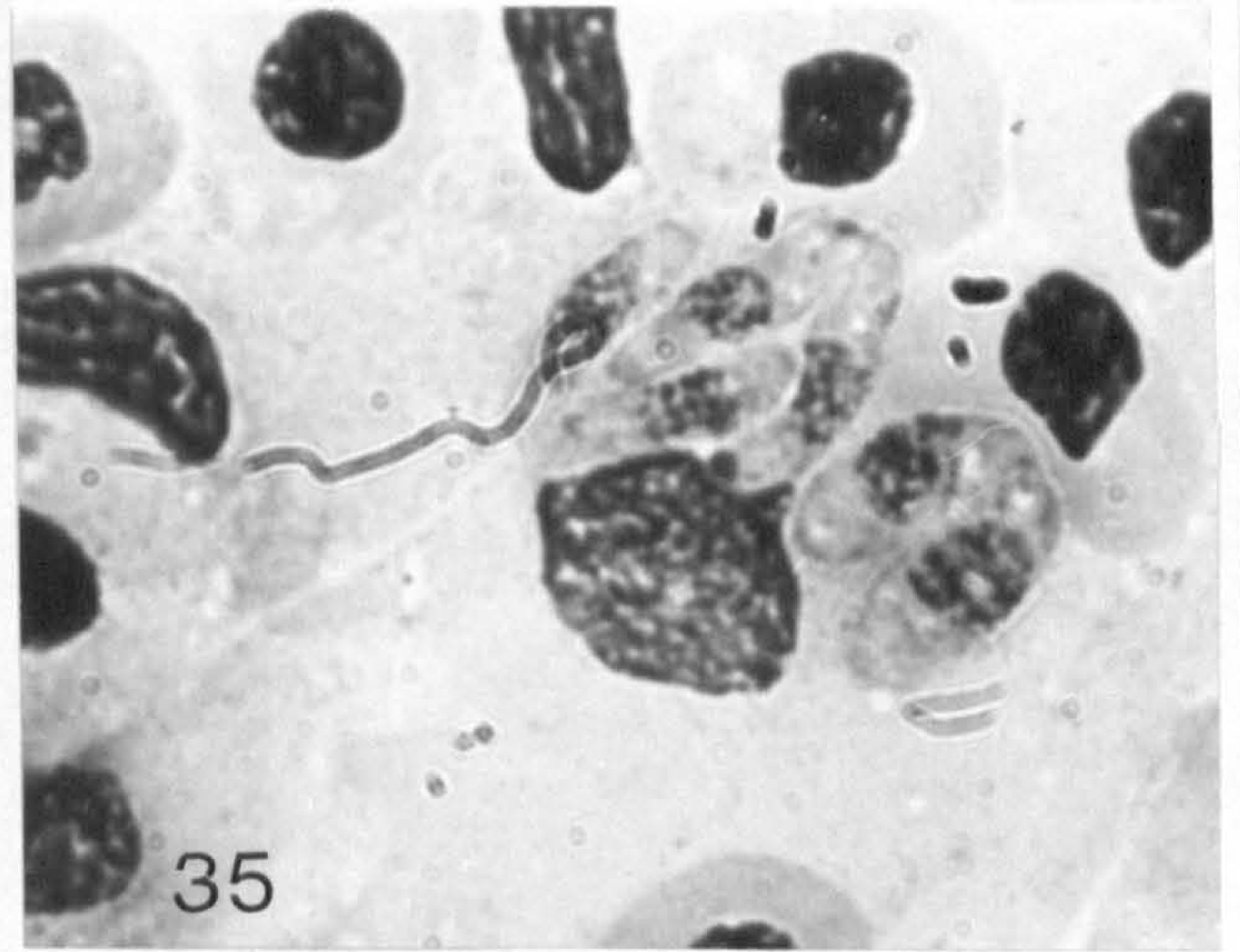
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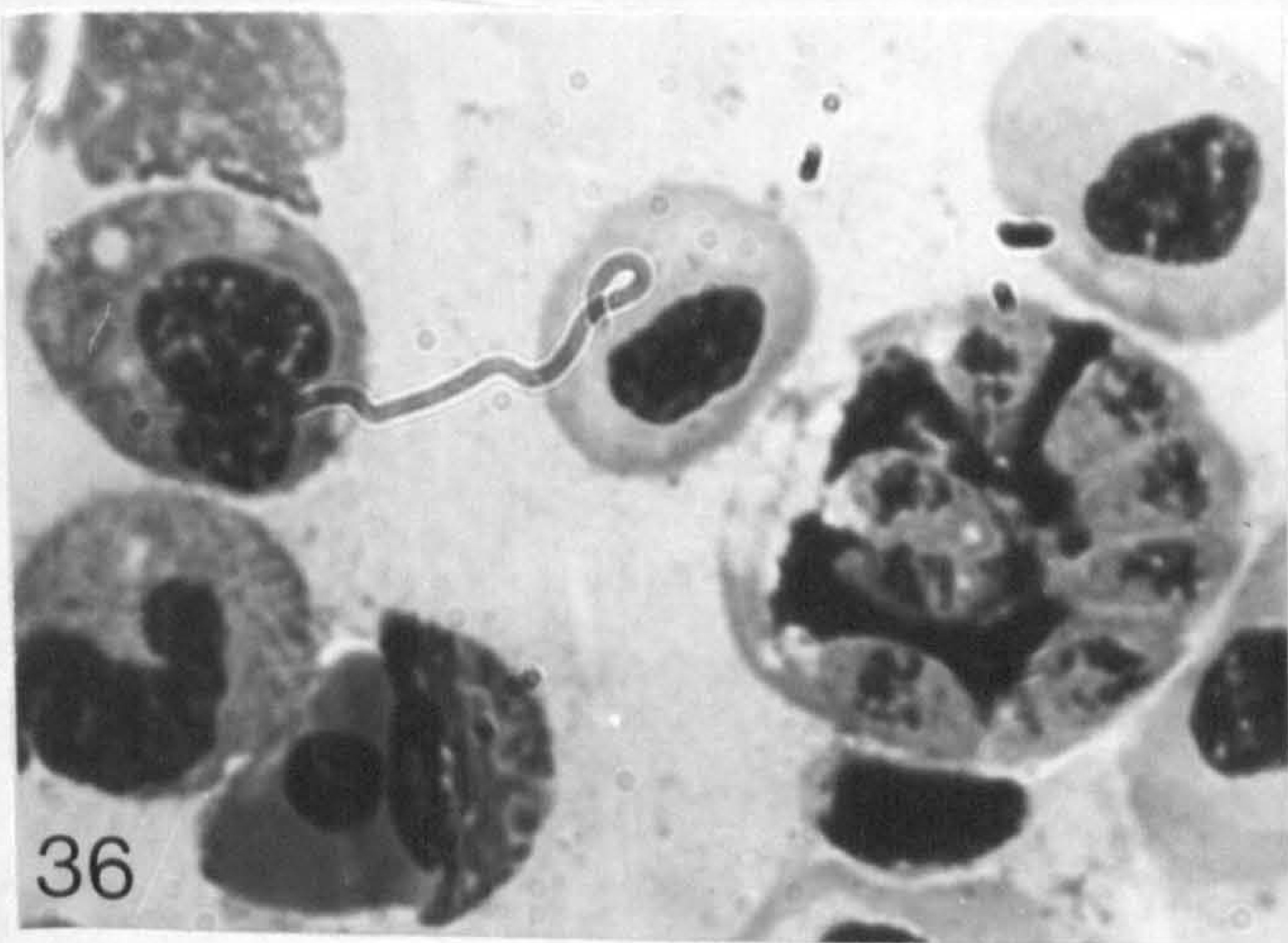
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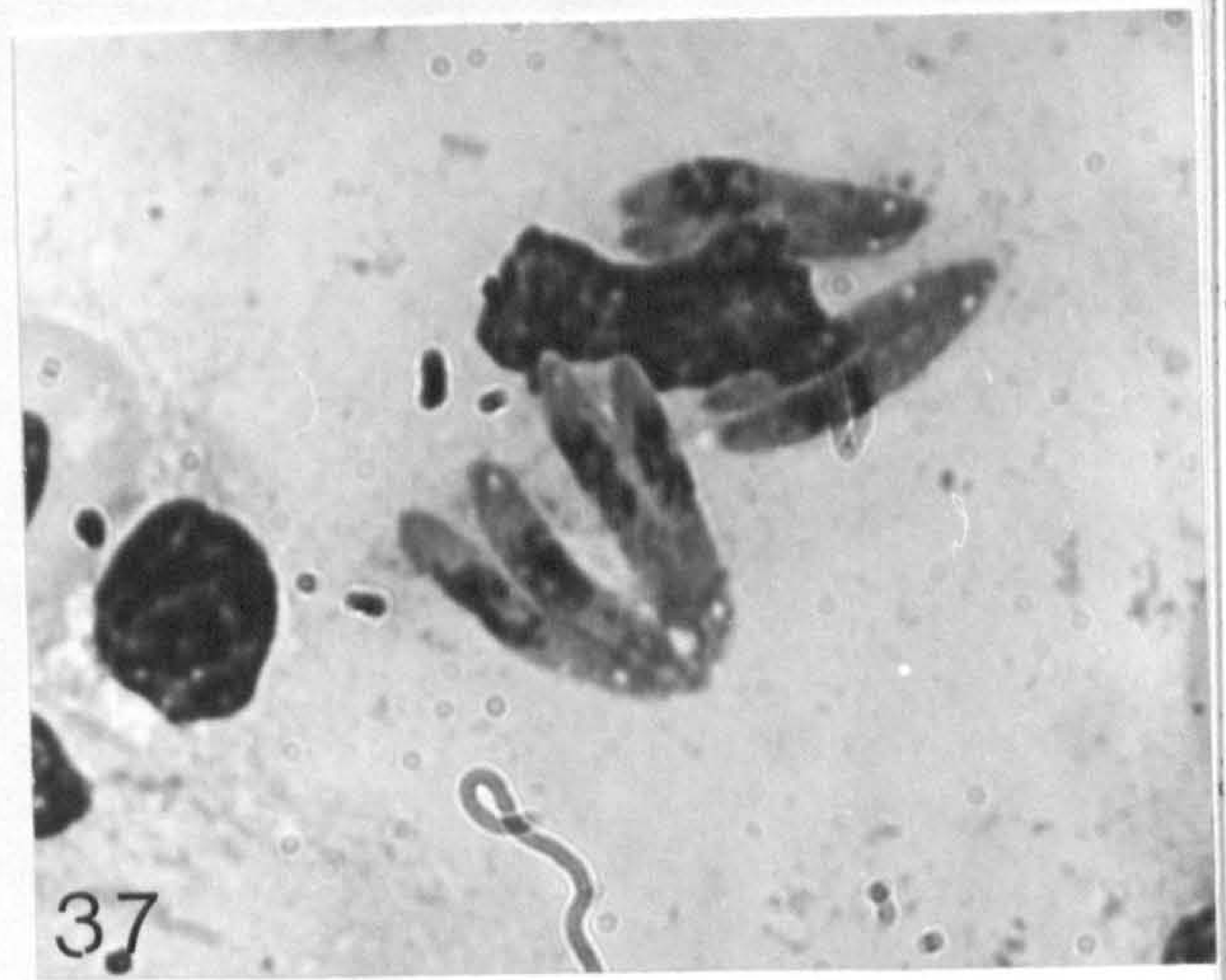
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38. Intraeryhtrocytic merozoite. Also "oval" and "spindle" type thrombocytes (Th). x 1,250

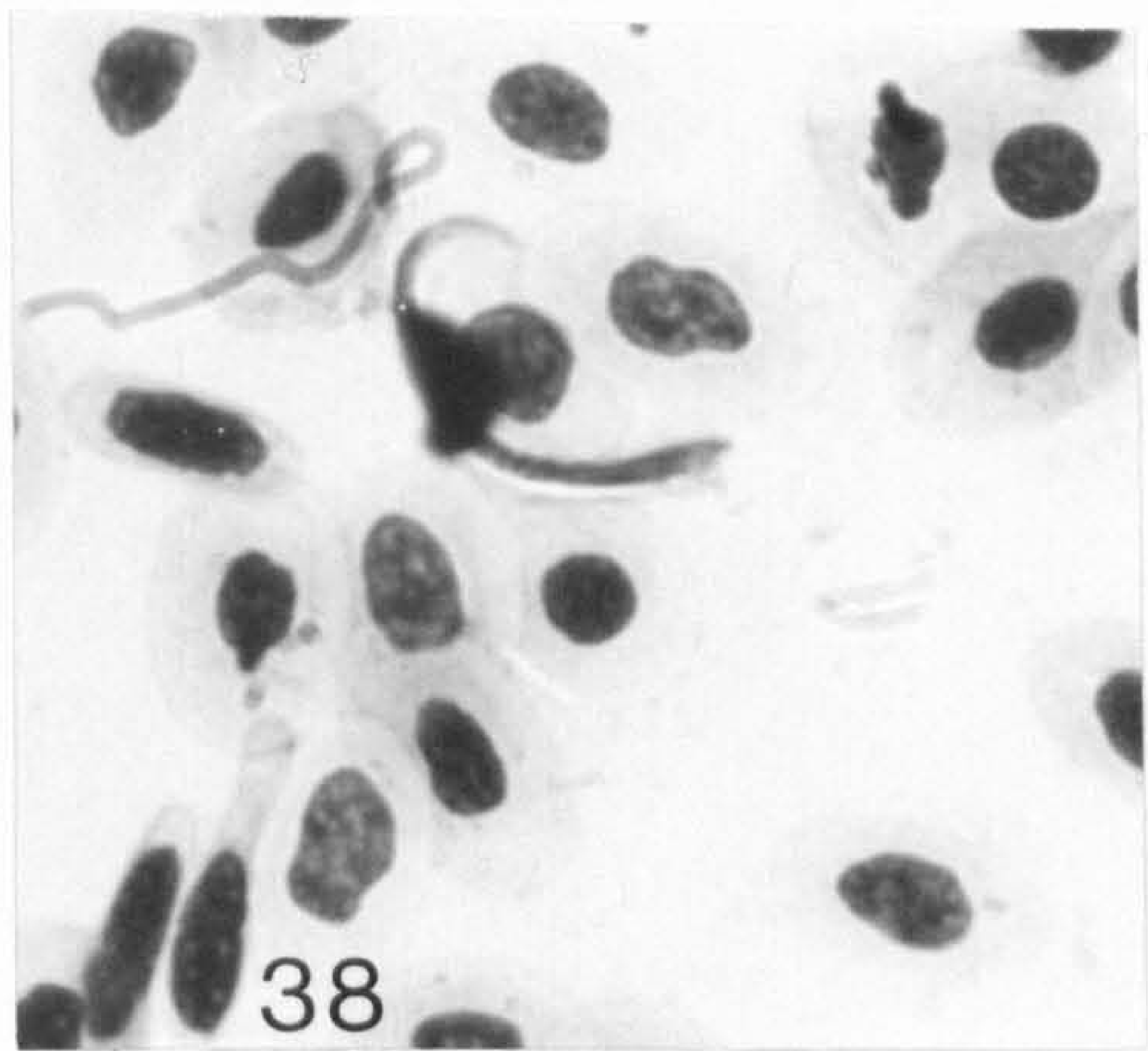
39. Intraerythrocytic merozoite with "tail" wrapped around host cell nucleus. Also "spiked" type thrombocyte (Th). x 1,600

40. Two intraerythrocytic merozoites. One large schizont. Small lymphocyte. x 2,500

41. Three intraerythrocytic merozoites. Large lymphocyte. x 2,500

42. Large intraerythrocytic schizont holding 8 merozoites. x 2,500

43. Breaking of an intraerythrocytic schizont releasing 8 individual merozoites. Note remainder of host cell nucleus and host cell cytoplasm (HCC). x 2,750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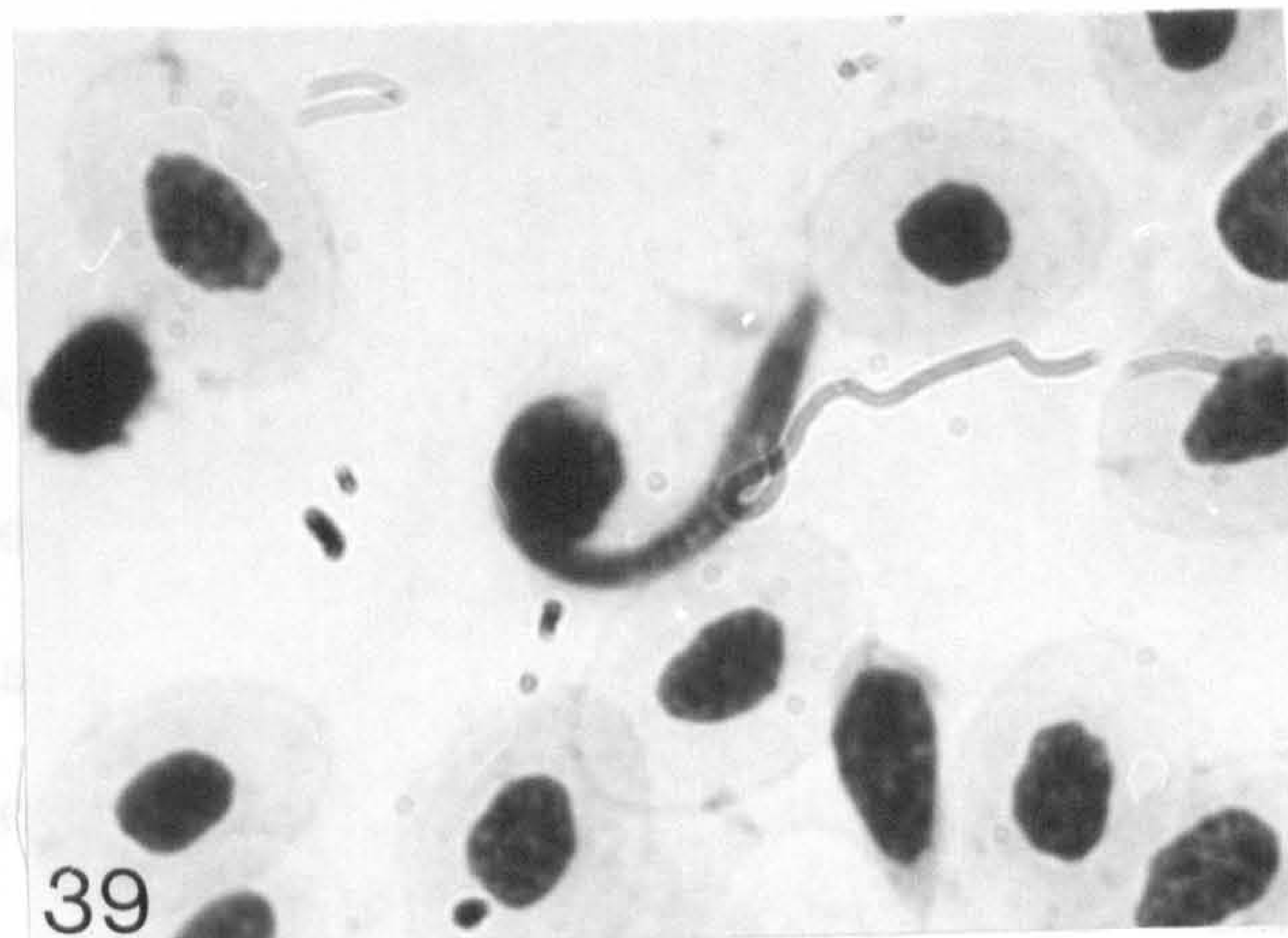
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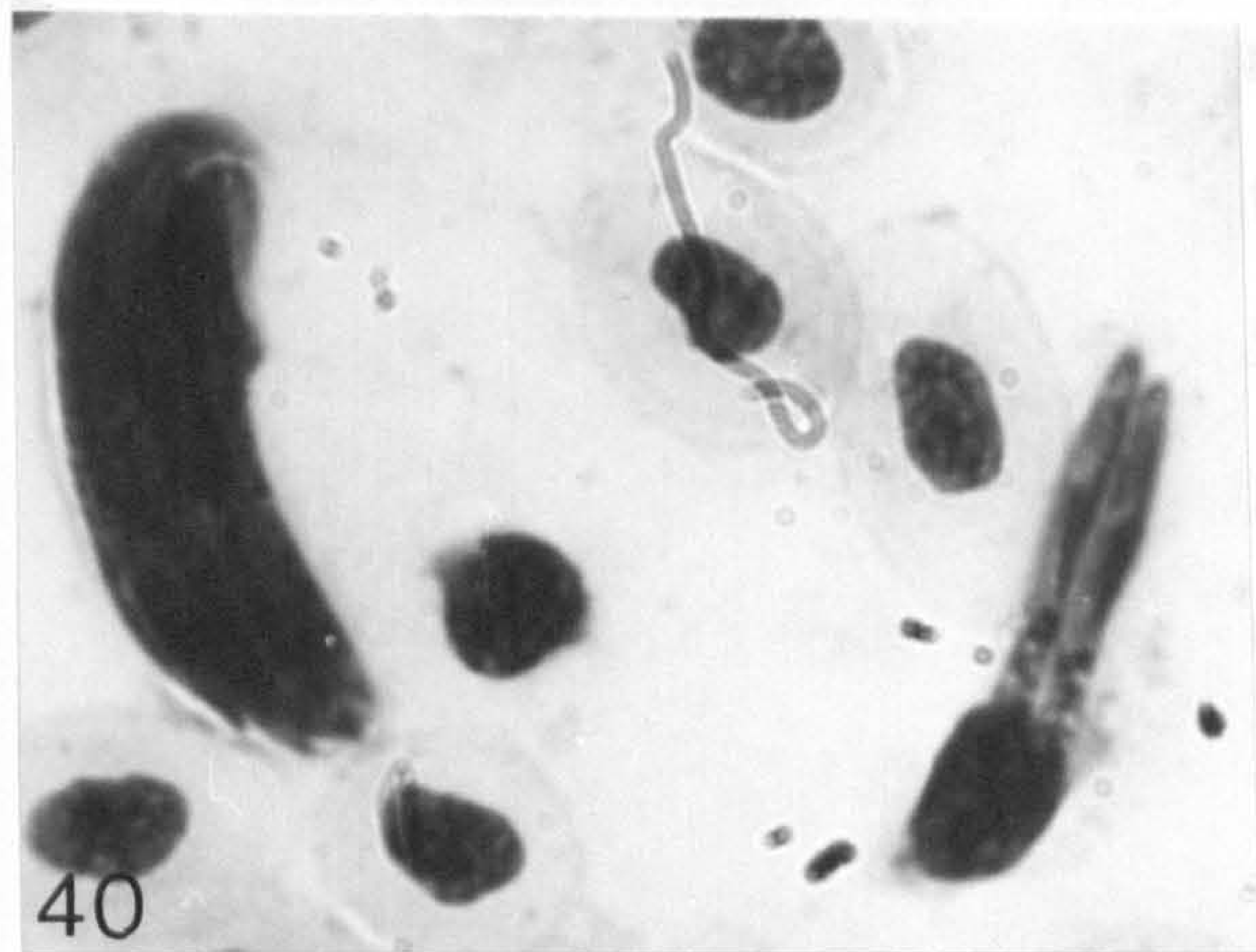
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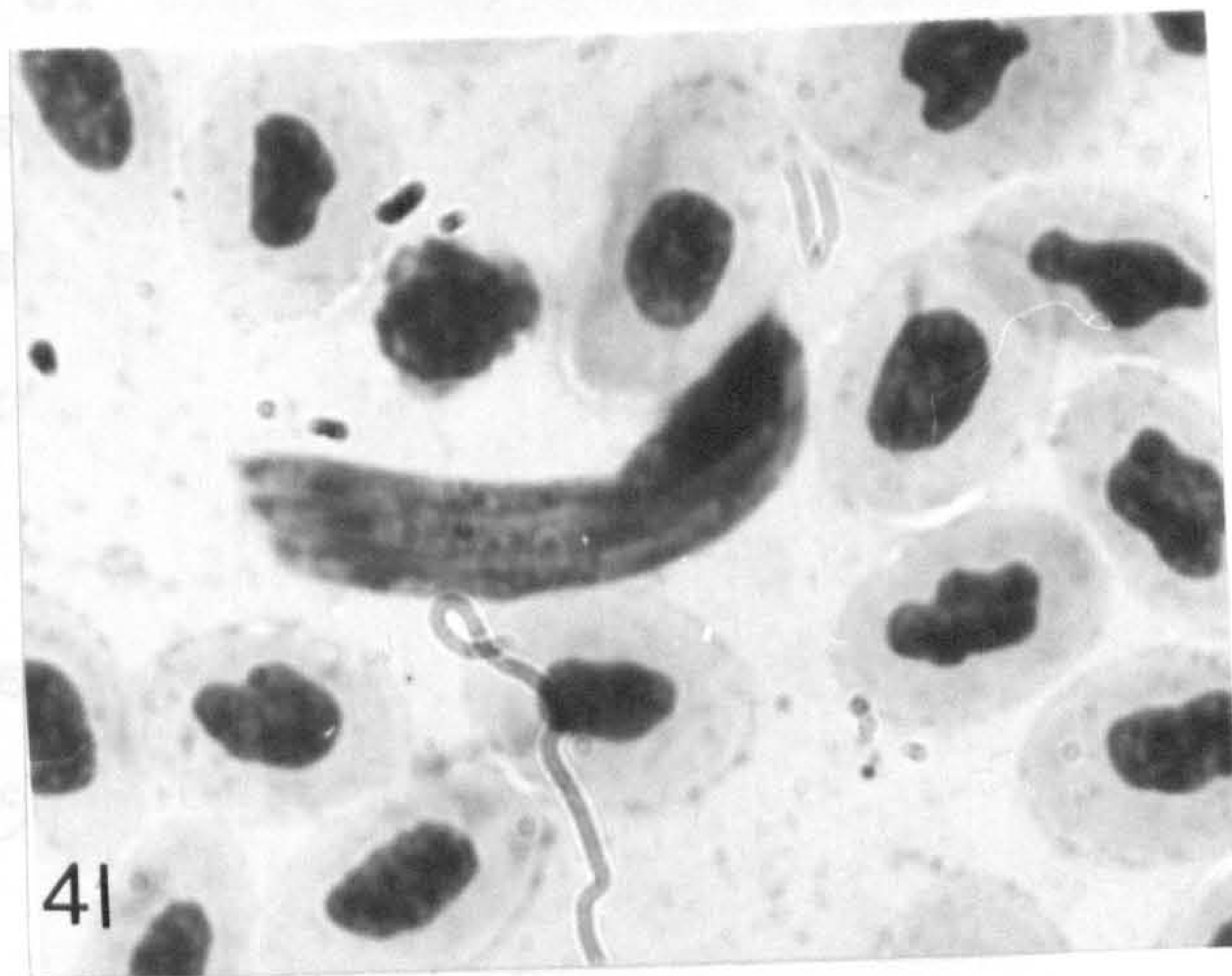
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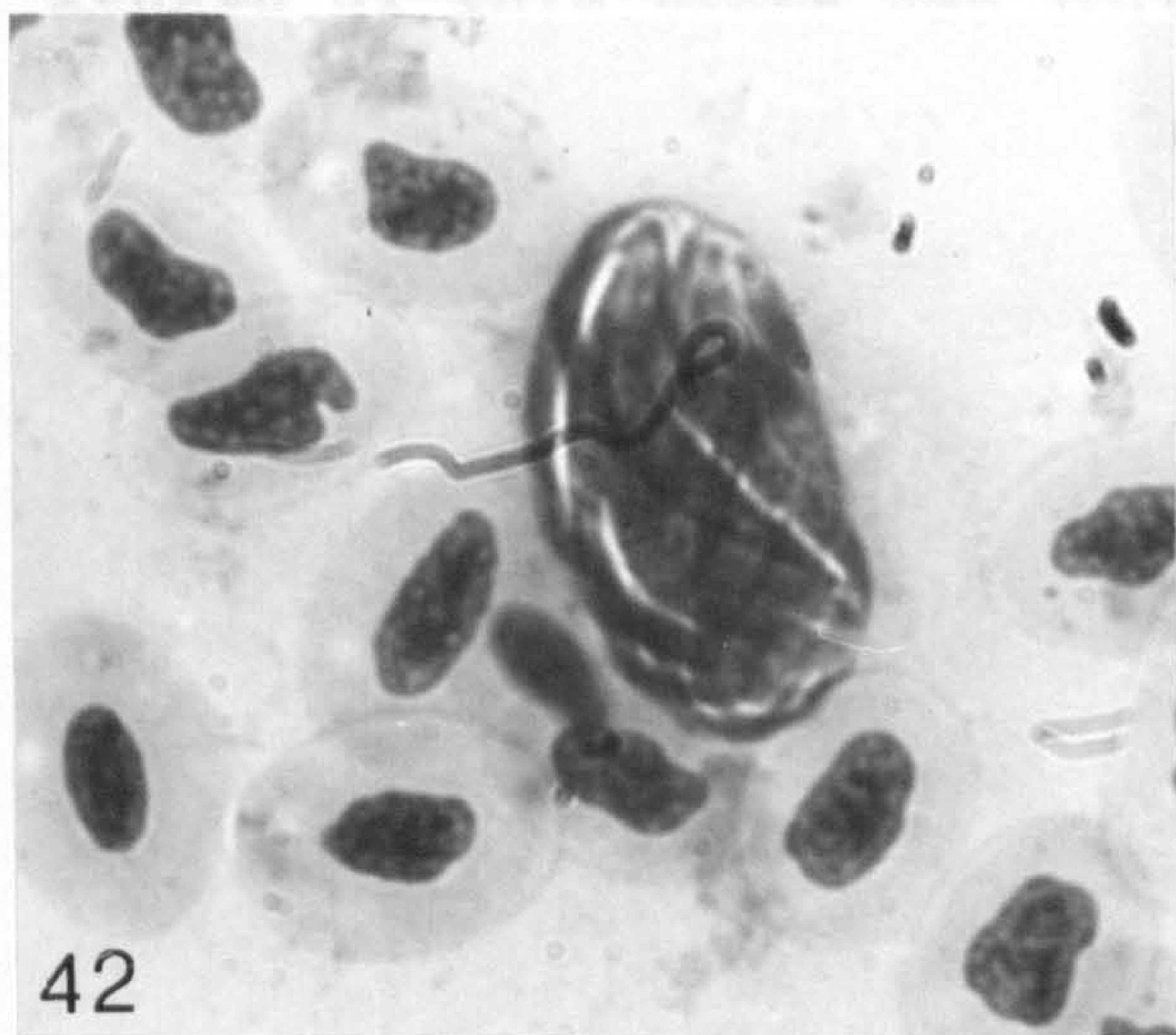
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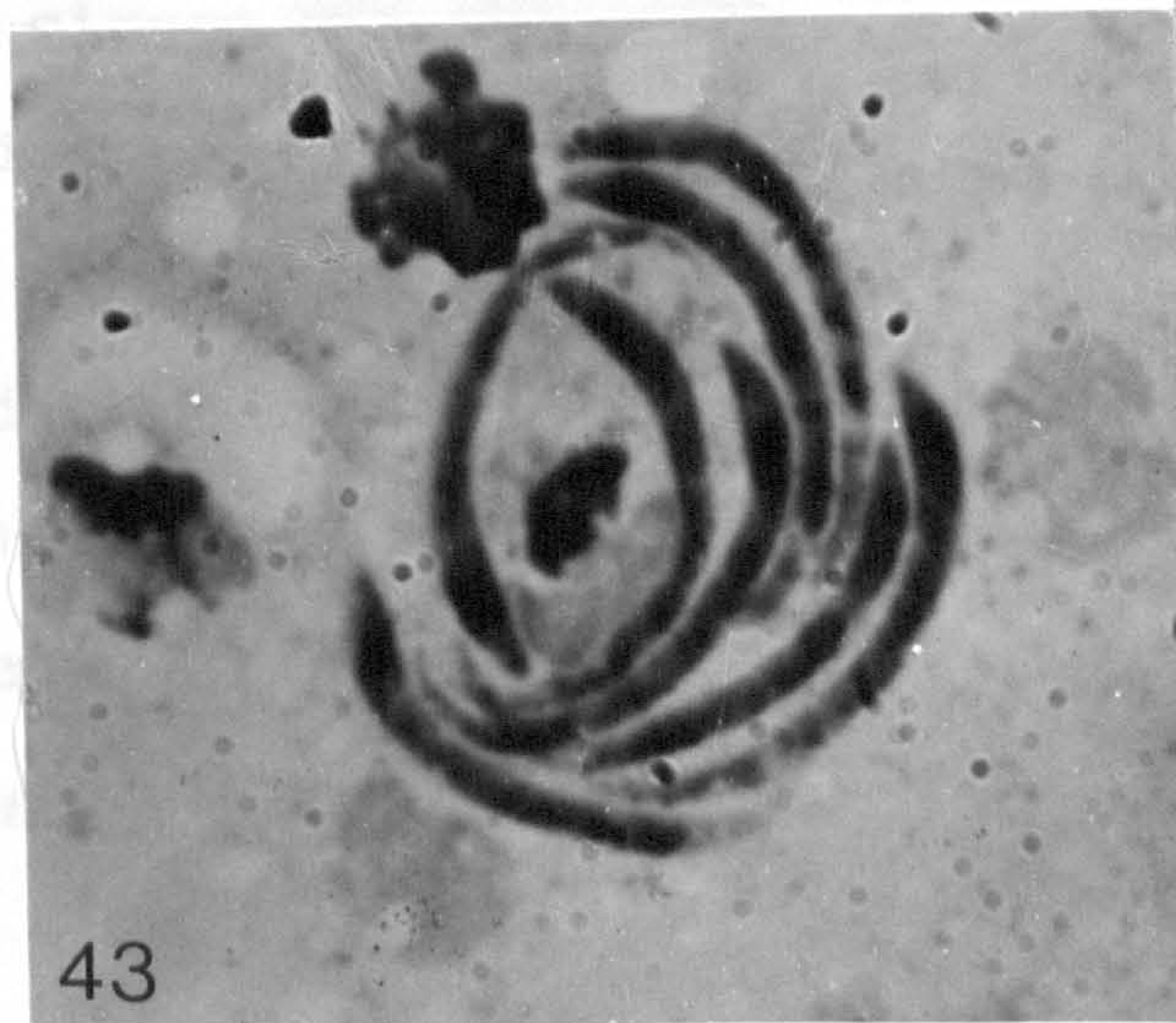
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was separated by a lighter area from a small dark and pointed apical pole with a prominent nipple (Fig.44, 45). These stages were considered to be micro-gametocytes. In wild fish from France the same stage measured 12.5-21.2 micron in length (average 16.8) and 1.4-1.9 micron in width (average 1.7).

In addition, large cucumber shaped stages were seen extracellularly in the plasma of the circulating blood of farmed fish. This stage was characterized by a pale blue cytoplasm, a rounded posterior end and a more blunt pointed anterior end containing several distinct vacuoles. Also present was occasionally a distinct large refractile body (Fig.46). The nucleus was large and of light pink color and measured 3.75 by 3.5 micron. A distinct and purple staining nucleolus was observed at the periphery of the nucleus bordering the outer pellicle of the parasite. Its dimensions were 1.25 by 1.9 micron. The overall length of this stage was 10.6 micron and the width 3.6 micron. Other parasites with similar morphological characteristics measured from 13.75-15.0 micron in length (average 14.3) and 3.75-5.0 micron in width (average 4.4). They had a large nucleus measuring from 4.37-6.25 micron in length (average 5.3) and from 3.1-3.75 micron in width (average 3.4). These free stages (Fig.46,47) were considered macro-gametocytes.

44. Free micro-gametocyte. x 2,500

45. Free micro-gametocyte. x 2,500

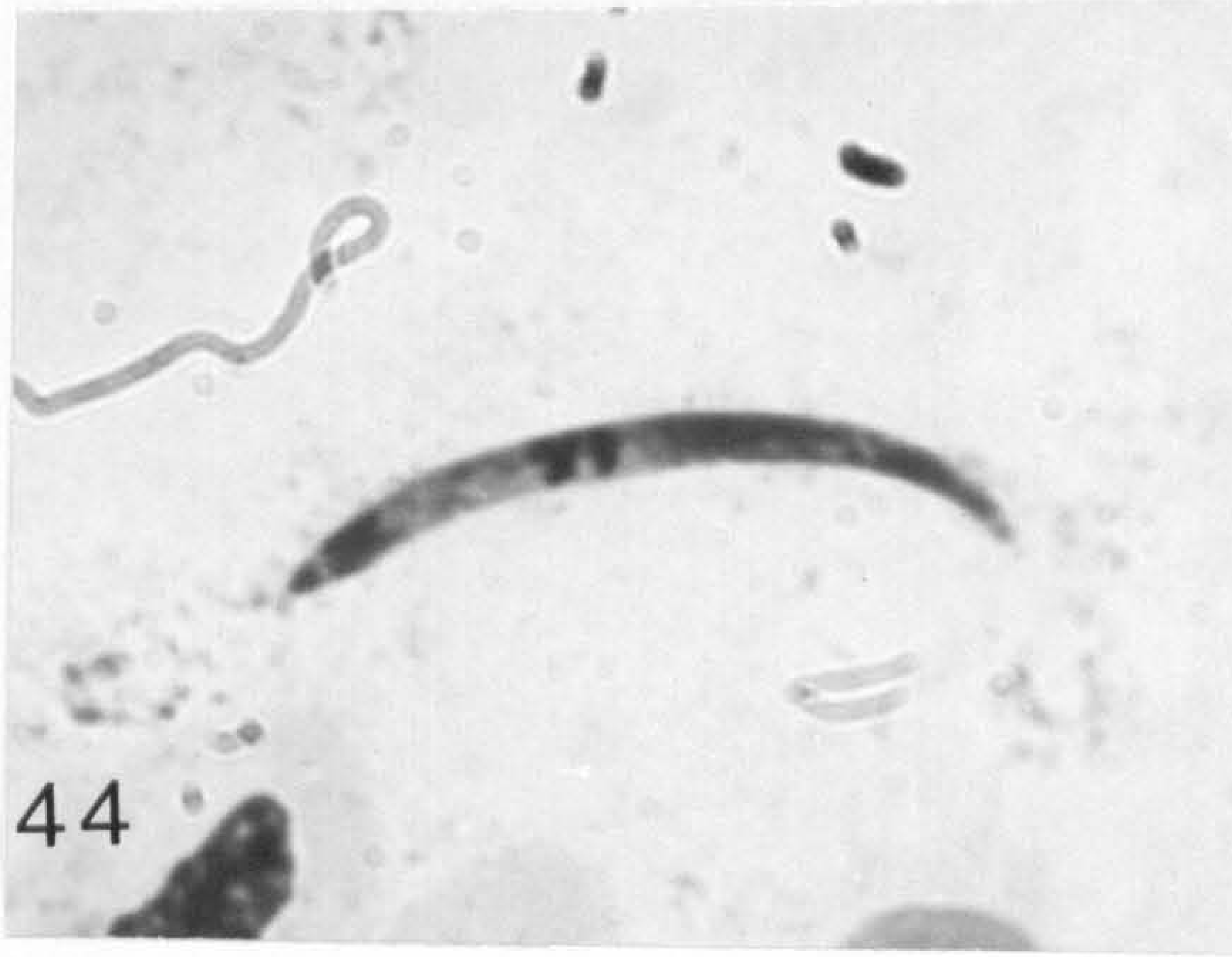
46. Free macro-gametocyte. x 3,200

47. Free macro-gametocyte. x 3,600

48. Four free merozoites partly dividing. Two large intraerythrocytic schizonts, one with a merozoite leaving the host cell. x 1,000

49. Five intraerythrocytic schizonts amongst erythrocytes. x 400

50. Four merozoites clumped to the host cell nucleus of a monocyte or neutrophil (arrow). Eight intraerythrocytic merozoites coiled around host cell nucleus at lower right of figure. Eight free slender merozoites derived from an intraerythrocytic schizont. Note absence of host cell nucleus and cytoplasm. x 1,250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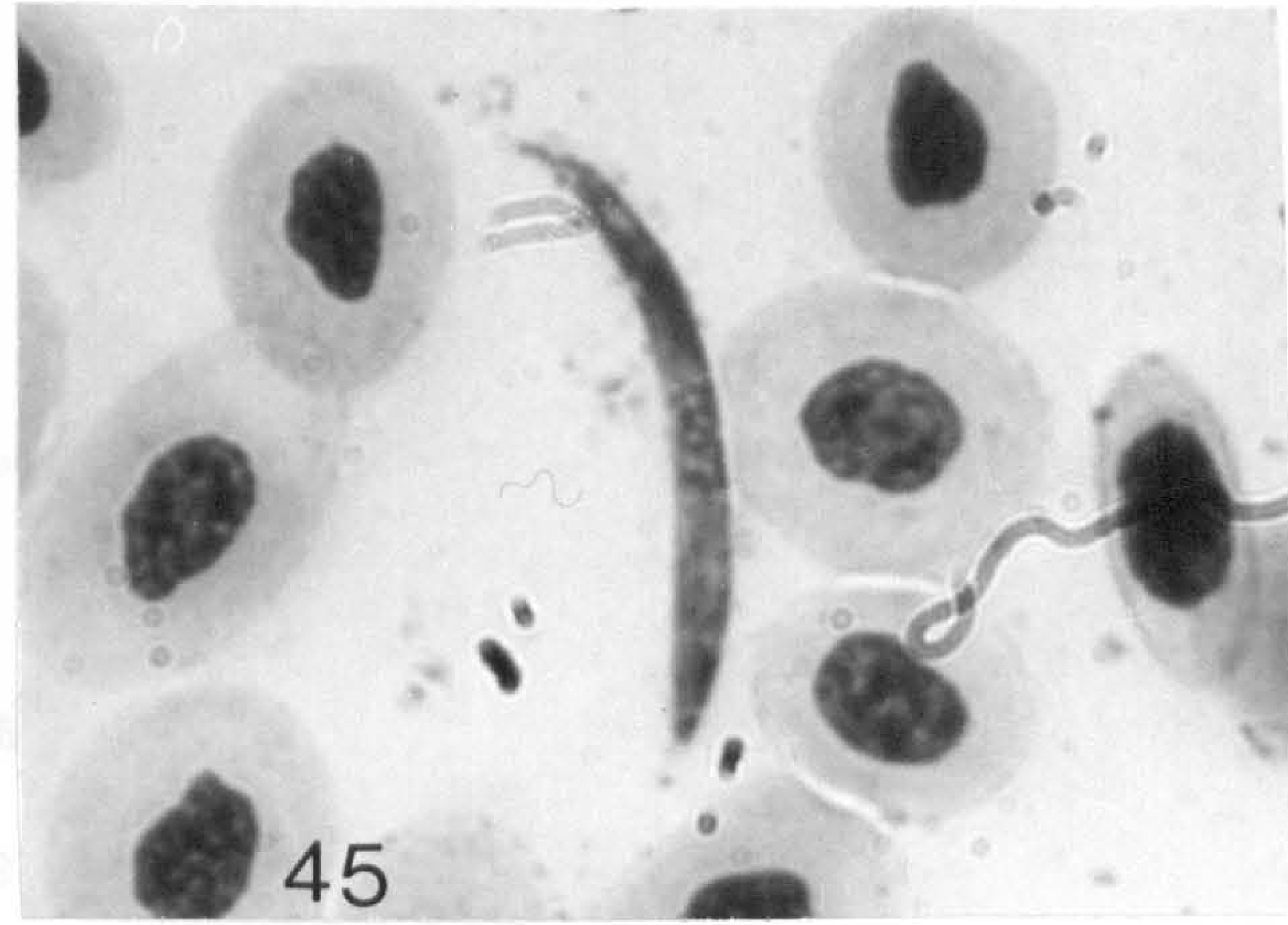
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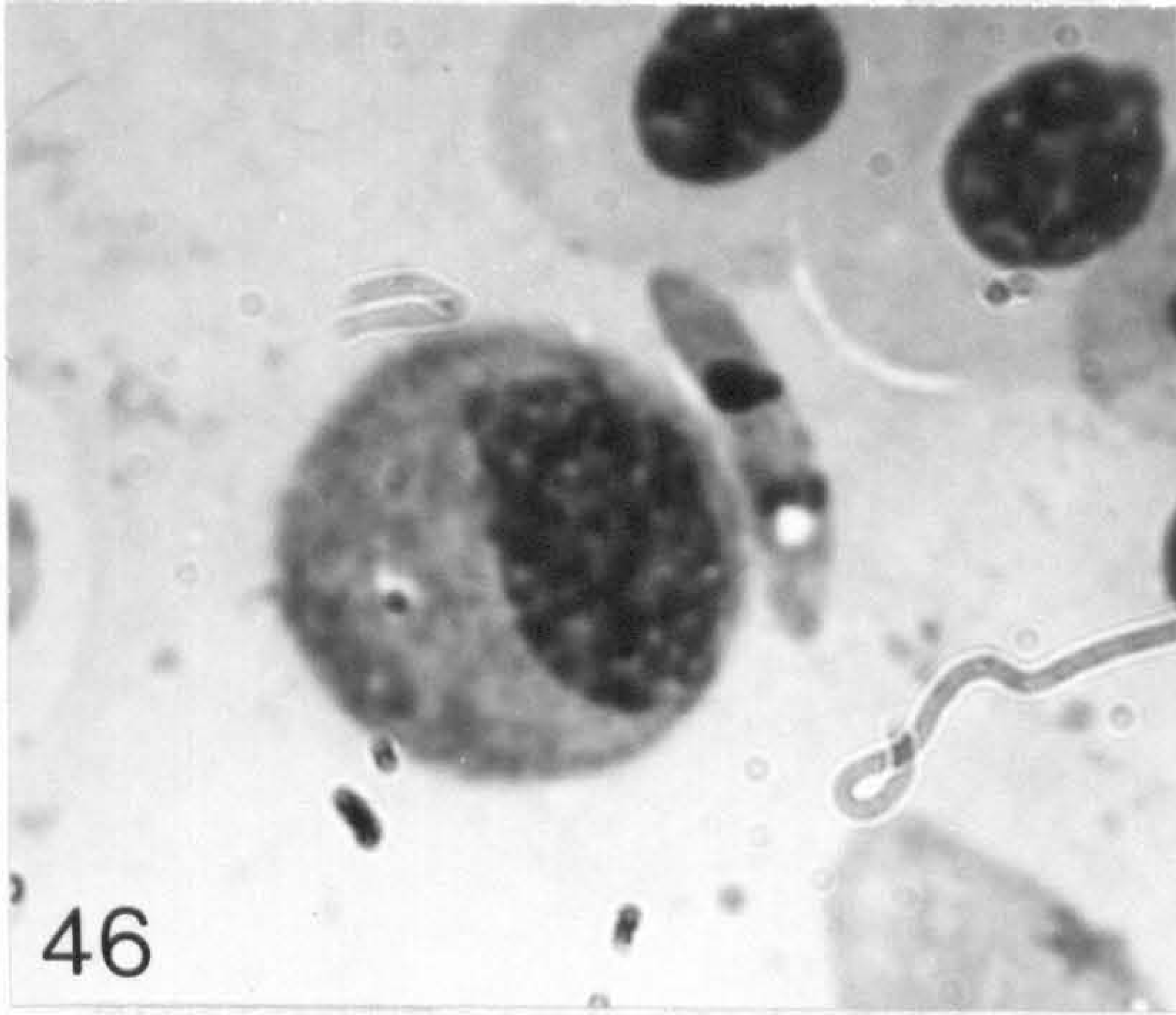
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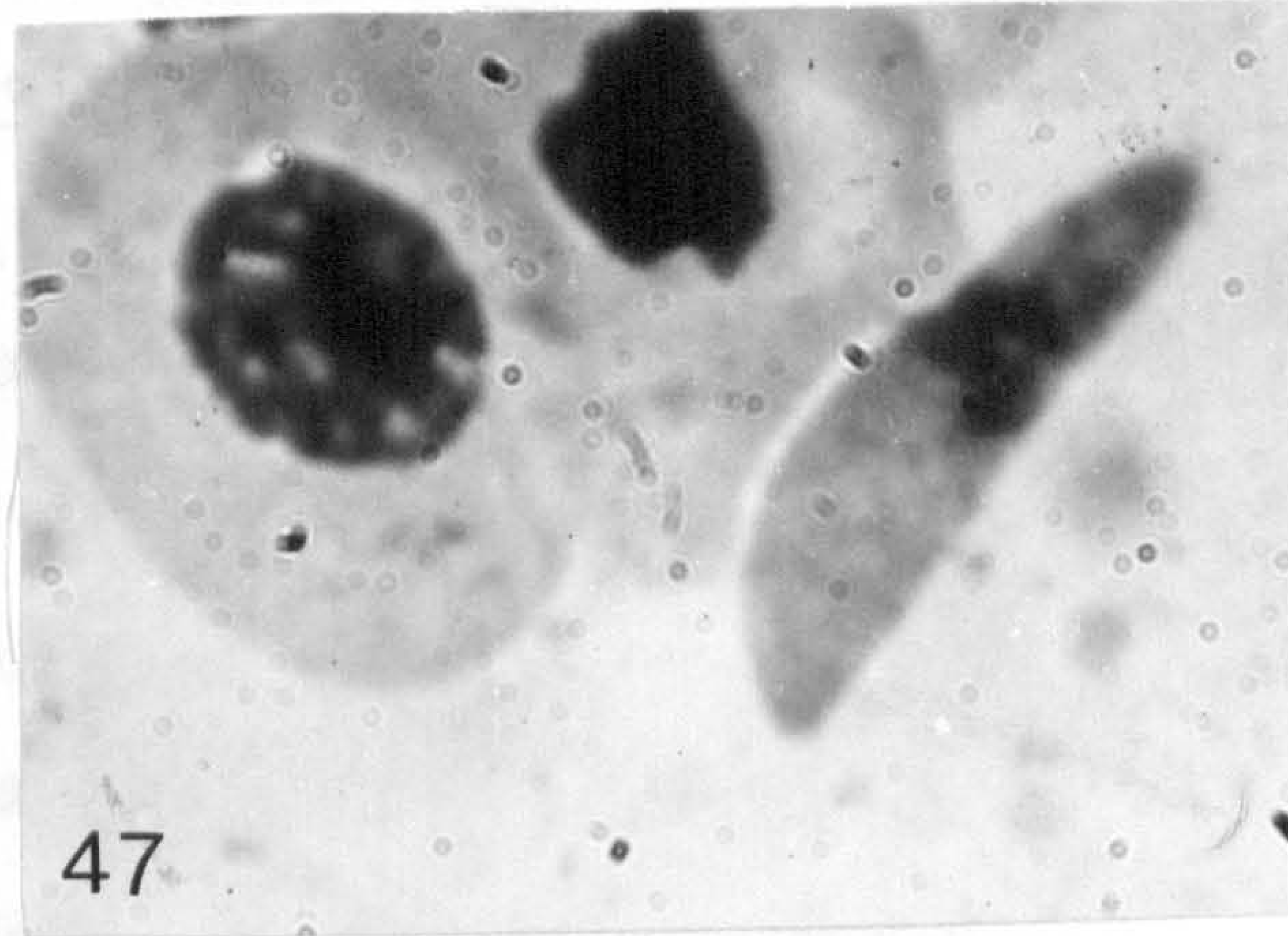
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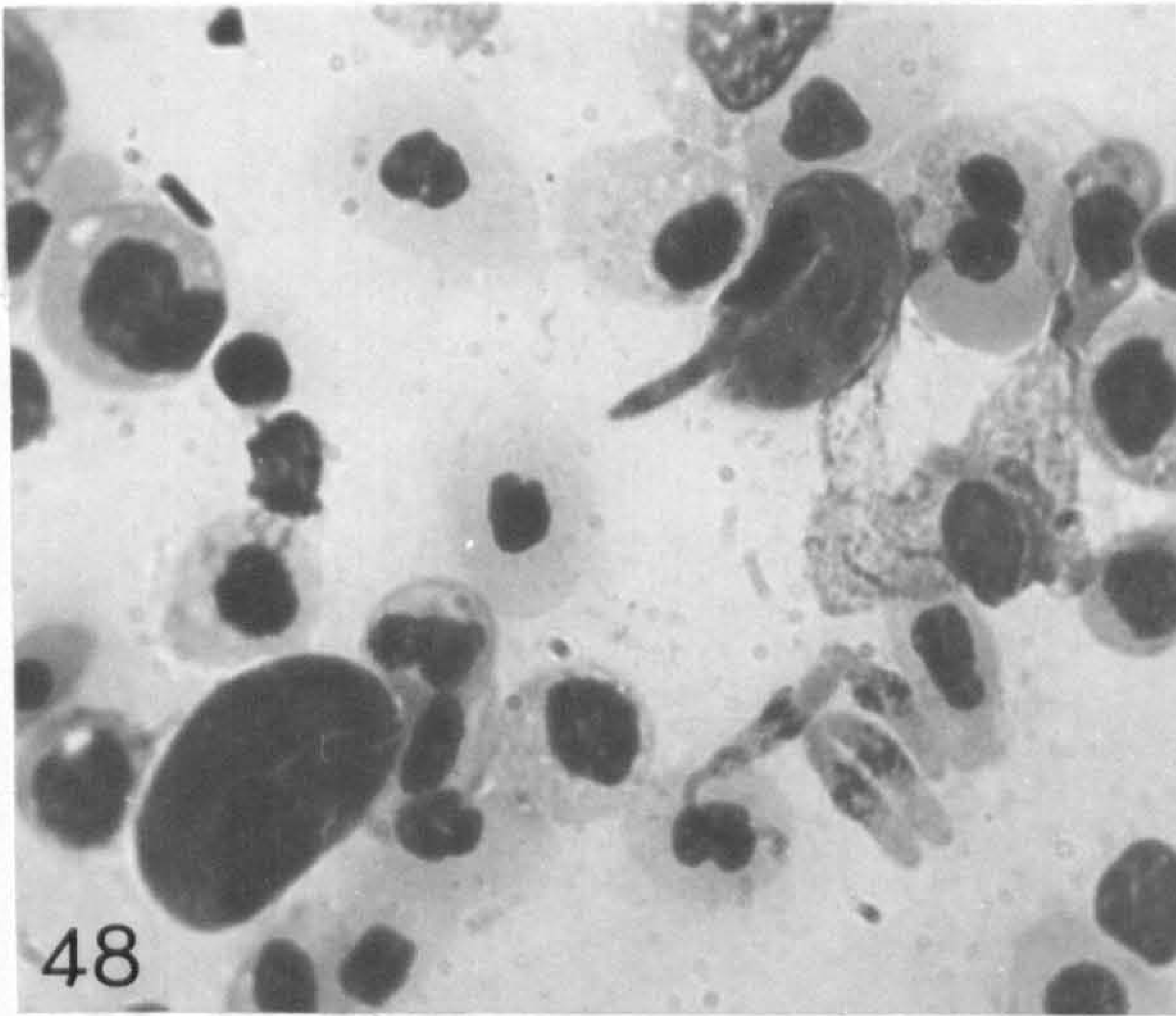
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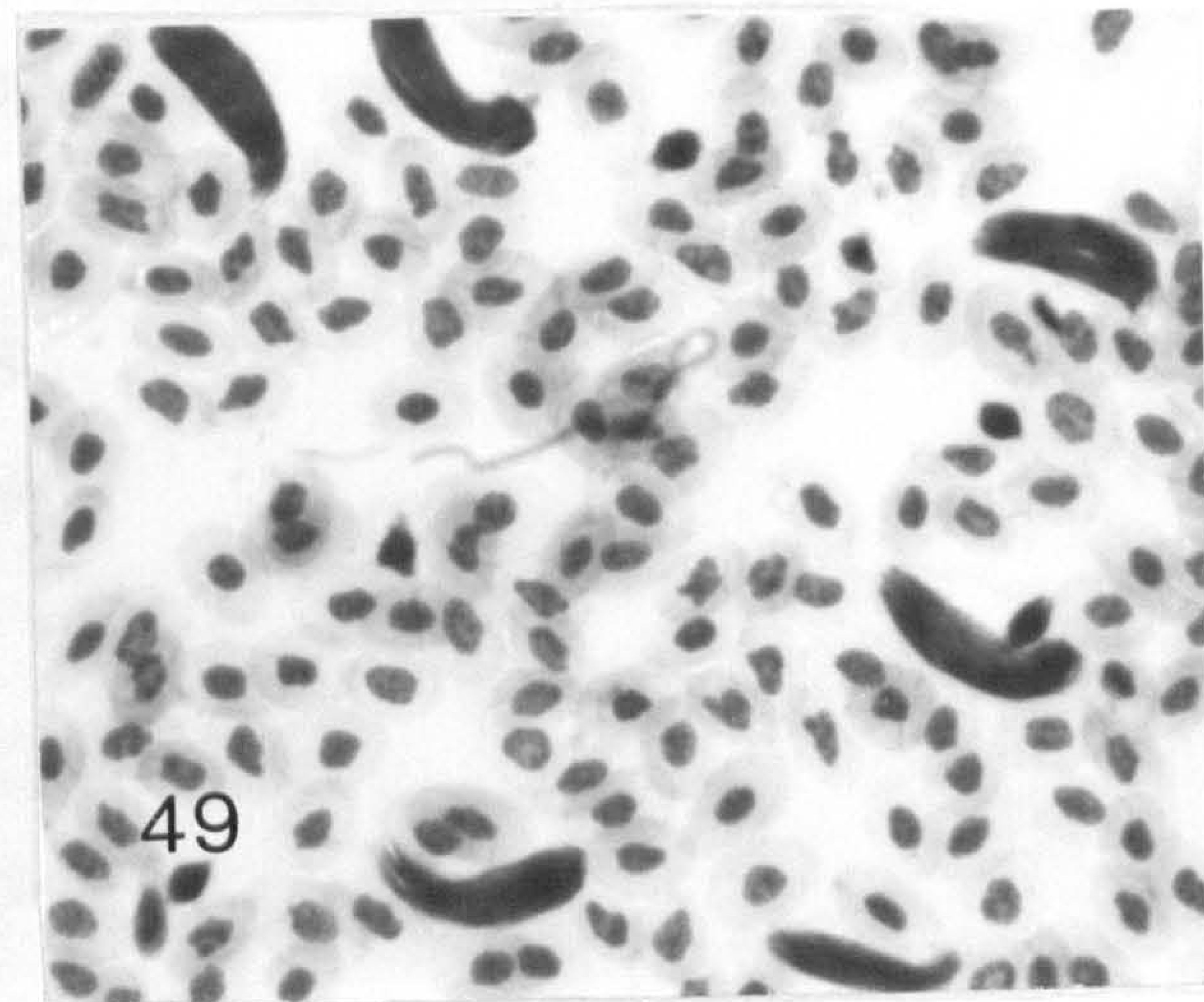
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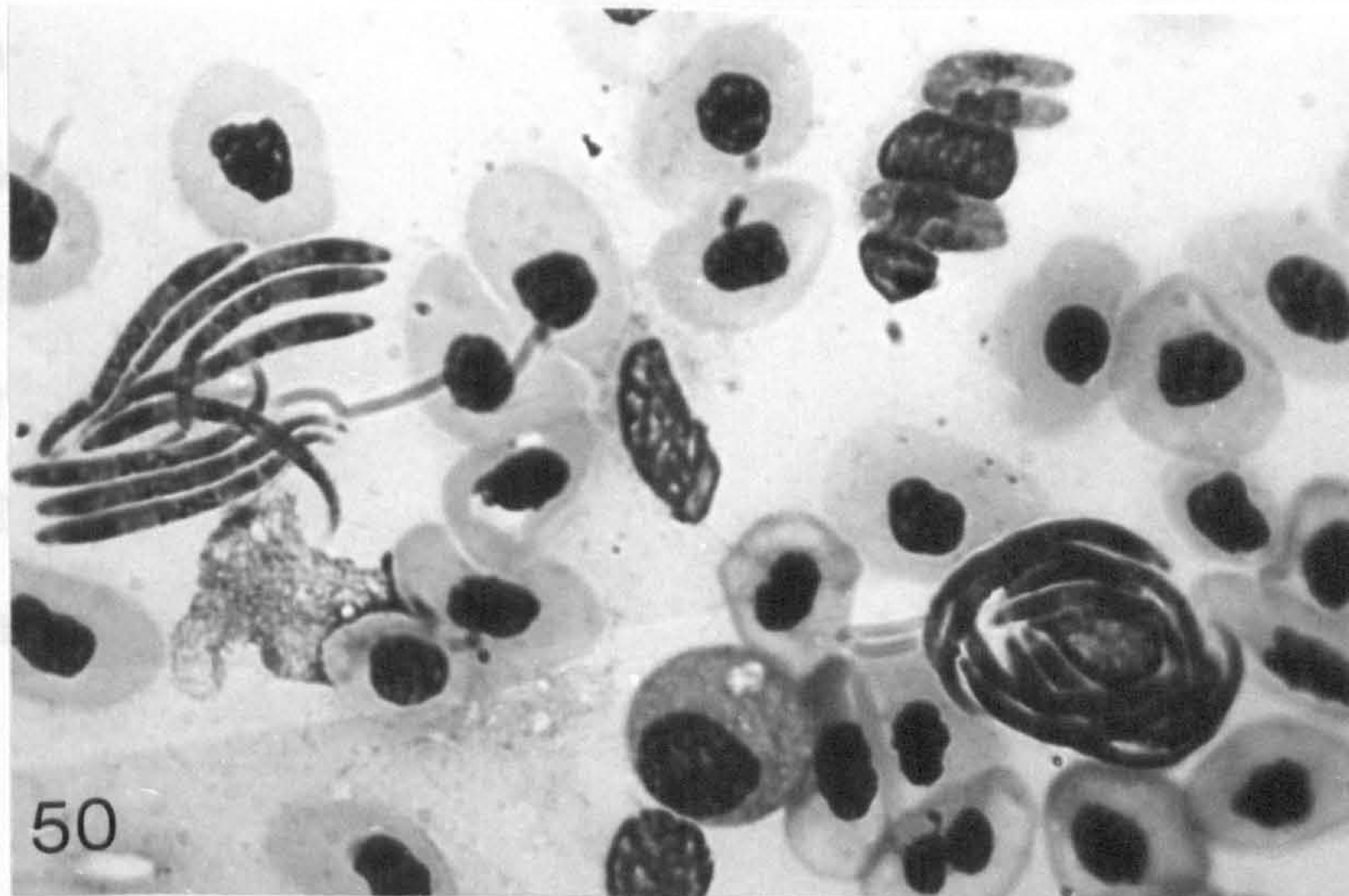
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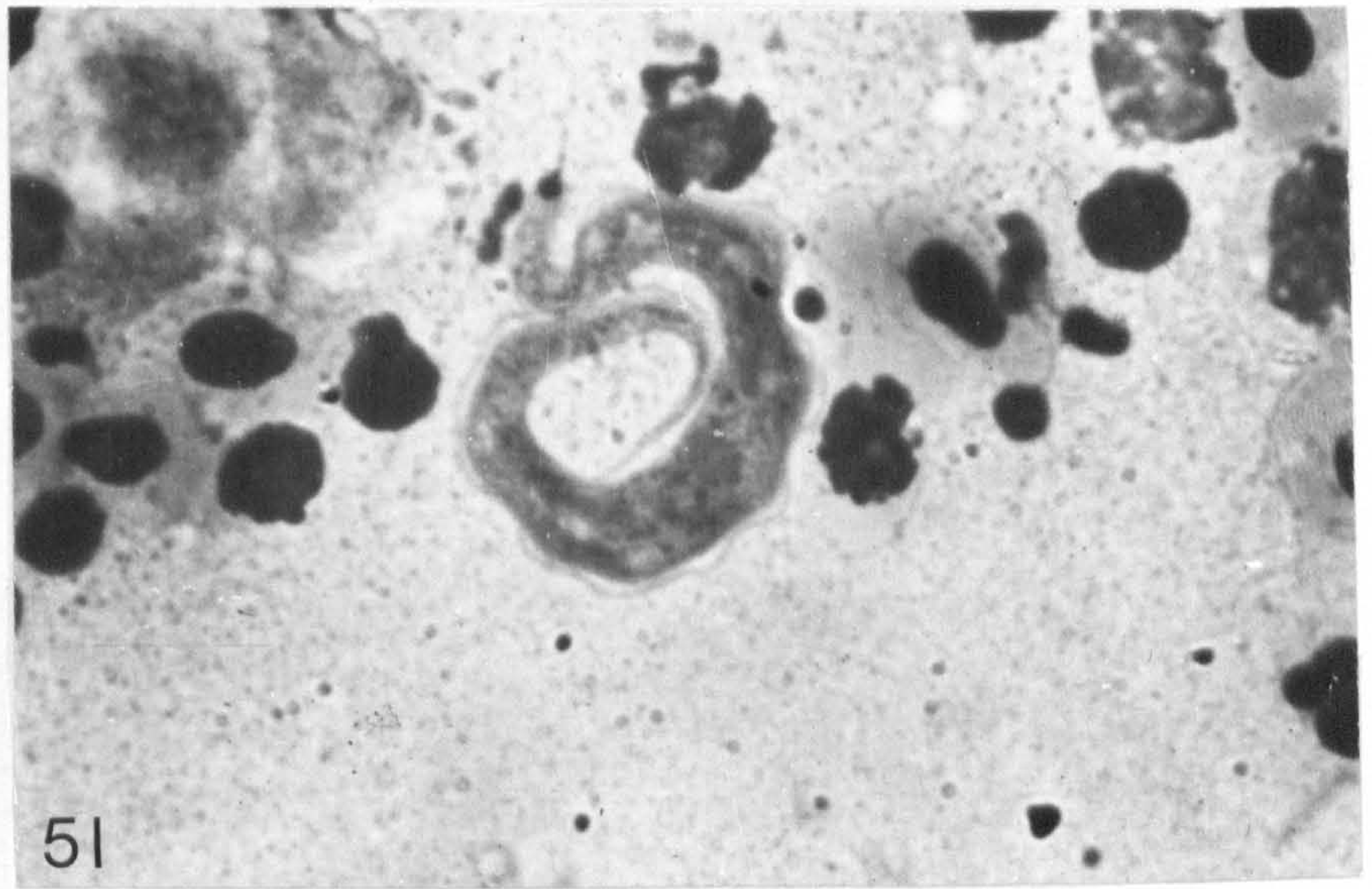
4.4.2. Trypanosoma soleae (Laveran and Mesnil,1902)

In all the blood smears examined of wild and farmed marine fish during the present study only one example of a trypanosome was encountered concurrently with an infection of Haemogregarina simondi in the same wild fish host, a Solea solea from the Atlantic in France. This single trypanosome was found in a curled-up position in the blood plasma (Fig.51). It_s total body length was 63.4 micron and its width at the site of the nucleus measured 5.0 micron. The eosinophilic nucleus was relatively large and round and measured 3.1 by 3.1 micron. It was centrally located and at a distance of 31.5 micron from the tapering,pointed end and 28.8 micron from the anterior end. The free flagellum was short and fine pointed and measured 6.25 micron, so that the overall length of the parasite came to 69.65 micron. The flagellum continued in the form of a distinct undulating membrane of an average width of 0.9 micron towards the posterior end where it appeared to originate from a minute blepharoplast at approximately 3-4 micron distance from the posterior end. A kinetoplast was hardly discernable and appeared to have an approximate size of 0.2 by 0.1 micron. The cytoplasm of this trypanosome was characterized by numerous smaller and larger

FIGURE 51. TRYPANOSOMA SOLEAE
in blood smear of wild *Solea solea*.
Nucleus (n), free flagellum (f) and
undulating membrane (um). x 1,500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vacuoles distributed throughout the body. Also a number of dark staining granules were observed, especially in the posterior part of the body, thus obscuring the kinetoplast. What appeared to be micronemes in parallel arrangement were seen in the posterior 1/3 of the body. This trypanosome was compared with earlier descriptions and identified as Trypanosoma soleae from the type host Solea solea.

4.5. Haemohormidium sp.(Henry,1910) from marine fish

Out of all the Scophthalmus maximus examined at various fish farms only a very small percentage (0.3%) were parasitized by this babesioid. Most of these infections appeared light and did not seem to have any effect on the host cells. The parasites were only found in erythrocytes. The majority of these cells harboured only one merozoite but cells with 2 or 3 parasites were also encountered. These amoeboid babesioids took up very little stain at all and therefore were difficult to visualize and were better observed in overstained preparations. That was probably the reason why they were not detected more often. The periphery of these parasites stained faint to medium blue with Giemsa but the central zone assumed a paler stain than the surrounding host cell

cytoplasm. Overall measurements ranged from 1.25 to 2.5 micron in length (average 1.63) and from 0.9 to 1.2 micron in width (average 1.25). Small chromatic granules often accompanied these amoeboid bodies whose shape was mainly oval,rounded or ellipsoid but did not show any division stages in the present case which might have been considered to be schizonts. In some infections every single erythrocyte seemed to contain from 1 to 3 merozoites. These babesioid parasites were considered to be *Haemohormidium* sp. and they were occasionally also observed in *Solea solea* and other marine fish.

5. DISCUSSION

5.1. Survey of wild fish populations

The present survey of wild fish populations was of necessity somewhat limited, but no more so than those of previous workers in the field (Tables 2-6). Only in one species of marine fish were sufficient numbers of parasites encountered to enable description although many of the fish examined, at two different seasons in France were recorded as harbouring haematoprotozoan parasites (Table 1). Most importantly no haematoprotozoan parasites were ever detected in juvenile or in adult Pleuronectes platessa although large numbers were examined from various locations (France:10; Oban:127; Millport:2; Aberdeen:50). That is completely in disagreement with the observations by Lebailly (1904); Robertson (1906) and Henry (1910) who regularly found Haemogregarina platessae even in their rather small samples. It is very difficult to explain such anomalies although migration of final host,or even vectors, as well as seasonal and zoogeographical factors may be involved.

The advent of mariculture, with its opportunities to study a fixed ecosystem, and to examine the same groups of fish over long seasonal periods should help to resolve the hitherto anomalous results which have emanated from spasmodic surveys of wild fish populations which have served to produce descriptions of isolated stages in life cycles with very little information on intermediate hosts or host and parasite relationships.

5.2. Life cycle of haemogregarines of marine fish in their final hosts

5.2.1. *Haemogregarina sachai* n.sp. in *Scophthalmus maximus*

In *Haemogregarina sachai* n.sp. schizogony occurred within leucocytes of the final host *Scophthalmus maximus* and was followed by a second schizogony within erythrocytes and erythroblasts leading to the production of gametocytes. The only similar development pattern in a haemogregarine is that described by Laird (1953) for *Haemogregarina bigemina* of the marine fish *Ericentrus rubrus*. He observed the production of merozoites within small and large lymphocytes and monocytes especially in

young fish. Gametocytes were rarely seen and only in erythrocytes of older fish. Such a difference in susceptibility according to age was not seen in the present study where all infected fish were in the age group 0-1 year. The earliest stages observed by Laird were ovoid merozoites of 3.0 by 2.0 micron lying free in the plasma of the host. These were followed by dividing stages within leucocytes measuring an average of 3.7 by 3.2 micron and thus appearing smaller than the more vermiform merozoites of H.sachai n.sp. within the small lymphocytes of Scophthalmus maximus (5.5 by 1.4 micron). Laird also observed vermiform merozoites resulting from intermediate schizogonies in leucocytes measuring an average of 5.5 by 0.7 micron as compared with the corresponding stage of H.sachai n.sp. which had an average size of 5.7 by 2.7 micron (small lymphocytes); 7.8 by 2.3 micron (neutrophils) and 8.1 by 1.3 micron (monocytes),respectively. In addition ovoid parasites were also observed in H.sachai n.sp. measuring 5.4 by 3.3 micron in neutrophils and 6.0 by 2.6 micron in monocytes and thus were considerably larger than the stages found in H.bigemina. The commonest number of intraleucocytic merozoites for both haemogregarines was 4 but the maximum number for H.bigemina was only 10 whereas H.sachai n.sp. had schizonts with up to 36 intracellular merozoites.

When these merozoites are freed, either as a result of their own active movement within the cell or after a breakdown of the host cell because of the large excessive number of parasites such as in H.sachai n.sp., they invade erythroblasts and erythrocytes. Intra-erythroblastic stages of H.sachai n.sp. measured an average of 6.2 by 1.6 micron with the corresponding stage in H.bigemina measuring 5.5 by 3.0 micron. Only one intracellular merozoite was observed in H.sachai n.sp. whereas Laird on rare occasions also observed two merozoites in a host cell.

In both H.bigemina and H.sachai n.sp. 2 gametocytes are eventually formed. A differentiation in micro-gametocytes (10.9 by 1.1 micron) and macro-gametocytes (5.5 by 2.0 micron),always produced in pairs, was seen in H.sachai n.sp., but Laird only mentioned an elongate crescentic stage of 11.38 by 1.25 micron, the "mature gametocyte" (presumably the micro-gametocyte). Similar dimensions were given by Laveran and Mesnil(1901) in their original description of H.bigemina of Blennius pholis and Blennius gattorugine,i.e. 12.0 by 1.75 micron.

5.2.2. *Haemogregarina simondi* in *Solea solea*

The life cycle of *H.simondi* as observed in the present study was similar to the description of *H.bigemina* (Laird,1953) and that of *H.sachai* n.sp. (*vide supra*). Although *H.simondi* also developed first by intraleucocytic schizogony followed by intraerythrocytic schizogony there was a difference in the number of merozoites produced during schizogony. In contrast to *H.bigemina* (10) and *H.sachai* n.sp.(36) only 8 merozoites were produced in intraleucocytic schizonts of *H.simondi*. The ensuing intraerythrocytic schizogony also produced 8 merozoites and no schizogony was ever observed within erythroblasts. The sequence of production of gametocytes,however, was more difficult to interpret. All of the stages of *H.simondi* found in the circulating blood of *Solea solea* were significantly larger than those observed in either *H.bigemina* or *H.sachai* n.sp. and the final intra-erythrocytic merozoites measured an average of 16.3 by 1.7 micron as compared with the corresponding stage from erythroblasts in *H.bigemina* (5.5 by 3.0 micron) and *H.sachai* n.sp. (6.2 by 1.6 micron). They were released after rupture of the host cell and appeared to differentiate again but it was impossible to determine here whether they all developed into

micro-gametocytes or whether some of these developed also into macro-gametocytes. Gametocytes were only encountered free in the plasma. There was a clear differentiation into slender,elongate micro-gametocytes of 21.2 by 1.4 micron in average and the rarely observed stouter macro-gametocytes of 12.45 by 4.45 micron in average. Neumann (1909) observed a similar dimorphism in H.torpedinis of the marine fish Torpedo ocellaris where micro-gametocytes measured in average 16.0 by 1.5 micron and the somewhat stouter macro-gametocytes 18.0 by 4.5 micron and believed that such a sexual differentiation might also be the case in H.polypartita of Gobius paganellus .Sexual dimorphism was also described by Minchin and Woodcock (1910) in H.rovignensis of Trigla lineata , the slender micro-gametocytes measuring 12.0 by 2.1 micron and the ovoid macro-gametocytes measuring 10.6 by 3.4 micron, and by Kohl-Yakimoff and Yakimoff (1915) in H.yakimovi-kohl of Gobius capito where micro-gametocytes measured 10.64 by 1.77 micron and macro-gametocytes measured 9.94 by 2.84 micron. In addition,Lebailly (1905) had suggested that the two haemogregarines of different generic status found at the same time in Callionymus lyra and described earlier by Brumpt and Lebailly (1904) might have been different sexual stages of the same haemogregarine. These were H.quadrigemina where free gametocytes measured 17.0 by 1.8 micron (probably the

micro-gametocytes) and H.callionymi where the corresponding stage measured 12.0 by 3.5 micron in average (probably the macro-gametocytes). From these few accounts and the results of the present study on H.simondi and H.sachai n.sp. it is assumed that in most,if not all,haemaogregarines of marine fish there exists indeed a sexual differentiation of the gametocytes. This was confirmed for other members of the genus Haemogregarina by Reichenow (1910,1921) who described it first in H.stepanowi of Emys orbicularis.

H.simondi was the first haemogregarine described from fishes and also the first one observed in a marine fish (Laveran and Mesnil,1901).Lebailly (1904, 1906) repeated the description of H.simondi and added a few of his own observations. Additional descriptions of this haemogregarine were given by Henry (1910,1913 a.and d.).All of these observations described only an intraerythrocytic schizogony with the production of 8 merozoites and free gametocytes measuring an average of 20.0 by 2.0 micron (presumably the micro-gametocytes) thus confirming the results of the present investigations for the schizogonic cycle of H.simondi in erythrocytes. Most of the other descriptions of haemogregarines from marine fish have been restricted to the intraerythrocytic and

gametocyte stage only and usually the macro-gametocytes were not detected.

5.3. Intra-leucocytic stages of haemogregarines

According to Wenyon (1926),the first record of an intraleucocytic parasite of marine fish was reported by Neumann (1909) who intended to found a new genus *Globidium* with the type species *Globidium multifidum* for a parasite which he discovered in the *mononuclear leucocytes* or *endothelial* cells of *Gadus aeglefinus*. Since this name had been given already to a totally distinct parasite, Brumpt(1913) ammended it to *Globidiellum*. Later,Laird (1953) compared the rounded and pyriform intraleucocytic schizonts of *H.bigemina* with certain of the life-history stages of *Globidiellum multifidum*. However, it is evident from the original publication by Neumann (1909) that he described this parasite *exclusively* from *erythrocytes* only and from two very different hosts,i.e. *Arnoglossus grohmanni* (syn.*A.kessleri* Schmidt,family *Bothidae*) and *Gobius minutus* (syn.*Pomatoschistus minutus* Pallas) and *not* from *Gadus aeglefinus*. In addition,Neumann had observed his *Globidium multifidum* together with *Haemogregarina minuta* in the same host *Gobius minutus* which made him assume that the new parasite might be representing a stage in the life cycle of the haemogregarine.

Henry (1913 c) was the first worker in this field who found parasites morphologically resembling haemogregarines within leucocytes of the circulating blood of Gadus aeglefinus and described them as Leucocytozoon sp. The youngest form observed measured 3.0-4.0 micron in length,was oval shaped and occupied the cytoplasm of a mononuclear cell,either a leucocyte or an endothelial cell. These intracellular merozoites grew to a length of 9.0 micron and divided actively and multinucleate forms with up to 56 merozoites per schizont were seen. When these schizonts ruptured free merozoites of 3.0 micron in length and 1.5-2.0 micron in width appeared in the plasma. In addition,Henry encountered a haemogregarine within erythrocytes but only one individual parasite was present intracellular. A differentiation of the parasite was apparent and Henry suggested that the elongate,slender form measuring an average of 9.4 micron (range 8.5-10.5) by 2.2 micron (range 2.0-3.0) might be the male form of the parasite (micro-gametocyte) and that the broad, stout forms measuring an average of 12.7 micron (range 11.0-15.0) by 3.6 micron (range 3.0-4.0) might be the female parasites (macro-gametocytes).He also found intermediate forms between the two stages.and he described this intraerythrocytic parasite as Haemogregarina aeglefini. Henry further compared the intraleucocytic stages with the Koch's bodies in

Theileria parva and the multinucleate plasmodial masses in enlarged leucocytes of *Haemoproteus columbae* and concluded that he might be dealing with the first schizogony undergone by the sporozoites of *H.aeglefini*. Since it is now secured that the haemogregarines of marine fish may have a life cycle in the final host with schizogony taking place within leucocytes as well as erythrocytes, such as was shown in *H.bigemina* (Laird,1953),*H.sachai* n.sp. and *H.simondi*, it is postulated that Henry (1913) was indeed the first person to observe the complete life cycle of a marine haemogregarine,i.e.*H.aeglefini* in the final host,i.e.the teleost *Gadus aeglefinus*.

Other authors have only occasionally encountered stages of haemogregarines within leucocytes. Yakimoff (1917) found only leucocytes infected in an unidentified fish from the river Kars-tschai in Russia. The parasite measured 9.0 by 4.5 micron and Yakimoff,completely ignoring the earlier report by Henry (1913 c),claimed this to be the first account of an intraleucocytic haemogregarine and designated it *Leucocytogregarina ninae-kohl-yakimovi*. Fantham *et al*.(1942) reported the occurrence of banana shaped intracellular organisms of an average size of 8.5 by 1.5-2.0 micron parasitizing predominantly polymorphnuclear leucocytes but also mononuclear cells in the blood of *Salvelinus fontinalis*.

Larger vermiform parasites measuring from 11.6-18.9 micron in length and from 1.8-3.7 micron in width were also seen and occasionally observed free in the plasma. A differentiation in micro-and macro-gametocytes was also suggestive but no measurements were given. The organism was placed in the genus Leucocytozoon and named Leucocytozoon salvelini. Laird (1952) found a large lymphocyte of the marine fish Coelorhynchus australis containing a mature gametocyte of H.coelorhynchi which had caused fission of the host cell nucleus but did not further comment on it. Saunders (1954) described a new Haemogregarina sp. from the fish host Cynoscion nebulosus. She found only one enlarged leucocyte of 44 by 20 micron containing 17 merozoites measuring from 9.0-10.0 micron in length and from 1.0-2.0 micron in width.Bullock(1958) detected a single reniform haemogregarine within the cytoplasm of a leucocyte of Spheroides maculatus. Laird and Bullock (1969) recorded numerous intra-leucocytic haemogregarines measuring 4.7-5.5 by 3.9-5.1 micron in a bloodsmear from Liparis atlanticus. All of these reported intraleucocytic stages of haemogregarines might have been part of the life cycle of these parasites,although Laird and Morgan(1973) also found intraleucocytic stages which they claimed had been phagocytosed by the leucocytes because they observed a nuclear and cytoplasmic breakdown of the parasites.

S E C T I O N B

VECTORS OF HAEMATOPROTOZOAN PARASITES OF MARINE FISHES

1. INTRODUCTION

During an investigation of a proliferative disease of farmed turbot (Scophthalmus maximus) caused by a hitherto undescribed haematoprotozoan parasite the question arose as to how this parasite was transmitted to such fishes. Attempted transmission of the parasite to apparently healthy turbot from various sources using different routes of inoculation and with either parasitized blood and lymph or with suspensions of "neoplastic" lesions was unsuccessful. However, no immunological tests were performed on these turbots prior to inoculation and so the immune status of the fish used in the experiments was unknown. Other experiments to stimulate or induce the disease, i.e. the use of cortisone to supress the immune response or stressing the fish were equally negative. The parasite was also found not to be pathogenic for small laboratory rodents and cats. Since the parasite could not be transmitted mechanically it appeared that perhaps an invertebrate or vertebrate vector might be involved. However, no ectoparasites except for Trichodina sp. on the gills were found during this study and planned experiments using marine leeches as intermediate hosts did not materialize (Kirmse, 1975).

There are several possibilities of a mechanism for transmission of the turbot parasite and other related haematoprotozoan parasites of marine fishes.

1. The parasite might either deliberately or accidentally enter the host orally by ingestion of a possible vector. This was demonstrated for mammalian trypanosomes (Kirmse and Taylor-Lewis, 1976) and can be found also in haemogregarines as for example in Hepatozoon sp. (Furman, 1966); Karyolysus sp. (Bergle, 1970, 1971); and Haemogregarina sp. (Garnham, 1954), as well as in the coccidian Sarcocystis sp. (Gestrich,Heydorn&Bayşu,1975) where free merozoites can be found also in the circulating blood.

2. An invasion of the infectious particles might occur through the bite of a bloodsucking vector, i.e. a marine leech or ectoparasitic copepod. Similar modes of infection are known with leeches: as vectors of mammalian trypanosomes (Soltys and Woo, 1968); marine fish trypanosomes (Robertson, 1907, 1909); and chelonid haemogregarines (Reichenow, 1910); with ticks: as vectors of trypanosomes (Cross and Patel, 1921); mammalian piroplasms (Shortt, 1973) and tortoise haemogregarines (Brumpt, 1938); with tsetse flies: as vectors of salivarian trypanosomes (Hoare, 1972) and haemogregarines (Chatton and Roubaud, 1913);

and with mosquitoes: as vectors of haemosporidians (Garnham, 1966) and haemogregarines (Booden *et al.*, 1970).

3. A contaminative route of transmission might be sought as a result of faecal contamination of the bite wound made by bloodsucking invertebrates. This mechanism is found for example in the case of *Trypanosoma cruzi* and the faeces of *Rodnius prolixus* (Hoare, 1972); or in *Haemogregarina triatomae* transmitted to the lizard *Tupinambis teguixin* by faeces of the bloodsucking reduviid *Triatoma rubrovaria* (Osimani, 1942). There might also occur an accidental or intentional ingestion by the host of faeces of the vector containing infectious stages of the parasite. This has been observed in *Toxoplasma gondii* (Frenkel and Dubey, 1972) which is sometimes found also in the circulating blood of the final host.

The trypanosomes, trypanoplasms and intracorpuscular haematoprotozoan parasites such as the haemogregarines, *Haemohormidium* sp. and *Dactylosoma* sp. found in the blood of fishes exhibit forms that are analogous to those found in warm blooded vertebrates. It can therefore be assumed that transmission takes place in fishes as in higher animals by means of a vector.

In the present situation it was considered most likely that a blood-sucking invertebrate was involved since this is the means by which at least some of the major groups of fish haematoprotozoans, e.g. trypanosomes and trypanoplasms, appear to be transmitted, namely by the blood-sucking piscicolid leeches. So far this route has been proven for trypanosomes and trypanoplasms but not for haemogregarines. Therefore an attempt was made to define the mode of transmission of the turbot parasite and other intracorpuscular haematoprotozoan parasites of marine fishes to ascertain that marine leeches were in fact the intermediate hosts for haemogregarines also.

2. REVIEW OF THE LITERATURE

2.1. Vectors of Trypanosomes

As early as 1857 Leydig encountered flagellate parasites with undulating membranes in the alimentary tract of the piscicolid leeches *Piscicola* sp. and *Pontobdella* sp. and in the chelonid tick *Ixodes testudinis*. Labbé (1894) found very fast moving stages of flagellates in the digestive tract of leeches. Doeflein (1901) based on Leydig's observations erected the hypothesis that these flagellates are transmitted to their vertebrate hosts by leeches in which they accomplish part of their life cycle. Laveran and Mesnil (1901) described the first trypanosomes from marine fishes and suggested that the intermediate host for the fish haematoprotozoan parasites might be sought among the blood-sucking ectoparasites.

In 1902, Laveran and Mesnil found numerous leeches of the species *Hemibdella soleae* on the skin of infected soles (*Solea solea*), which supported their view that the Ichthyobdellidae, leeches of freshwater

and marine fish, played a major role in the transmission of these blood parasites. However, they never found any leeches on the skin of the numerous infected blennies (Blennius pholis; Blennius montagui) which they examined and they suggested that perhaps parasites of the gills might be serving as intermediate hosts. They strongly recommended that research on the vectors of these haematoprotozoan parasites should be carried out and postulated the importance of such vector studies in fish. Around this time Siegel (1903) showed for the first time the definite role of leeches in the transmission of the haemogregarines of turtles.

Hofer (1904) supported Leydig's view that leeches are carrier of trypanosomes ingested from an infected fish and suggested that the eradication of fish leeches could become a major task for fish farming since leeches appear to be quite abundant in their distribution among fishes in culture and could serve as vectors for many hitherto unknown fish diseases. Léger (1904) fed Piscicola sp. on the blood of Cobitis barbatulae exclusively infected with Trypanosoma barbatulae and observed in their intestinal tract stages which belonged apparently to the life cycle of these parasites. But he did not know how these flagellates were inoculated

into the fish. In Hemiclepsis marginata, another leech of the family Glossiphoniidae, which had fed on the blood of the same fish species, the latter being infected with Trypanosoma varium, he found after several days, numerous trypanosomes in various stages of development.

Brumpt (1904) in a preliminary note gave some consideration to the evolution of the haematoprotozoan parasites of fishes. He investigated a number of species of the Hirudinea from amphibians, reptiles, birds and a variety of fishes e.g. Hemiclepsis marginata, Platybdella soleae (= Hemibdella soleae), Platybdella scorpii, Pontobdella muricata and Piscicola geometra. In the digestive tract of Hemiclepsis marginata numerous trypanosomes, smaller and with different morphological structure from those of the fishes were seen. He also studied the progeny of these heavily parasitized leeches but never encountered any trypanosomes, which lead him to the assumption that the infection was not hereditary and that transovarian transmission did not take place. These small trypanosomes from the leeches were experimentally inoculated into other marine fishes but although observed for more than 16 days these fishes never exhibited any flagellates in their blood. Brumpt (1905) remarked that some species of trypanosomes develop exclusively in

the digestive tract of Clepsines whereas others only develop in Piscicola sp. He found that during their multiplication in the digestive tract of the leeches the trypanosomes take on the "herpetomonas" form. When they stay longer in the intestine they settle in the mucosa of the gut and take the shape of "gregarines". The inoculation into the fish follows the passage of parasites from intestine to the proboscis.

Brumpt (1906 a) first definitely established the evolution of trypanosomes in fishes when he described the life cycle for Trypanosoma granulosum of the eel Anguilla anguilla taking place exclusively in Hemiclepsis marginata. The parasite lost its flagellum in the stomach of the vector, active division took place and later it re-acquired the flagellum. Then it developed into very small trypanosomes which were inoculated into the final host where they grew to normal size.

His subsequent experiments with other leeches showed that not every leech was a suitable host for a given trypanosome and also that different trypanosomes could develop in the same leech. Thus the trypanosomes Trypanosoma soleae and Trypanosoma cotti developed only in the stomach of Calliobdella punctata and never reached the proboscis of the intermediate host. The trypanosomes Trypanosoma scyllii and Trypanosoma rajae

first developed in the stomach of *Pontobdella muricata*, then they entered the intestine where they re-acquired flagella and were actively dividing but they were never found in the proboscis, nor in the stomach again and it remained unknown how they were inoculated from the intestine into the fishes.

Brumpt (1906 b) distinguished three major groups of development in the intermediate host of freshwater fishes. The first group developed exclusively in the stomach and never reached the intestine or proboscis. At the moment when a leech sucks blood from a fish, the parasites leave the stomach and pass actively through the proboscis to infect the new host. This applies to *T. barbi*, *T. percae*, *T. acerinae*, *T. squalii* and others.

In the second group the trypanosomes begin their life cycle in the stomach,pass from there to the intestine, return to the stomach and enter from there into the proboscis, e. g. *T. granulosum* of the eel *Anguilla anguilla*.

In the third group the parasites develop in the stomach and enter from there into the proboscis from where they are inoculated into the final host. This was found to be the case in *T. cyprini* and *T. phoxini*.

A complete life cycle was apparently only observed in *Hemiclepsis marginata*. In *Calliobdella punctata*, *Hirudo troctina* and *Piscicola geometra* the cycle appeared incomplete and the infective stages never reached the proboscis.

Lebailly (1906) examined the digestive tract of several *Platybdella soleae* (= *Hemibdella soleae*) fixed on the back of *Solea solea* and found broad and vacuolated trypanosomes in smears of these leeches. He concluded that for marine fishes it was probably apparent that leeches served as intermediate hosts. Robertson (1907) made intensive studies on the evolution of a trypanosome found in the alimentary canal of *Pontobdella muricata* parasitic on skates (*Raja* sp.). The trypanosomes were as a rule in the intestine but were also found in the crop and proboscis in large numbers. Towards the end of digestion only long, slender trypanosomes were seen which migrated up into the proboscis and were considered the infective stages. The author found a very slow development of the trypanosomes which she linked with the extreme slowness of the process of digestion by the leech. Her work supported the opinion of Brumpt (1906 b) who had earlier suggested that the trypanosome in this leech formed part of the life cycle of *Trypanosoma rajae*. Robertson (1909) gave conclusive evidence that the

trypanosome stages appearing in the leech Pontobdella muricata were really Trypanosoma rajae. Uninfected leeches were hatched from a number of cocoons collected in the wild and were fed on skate (Raja sp.) infected with T. rajae. The skate trypanosome demonstrated the same stages in the leech as had been previously reported from leeches infected in nature. However, no transmission experiments with infected leeches to uninfected skates were performed.

Neumann (1909) investigating the possible vectors of haematoprotozoan parasites of fishes in the Mediterranean Sea was able to prove that the marine leech Pontobdella muricata was the vector for the skate trypanosome Trypanosoma giganteum from Raja oxyrhynchus. He also successfully transmitted Trypanosoma variabile with the same vector leech to apparently uninfected Raja punctata. The first developmental stages were seen after 10 days. This was the first experimental proof that trypanosomes from marine fish can be transmitted by marine piscicolid leeches. All stages of the two different trypanosomes seen in the leech corresponded to those observed by Robertson (1907, 1909). On Torpedo ocellata two different leeches were found: Pontobdella muricata and Branchellion torpedinis. Since both of these trypanosomes, T. giganteum and T. variabile,

make their secondary life cycle in *Pontobdella muricata* and since this leech and *Branchellion torpedinis* are the only leeches found in the Gulf of Naples the author suggested that it might be possible for one leech to serve as the host for the development of different trypanosomes. He also voiced the opinion that the major vectors of the trypanosomes of marine fishes could be various *Ichthyobdellidae* and the ectoparasitic isopods, copepods, trematodes or fish lice were probably only of secondary significance. Minchin (1909) found leeches attached to freshwater fishes only rarely. In the few specimens of *Piscicola* sp. he examined he did not find any stages of haemoflagellates.

Robertson (1911) demonstrated that leeches infected several species of freshwater fishes in one particular pond with trypanosomes after they were allowed to feed on other species of infected fish. Thus she transmitted trypanosomes from *Carassius auratus* with the leech *Hemiclepsis marginata* as vector to apparently healthy fish of the same species but also to *Perca fluviatilis* and *Abramis brama*. This demonstrated a lack of specificity in freshwater fish trypanosomes after leech passage. The trypanosomes found in *Hemiclepsis marginata* were not transmitted from parent to offspring.

Tanabe (1924) noted that the trypanosome of Misgurnus anguillicaudatus multiplied for a period of 3-4 days in the intestine of the leech Hirudo nipponica. However, no transmission experiments were carried out. Laird (1951) described Trypanosoma heptatreti from the hagfish Heptatretus cirrhatus but did not find any ectoparasites on them. However, in the Victoria College collection in New Zealand were some undescribed platybdellid-like leeches collected earlier from this fish species. The blood was not suitable any more for satisfactory blood smears of these leeches. The author nevertheless suggested that the hagfish leech might prove to be the vector for this trypanosome. He also described T.gargantua from the skate Raja nasuta and although no leeches were found it was assumed that a pontobdellid leech recorded from this skate in the past might prove to be the intermediate host.

Mackerras and Mackerras (1961) did not see any leeches on the shark Hemiscyllium ocellatum infected with T. gargantua which also parasitizes Raja nasuta. Khan (1974) has shown conclusively that trypanosomes of marine fishes are transmitted by leeches. He was able to transmit Trypanosoma murmanensis from the Atlantic cod (Gadus morhua) by a marine leech of the genus Myzobdella to apparently uninfected cod. Matthews pers. com. (1977) investigating T. platessae from the plaice

Pleuronectes platessa examined large numbers of plaice and turbot (Scophthalmus maximus) from the English Channel but was never fortunate enough to find any leeches on these fishes. Also Kirmse (1975) and Slinn (1977)pers. com. never encountered any leeches on turbot although the leech Branchellion torpedinis normally an ectoparasite of Torpedo ocellata has been recorded from turbot(Mann,1964).

2.2. Vectors of Trypanoplasms

Laveran and Mesnil (1902) were the first to describe the new genus Trypanoplasma but did not elaborate on the mode of transmission of this haemoflaggellate. Léger (1904) found Trypanoplasma varium frequently in the blood of Cobitis barbatula. He had on several occasions observed leeches of the Clepsinae (Hemiclepsis marginata) feed on infected fish and also various developing stages of the trypanoplasm in the intestinal tract of the leech. Tr. varium apparently developed in Piscicola sp. which had fed on the same species of infected fish. Brumpt (1906) studying the evolution of trypanoplasms in leeches found these haemoflagellates quite hostspecific. Thus Tr. abramidis of Abramis brama had a complete life cycle in Hemiclepsis sp. and Tr. barbi of Barbus fluviatilis developed exclusively in Piscicola sp.

However, *Tr. truttae* from *Salmo truttae* developed neither in *Hemiclepsis* nor *Hirudo* sp. but might have developed possibly in *Piscicola* sp.

The first real evidence that the life cycle of trypanoplasms depended on a leech vector for its completion was supplied by Keysselitz (1906). He demonstrated the different developmental stages of *Tr. borreli* in the leech *Piscicola* sp. The eggs of infected leeches were never found infected. But trypanoplasms were encountered in approximately every 25th cocoon examined, inside the yolk surrounding the egg. Surprisingly enough the author did not find any infection in newly hatched leeches. The search for these bloodflagellates in such ectoparasites of marine fishes as the crustaceans *Argulus foliaceus* and *Argulus coregoni* was unsuccessful but they were frequently found in leeches. The author never succeded in reproducing an infection in fishes with the help of infected leeches and assumed that this did not take place in nature. However, he did not suggest any alternative way of transmission although he believed that *Piscicola* sp. was a genuine intermediate host. *Tr. borreli* can develop also in other leeches and the beginning of such development was observed in *Hirudo medicinalis* which had fed on infected fish.

The life cycle of *Trypanoplasma* is considered to develop in two phases, a schizogony in the blood of fishes and a sexual phase which takes place in the intermediate host, the leech. Robertson (1911) was able to transmit trypanoplasms by means of the leech *Hemiclepsis marginata* from infected *Carassius auratus* to apparently healthy ones. The trypanoplasms enter the crop with the blood, multiply and produce slender forms, which pass forward into the proboscis-sheath from where they are injected into the fish. A healthy fish thus showed parasites in the blood 4 days after initial infection. Mavor (1915) suggested a leech as the probable vector for *Tr. borreli* from *Catostomus commersoni* a distant relative of European carp (*Cyprinus carpio*) and postulated that the blood parasite might have been introduced to Canada with carp brought from Europe for stocking purposes. Tanabe (1924) gave an acçount of the development of the trypanoplasms of *Misgurnus anguillicaudatus* in *Hirudo nipponica*. Katz (1951) dissected several *Piscicola salmositica*, ectoparasitic leeches of apparently trypanoplasma-free *Salmo gairdnerii* and found these full of several developmental stages of *Trypanoplasma* sp. Laird and Bullock (1969) also suggested leeches for transmission of *Tr. bullocki* even though the New England fish hosts *Liopsetta putnami* and *Pseudopleuronectes americanus* never bore leeches.

Trypanoplasma infections whether leech to flounder or flounder to leech were never achieved experimentally but trypanoplasma-positive leeches were collected from trypanoplasma-positive fishes. Kirmse (1975) describing *Trypanoplasma* sp. from *Scophthalmus maximus* suggested a leech vector as an intermediate host although the infected fish did not have any ectoparasites.

2.3. Vectors of Haemogregarines

To date no complete life cycle of a marine fish haemogregarine in an intermediate host has been described. However, a number of speculations have been made concerning the possible nature of such a vector. Laveran and Mesnil (1901) suggested that fish leeches are responsible for transmission because they had found numerous *Platybdella soleae* (= *Hemibdella soleae*) on all the *Solea solea* infected with *Haemogregarina simondi* which they examined. The first conclusive evidence for the hypothesis that leeches can serve also as vectors for haemogregarines was given by Siegel (1903) who described the life cycle of *Haemogregarina stepanovi* in the leech *Placobdella catenigera*. Similar observations were made by Brumpt (1904) who saw apparently developmental stages in the digestive tract of several leeches which

parasitized marine fishes: Platybdella soleae (=Hemibdella soleae) parasite of Solea solea; Platybdella scorpii parasite of Cottus scorpius; Trachelobdella lubrica parasite of Scorpaena porcus and Branchellion torpedinis parasitizing Squatina angelus and Trygon pastinaca. Stebbins (1904) however, showed that an intestinal mode of transmission might also be possible because he was able to transmit Haemogregarina catesbianae from the frog Rana catesbiana by contact between infected and apparently healthy frogs the latter probably infected by intestinal invasion of sporozoites. Lebailly (1906) found in Platybdella soleae (=Hemibdella soleae) from infected Solea solea typical forms of H. simondi. Also in smears he observed other stages which measured 22 by 2 micron and had 2 nuclei. Robertson (1907) occasionally encountered a haemogregarine, closely resembling H. delagei parasitic in the erythrocytes of skate (Raja sp.)e.g. Raja punctata,Raja mosaica in the intestine of Pontobdella muricata. However, the life cycle was not further elucidated. Neumann (1909) found occasionally ectoparasites which were bloodfeeders on fishes,e.g. Anilocra mediterranea on the skin of Sargus annularis and on Smaris vulgaris but no haematoprotozoan parasites were found in them. The free living forms of H. simondi were found in the digestive tract of Platybdella soleae (= Hemibdella soleae).

Henry (1912) found numerous leeches tentatively diagnosed as *Trachelobdella lubrica* on the gills of all the *Anarrhichas lupus* infected with *H. anarrhichadis*. The dissection of several of these leeches did not reveal any developmental stages of the haemogregarine though it was assumed that this leech was the actual carrier of the infection. Fantham, Porter and Richardson (1942) did not find any ectoparasites on marine fishes infected with haemogregarines. As late as in 1953 Laird stated: " Nothing is yet known of the means of transmission of the schizohaemogregarines or indeed piscine haemogregarines in general". Saunders (1958) reporting the occurrence of *H. bigemina* in marine fishes from the Bahamas could not find the invertebrate host from which the sporozoites invaded the fish hosts. The only ectoparasite she could find was a firmly embedded isopod on the gill-cover of one infected *Calamus bajanado*. The same author when investigating the haematoprotozoan parasites of marine fishes in Puerto Rico (Saunders, 1966) observed large numbers of ectoparasitic isopods clinging to these fishes. However, only 2 fishes of 174 members of the *Pomadasyidae* which he examined were parasitized by *H. bigemina* and neither of these had any ectoparasites. Three small, red copepods were found on the body of one out of 5 *Euthynnus alletteratus* examined but none of these fish contained any bloodparasites. In a further

study on fishes from the Red Sea, Saunders (1960) found only coral reef species infected with *H. bigemina* and suggested that the vectors of fish haemogregarines might be found to live in that habitat. So (1972) investigated marine fish haematoprotozoans from Newfoundland and found piscicolid leeches of the species *Malmiana nuda* on *Myoxocephalus scorpius*. No blood smears were taken from the fishes bearing these parasites but sporozoites of haemogregarines were seen in 8 out of 10 leeches on examination of gut smears. Since *H. myoxocephali* was reported earlier from a related species of fish, i.e. *Myoxocephalus octodecemspinosus* (Fantham *et al*. 1942) the author found presumptive evidence to suggest that *Malmiana nuda* was a possible vector for the haemogregarine although experimental proof of this hypothesis was not given. Kirmse (1975) also postulated marine leeches as possible vectors for a pathogenic haematoprotozoan parasite of *Scophthalmus* maximus because of the weight of evidence from previous work although leeches were never found on these infected fish nor indeed were any other ectoparasites except for *Trichodina* sp.

2.4. Vectors of Haemohormidium

Though this blood parasite is occasionally described during surveys for haematoprotozoan parasites of fish

and has been found sometimes together with other bloodprotozoans (Saunders, 1966), its vector is not known. The fact that it has been described in connection with haemogregarines suggests that they might have a common intermediate host.

2.5. Vectors of Dactylosoma

Only very few records of *Dactylosoma* sp. have been found in fish and little is known about the vectors of this group of haematoprotozoan parasites. Nöller (1913) was unable to obtain transmission of *Dactylosoma ranarum* with the leech *Hemiclepsis marginata*. Also Fantham *et al.*(1942) describing *Dactylosoma fontinalis* in Canada did not observe any ectoparasites on the infected trout (*Salvelinus fontinalis*) nor did they find any leeches in the neighbourhood. The dissection of various aquatic *Hemiptera* revealed no stages of *Dactylosoma*. Jakowska and Nigrelli (1956) suggested in view of these negative results that the fish louse *Argulus foliaceus* should be considered as a likely vector for these blood parasites.

3. MATERIAL AND METHODS

3.1. Survey for possible invertebrate vectors of haematoprotozoan parasites of marine fish

3.1.1. Copepods and amphipods

A number of ectoparasitic copepods,i.e. 26 *Caligus* sp.; 7 *Clavella* sp. and 1 *Lernaea* sp. and amphipods, i.e. 24 *Gammarus* sp. were collected by Mr.Finlayson of the Marine Laboratory at Millport from various marine fish species such as *Gadus morhua*, *Cottus* sp. and *Merluccius merluccius* from the Clyde area during 1975/76. These were preserved alive on moist seaweed and shipped by rail express to the Unit of Aquatic Pathobiology. On arrival smears of these invertebrates were made on glass slides and these were dried, fixed with methanol and stained with Giemsa. The slides were screened for haematoprotozoan parasites under high power oil immersion. In addition a number of the ectoparasitic copepod *Lernaeocera* sp. found on farmed *Solea solea* from the W.F.A. Hunterston were also examined for developing stages of haematoprotozoan parasites.

3.1.2. Marine piscicolid leeches

3.1.2.1 *Calliobdella nodulifera*

During 1975 to 1977 a survey was made on the haematoprotozoan fauna of the marine leech *C.nodulifera*. The only specimens of this leech obtained,were collected by otter trawl from *Gadus morhua* in the outer Clyde basin from Garroch Head to Ascog (Rothesay) from a depth of approximately 45-60 fathoms. The leeches were mainly found on the belly and around the gills of these fish. They were shipped on moist seaweed in seawater sealed in small polythene bags. Their morphological anatomy was studied in physiological Ringer's solution under the dissecting microscope. Specimens were also contrasted in 28.5% lacto-phenol and mounted in cedar oil and in addition serial histological sections were made and routinely stained with haematoxylin-eosin for further identification of the internal organs. Leeches were identified as *C.nodulifera* according to descriptions by Leigh-Sharpe (1916-25),Soos (1965) and Mann (1962) and this was later confirmed by Mr.E.G.Easton,Annelid Section, British Museum of Natural History. A total of 193 specimens of *C.nodulifera* were examined for the presence

of haematoprotozoan parasites in their digestive tract. They were dissected and smears made from the anterior part (proboscis, salivary glands and oesophagus),centre part (crop or stomach) and posterior part (intestine) onto glass slides which were either viewed as wet preparations under phase contrast or after they had been air dried, fixed with methanol and stained with Giemsa. Some of the leeches were preserved in 2.5% glutaraldehyde for electron microscopy or in 10% formalin for histological sections.Others of them,from supposedly infected batches, were kept alive in clean seawater in large glass containers for further transmission studies. In late February 1977 they deposited their cocoons on the inside of the glass walls but these never hatched.

3.1.2.2. Hemibdella soleae

These leeches derived from adult Solea solea of the W.F.A. fish farm at Hunterston and were collected with a pair of forceps from the backs of anaesthetized fish. They were examined regularly for the presence of haematoprotozoan parasites in their digestive tract by the methods already described under C.nodulifera. Their dissection was very difficult because of their small size, so that all smears were made simply by

cutting the leech into 3 parts,as before,and squashing rather than dissecting. This made the exact localization of parasite stages rather more difficult than in *C.nodulifera*. Live specimens of these leeches and kept in seawater for transmission experiments deposited cocoons at the end of July 1977 but again no young emerged.

3.2. Transmission experiments using *Calliobdella nodulifera*

To test the hypothesis that *Haemogregarina sachai* n.sp. might be transmissable via the bite of marine leeches acting as intermediate hosts, apparently uninfected *C.nodulifera* were fed on several *Scophthalmus maximus* with high parasitaemias. Since the leeches appeared to find it difficult to feed off the upper surface of the test fish, they were placed with the lower surface up in shallow containers filled with aerated fresh seawater. The leeches were deposited on this lower surface which was just barely covered with water and after several minutes the majority had attached to the skin by their posterior suckers. The leeches searched for about 20 minutes with the anterior sucker and the proboscis before attaching and feeding, in a reversed u-shaped position. Blood soon appeared in the crop (stomach),outlining the different segments

clearly,and indicating that the lecch was feeding. Most of the leeches were attached to the body skin but a few fed on the pectoral and lateral fins. After a total feeding time of 25 minutes the blood meal of the leeches was interrupted and they were all removed. The first leech was killed after 24 hours to check for *H.sachai* n.sp. in its crop and when intracellular and free stages of the parasite were detected, further individual leeches were dissected after 48,72 and 96 hours. After 96 hours all the remaining leeches were fed again on apparently healthy hatchery reared or wild *Scophthalmus maximus* from Ardtoe to attempt transmission mechanically. Leeches were again examined on the 10th day after feeding on infected fish, when numerous sporozoites began to appear on smears of the leech digestive tract. A number of leeches was again fed on apparently healthy fish. The same procedure was repeated on the 20th day after the initial feeding of the leeches on infected fish. During this time all the experimental fish were examined at intervals for haematoprotozoan parasites in their blood. The experiment could not be conducted beyond the 20th day because of the accidental loss of both leech vectors and test fish.

In another experiment some of the successfully infected leeches were force-fed to apparently healthy Scophthalmus maximus either as a whole or in ground up saline suspensions. The material was placed in the stomach of the fish by plastic tube attached to a 1 or 2 ml syringe.

3.3. Transmission experiments using Hemibdella soleae

Leeches highly parasitized by Haemogregarina simondi schizonts were collected from Solea solea at Hunterston and were immediately ground up with phosphor-buffered-saline (PBS) pH 7.2 and force-fed by stomach tube to 2 Scophthalmus maximus of age group 1+ and two fishes of age group 0+.All were hatchery derived.

In addition an attempt was made to make live Hemibdella soleae infected with H.simondi feed on Pleuronectes platessa, Scophthalmus maximus and hatchery derived Solea solea. The Scophthalmus maximus were observed for 6-8 hours but only two leeches attached ,with their posterior suckers on the fin rays of the lateral fins. When the leeches were finally removed very little fresh blood appeared to have been ingested although by this time both suckers were attached to the fish.

No leeches ever attached to Pleuronectes platessa. However, Hemibdella soleae attached readily to the upper surfaces of the two hatchery Solea solea and were observed feeding there.

Two hatchery Solea solea were also placed as sentinels in one of the tanks at Hunterston known to contain a substantial number of Hemibdella soleae and fish infected with Haemogregarina simondi. These test fish were monitored over an extended period of time at irregular intervals for haematoprotozoan parasites in their blood circulation.

3.4. Transmission experiments using Lernaeocera sp.

Adult female Lernaeocera sp. having fed blood from haemogregarine infected Solea solea and found to contain developing stages of Haemogregarina simondi were ground up in sterile PBS pH 7.2 and 1 ml of this suspension,which had been examined in wet and dry smears for the presence of parasites, was then force-fed to 3 Scophthalmus maximus,apparently free of blood parasites,as described above.

4. RESULTS

4.1. Survey for possible invertebrate vectors of haematoprotozoan parasites of marine fish

4.1.1. Copepods and amphipods

The number of ectoparasitic copepods and amphipods examined was comparatively minimal and no haematoprotozoan parasites were detected in any of the *Caligus* sp.; *Lernaea* sp.; *Gammarus* sp.; and *Clavella* sp. from the Clyde. However, all of the *Lernaeocera* sp. examined from farmed *Solea solea* at Hunterston, known to be infected with *Haemogregarina simondi*, were found to harbour various stages of this haemogregarine. It is assumed that all of these stages observed were taken up by the *Lernaeocera* sp. with the blood meal from the infected fish hosts. What appeared to be *macro-gametocytes* of *H.simondi* had an average length of 14.4 micron (range 13.75-15.0) and an average width of 4.4 micron (range 3.75-5.0) and thus compared well with the corresponding stage found in the fish host (14.3 by 4.4 micron) and described in Section A-4.4.1. The cytoplasm stained dark blue, the nucleus stained

bright red and occupied the central 1/3 of the parasite. It measured an average of 5.3 (range 4.37-6.25) by 3.4 (range 3.1-3.75) micron. Adjacent to the nucleus and covering most of the rounded end of the parasite was a distinctly pink staining area of 1.87 by 1.5 micron. The other end of the parasite was tapering into a point. In addition there were observed what were considered to be micro-gametocytes of 22.5 by 1.37 micron in average size. Also observed were very fine elongate stages with a bright red nucleus of granular appearance and almost filling the total length of the parasite. This nucleus was bordered at each end by a distinct refractile body. It is possible that these were sporozoites but no intermediate forms of development were ever observed.

4.1.2. Marine piscicolid leeches

4.1.2.1. Calliobdella nodulifera

The results of this survey of C.nodulifera leeches from the Clyde are summarized in Table 8 .The first leeches appeared infected in March when 8 out of 38 leeches examined harboured trypanosomes. Infection appeared to be highest in summer when 17 out of 24 leeches were found parasitized by trypanosomes.

TABLE 8

OCCURRENCE OF *TRYPANOSOMA* SP. IN THE MARINE LEECH *CALLIOBDELLA NODULIFERA*

No.infected/total No.examined	Date
0 / 30	January
0 / 31	February
8 / 38	March
4 / 4	April
9 / 18	May
17 / 24	June
9 / 12	July
5 / 15	September
1 / 21	November
tot. 53 /193	

This was followed by a decrease in the number of leeches infected until in November only 1 out of 21 leeches showed trypanosomes in its alimentary tract when examined. A description of the various developmental stages of this trypanosome is given in 4.2.

In addition an occasional haemogregarine was also detected (Fig.66) and sometimes other developmental stages of haemogregarines and considered to be large oocysts were also discovered in leeches collected in June and concurrent with developmental stages of trypanosomes. What appeared to be a sporocyst with 22 spheric, dark purple staining nuclei and measuring 12.5 by 12.5 micron was also seen. The nuclei measured 1.9 by 1.9 micron in average and presumably develop into sporozoites. A young zygote of 16.8 by 8.3 micron contained two large and dark staining nuclei of 6.25 by 9.4 micron and 4.4 by 3.75 micron, respectively. Another early zygote had a size of 12.5 by 9.4 micron. However, most of the stages of haemogregarines observed were so scanty that no proper identification was possible and a life cycle of this haemogregarine in *C. nodulifera* can only be hypothesized.

4.1.2.2. *Hemibdella soleae*

Only a limited number of leeches were available because of the delicate way *Solea solea* brood stock had to be

handled. All of these leeches were collected from fish hosts with moderate to heavy infection with Haemogregarina simondi and all had taken up various stages of this haemogregarine with their blood meal so that the development of this haematoprotozoan parasite could be observed throughout the alimentary tract of these leeches.A detailed description is given in 4.4.

4.2. Description of different developmental stages of a trypanosome found in the alimentary tract of the marine leech Calliobdella nodulifera

A striking feature in the development of this trypanosome was the number of different stages identifiable at any one time in the leech.On one preparation the entire range of morphological forms could be observed from the rarely encountered promastigote form and a more common rounded or irregularly shaped organism with a thick short flagellum (sphaeromastigote) via a round organism without flagellum (amastigote) to a fully developed parasite with a kinetoplast in juxta-position with the nucleus(epimastigote) and up to a very slender,spirochaete-like form(trypomastigote), e.g. the infective stage(Fig.64).The earliest stages of development were seen when the ingested blood of the fish host in the leech's digestive tract still appeared fresh. These were irregular shaped stages with an immensely long, thick flagellum and pronounced undulating membrane and were

FIGURES 52 - 66.

Developmental stages of TRYPANOSOMA SP.from the alimentary tract of the marine leech CALLIOBDELLA NODULIFERA

52. Trypomastigote form with distinct undulating membrane thought to be derived from the fish host Gadus morhua x 1,000

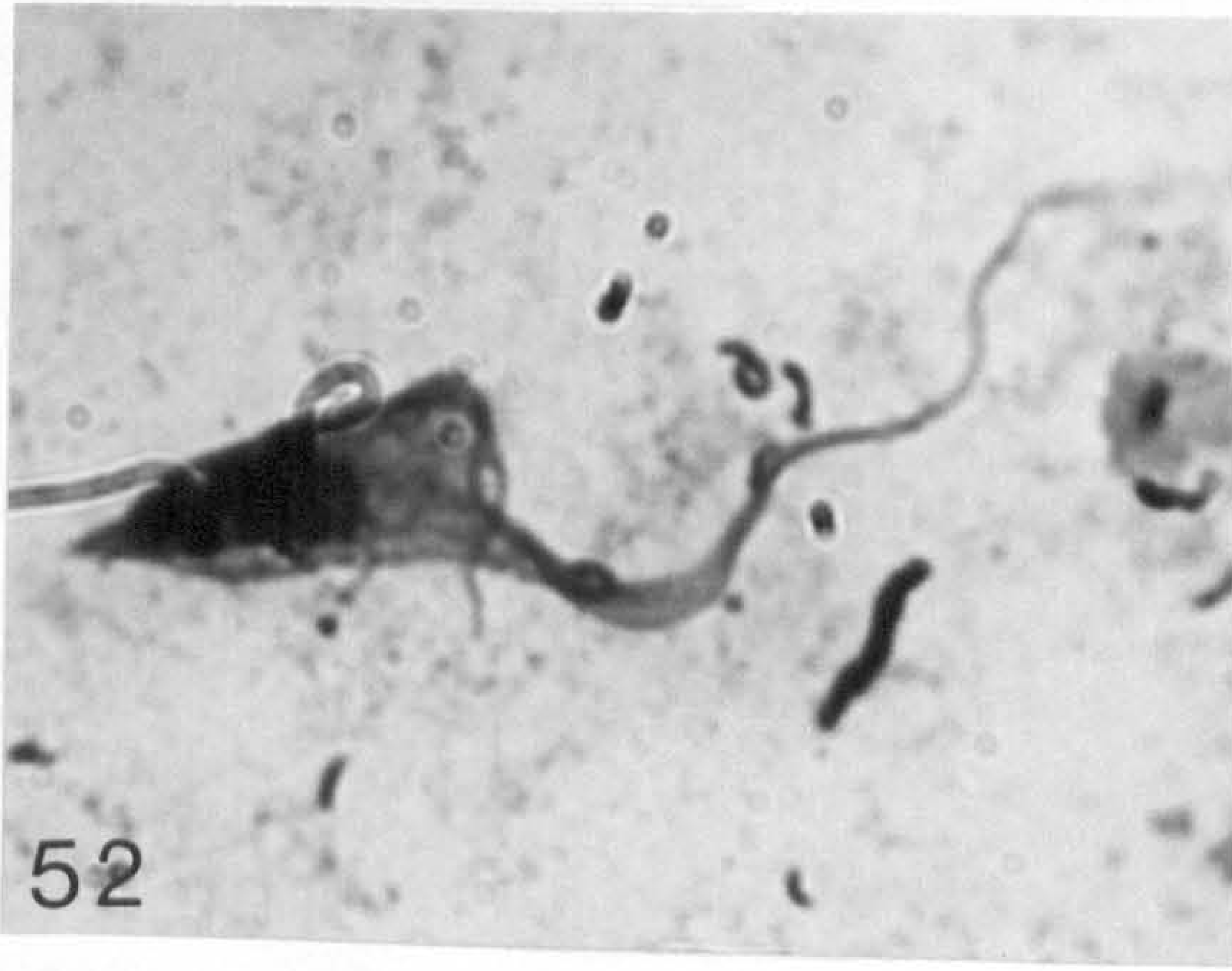
53. Dividing form from the intestine x 1,600

54. Pyriform amastigote form(a) and intermediate stages with growing flagella(f) x 1,000

55. Multiple division(arrow) and epimastigote(e) forms x 1,000

56. Epimastigote form with rod-shaped kinetoplast and medium sized free flagellum x 1,600

57. Epimastigote form with short,straight free flagellum x 1,000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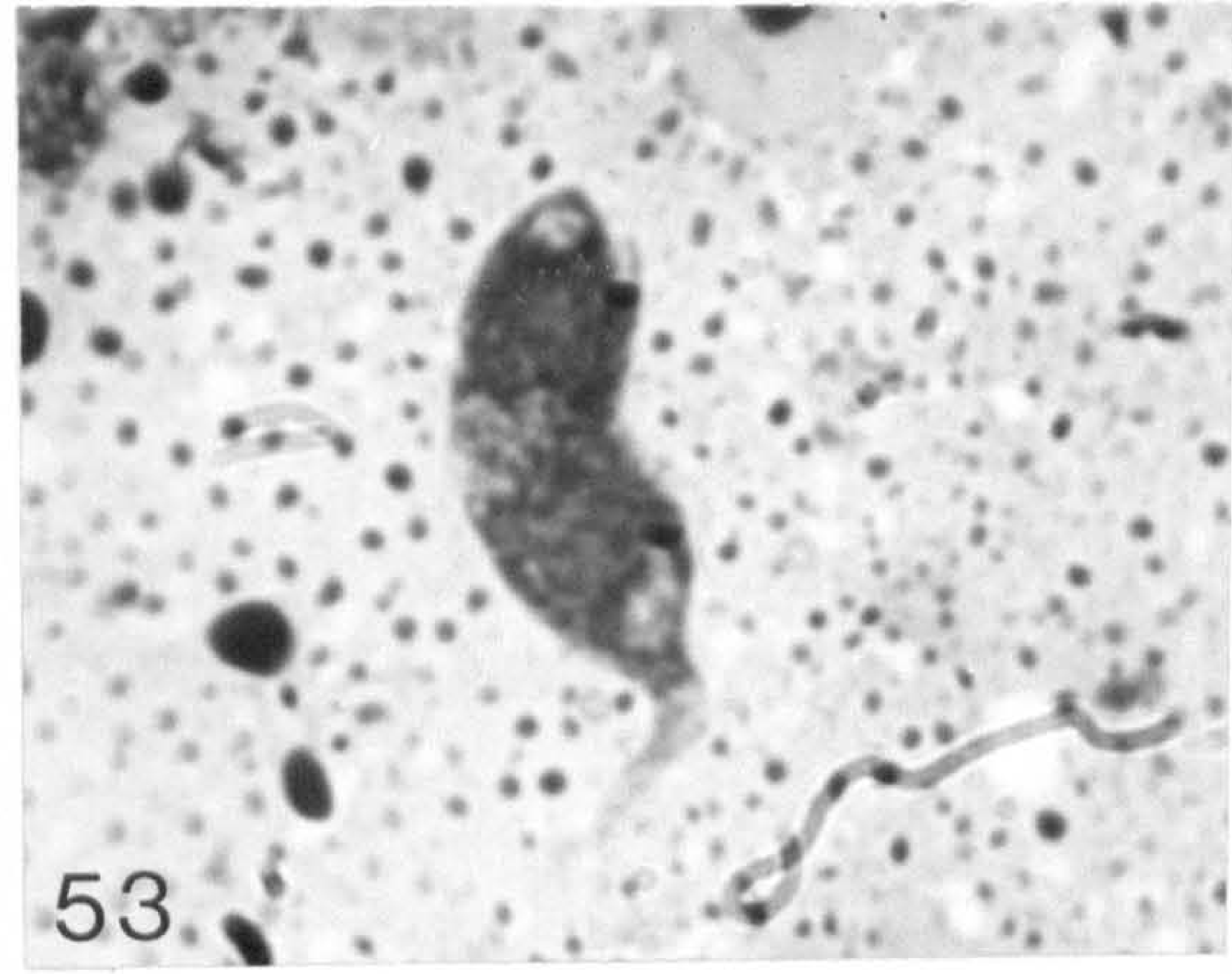
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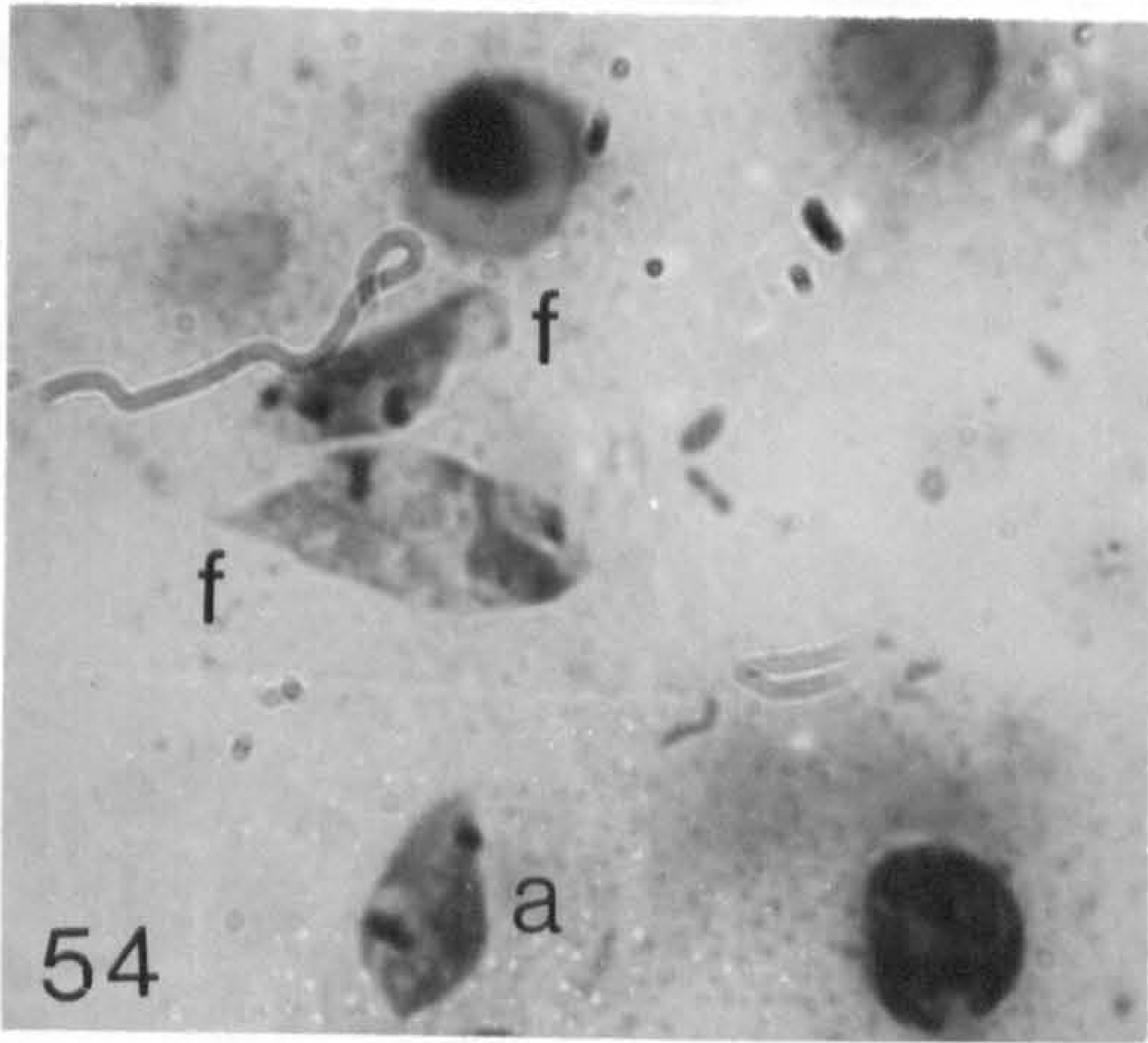
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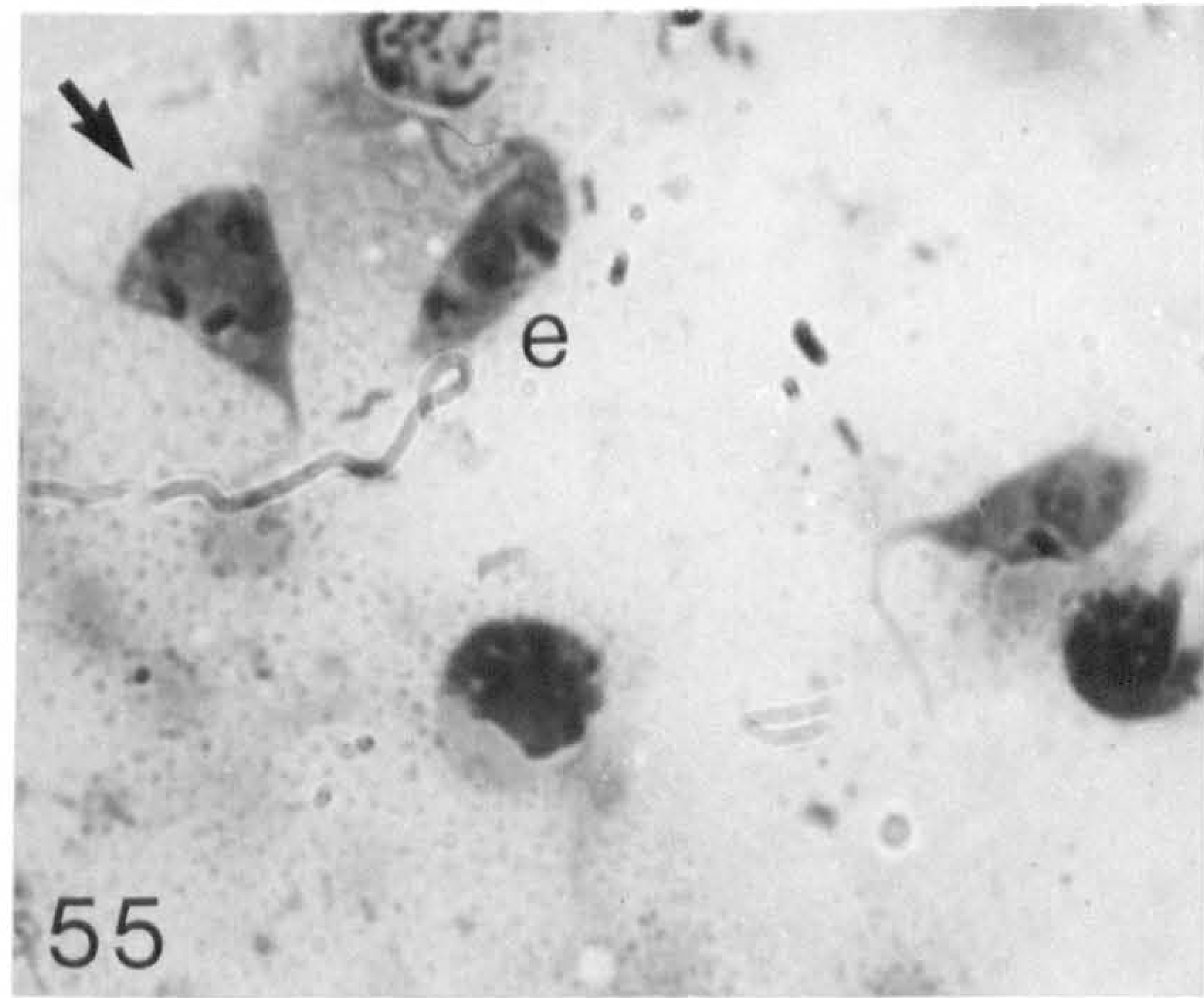
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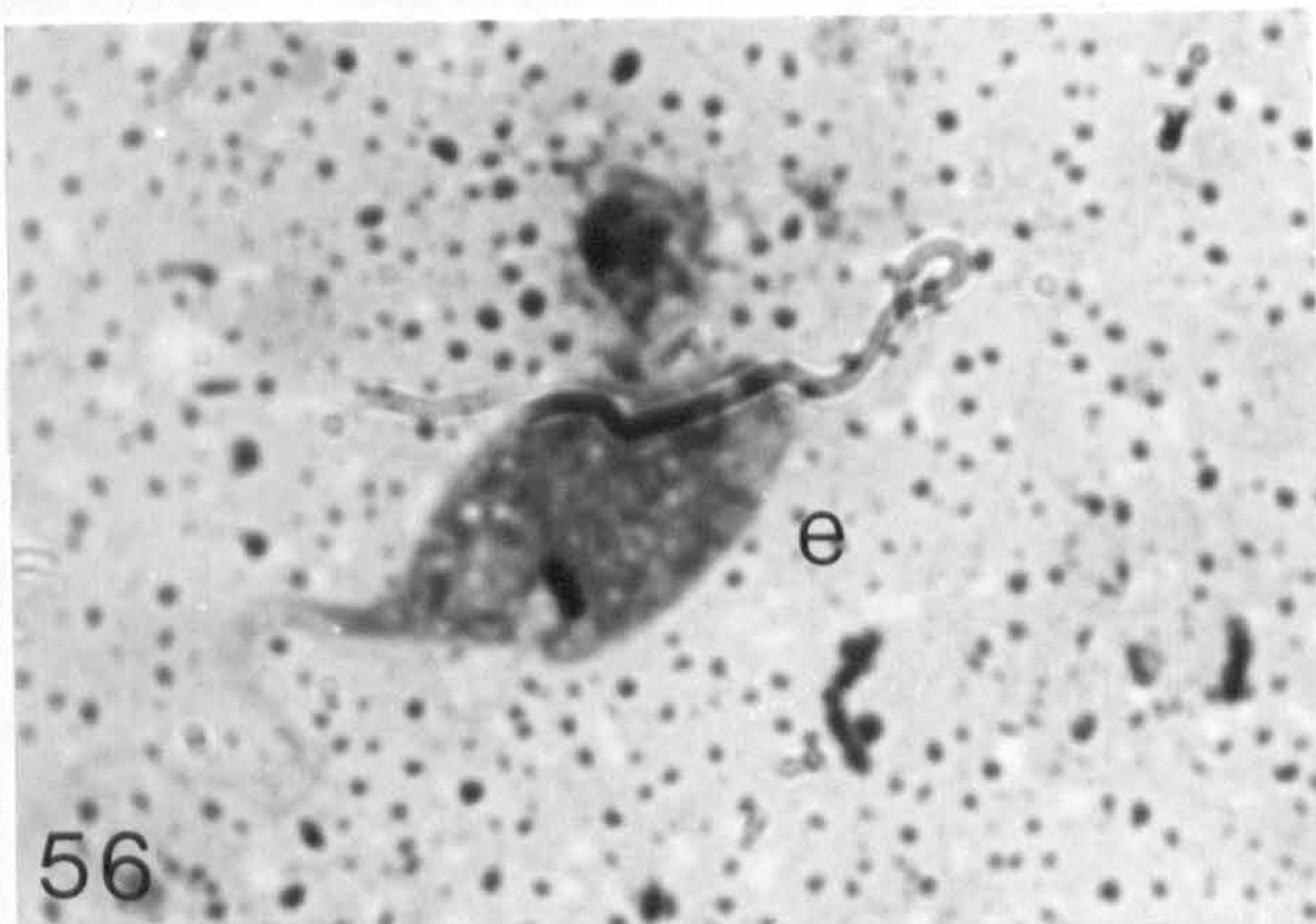
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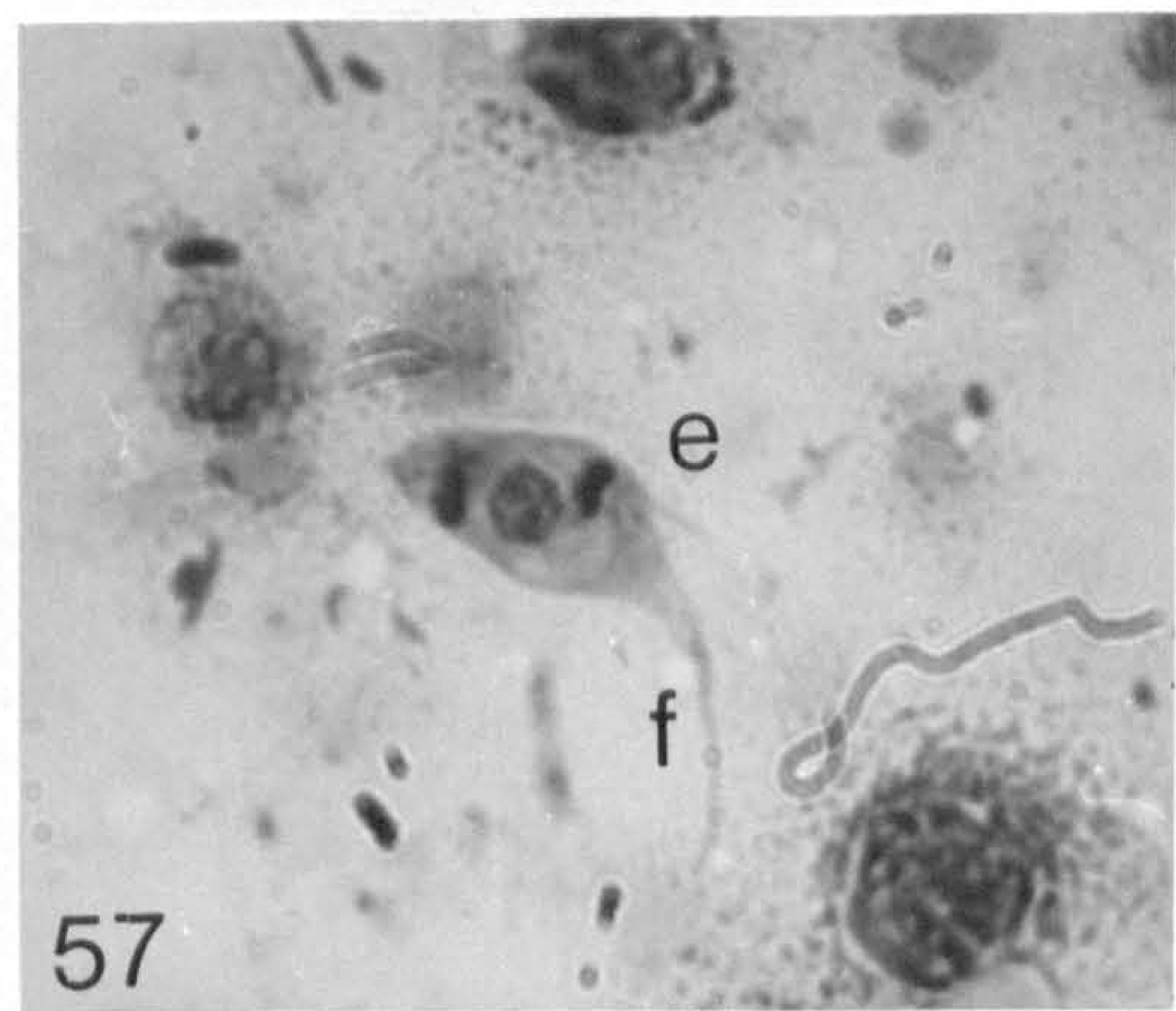
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considered to be perhaps the first forms appearing in the alimentary tract of the leech directly derived from the fish host because of their similar structure as compared with the blood form from fish. They were rarely observed and measured 12.5 micron in length and 3.75 micron in width with a free flagellum of 23.8 micron. The width of the undulating membrane was 1.25 micron. The nucleus was very dark staining measuring 2.5 by 2.3 micron and the kinetoplast was a small dark purple dot and measured 1.1 by 0.6 micron (Fig.52,61).This stage might have also just been an intermediate stage on the way to the final infective trypomastigote form. At about the same time there appeared occasionally another intermediate stage pointed posteriorly and appearing highly vacuolized (Fig.60).The compact nucleus of 3.1 by 1.8 micron was centrally located and posteriorly there were 3 distinct dark staining granules. The small kinetoplast (0.6 by 0.6 micron) was situated at a distance of 2.2 micron from the nucleus in the anterior half of the parasite. This promastigote form measured 21.2 (17.5-27.5) micron in length and only 2.3 (1.9-2.5) micron in width and exhibited a rather long and thin free flagellum of 29.1 (21.3-40.1) micron in length. These stages were undergoing active division and were then showing straight flagella of considerably reduced length (Fig.60).

58. Two epimastigote forms with pronounced rod-shaped kinetoplasts and free flagella of different length. x 1,000

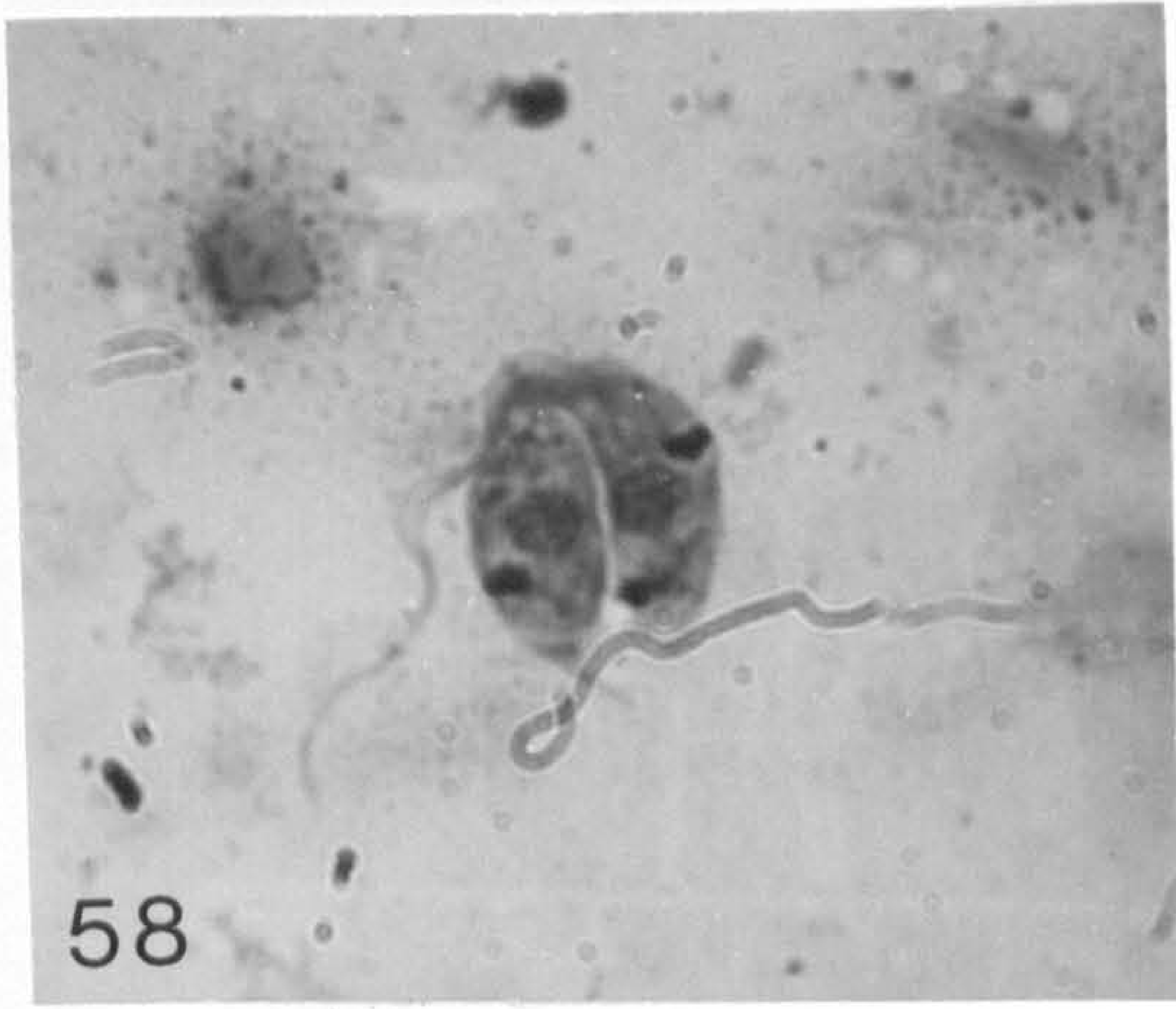
59. Intermediate forms still attached by their posterior ends while the flagella have grown to different length. x 1,000

60. Elongate intermediate form (promastigote) with comparatively short,straight flagellum. x 1,000

61. Intermediate stage with pronounced undulating membrane. x 1,000 (possibly similar to Fig.52)

62. Very thin and long trypomastigote form with extremely elongate free flagellum encountered mainly in proboscis of leech. x 400

63. Thread-like infective stage (trypomastigote form) found predominantly in the proboscis of the vector leech. x 400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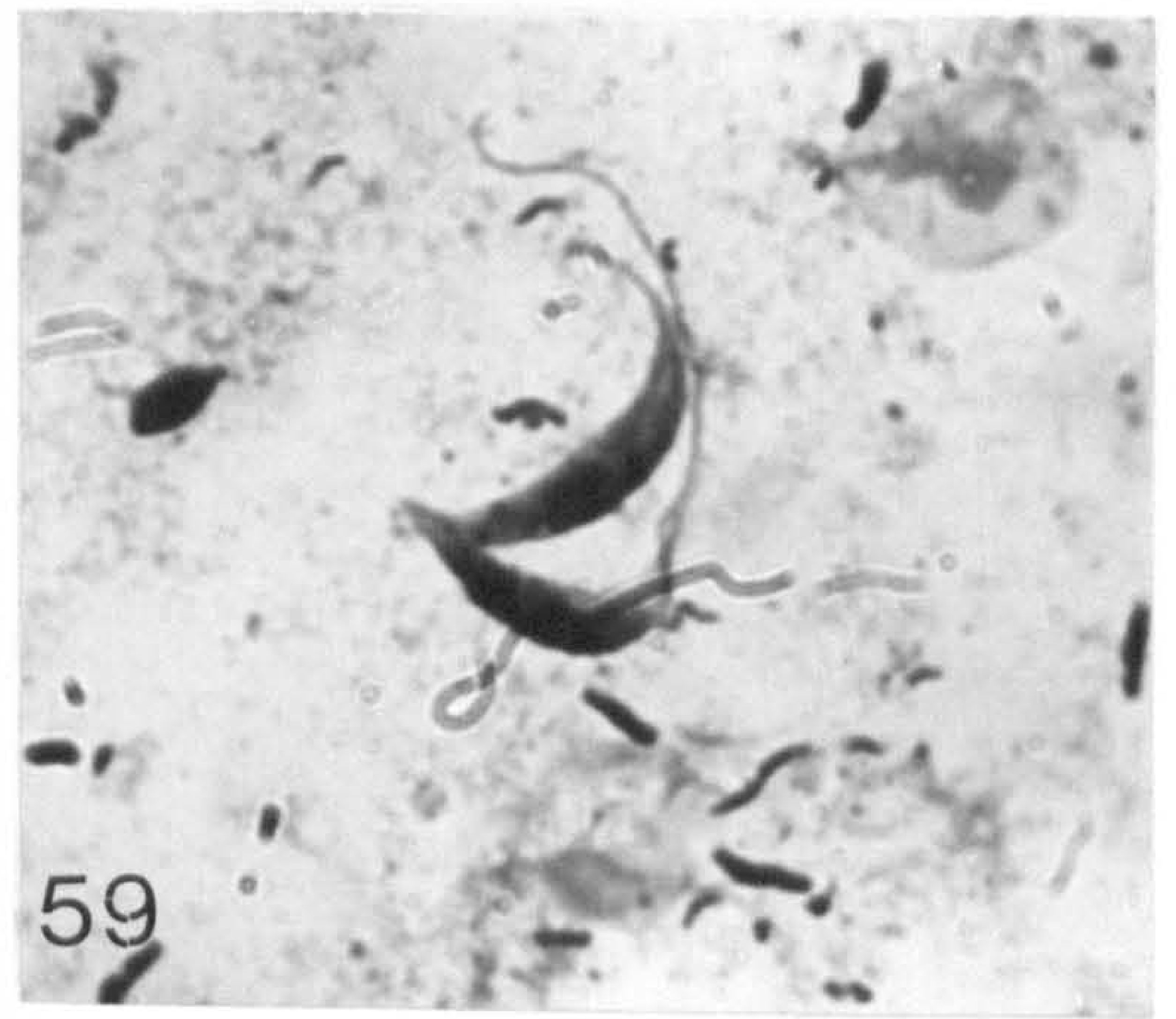
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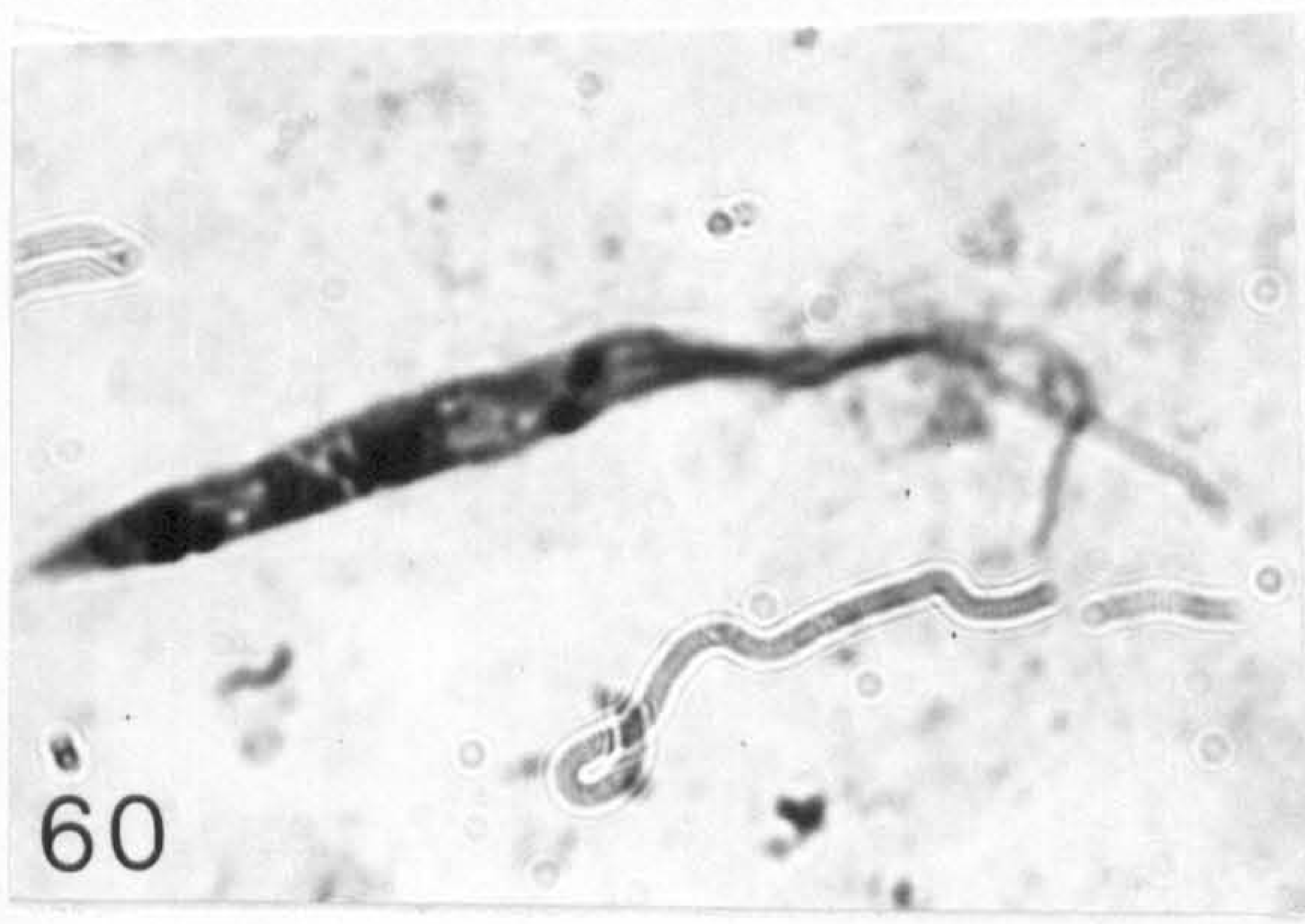
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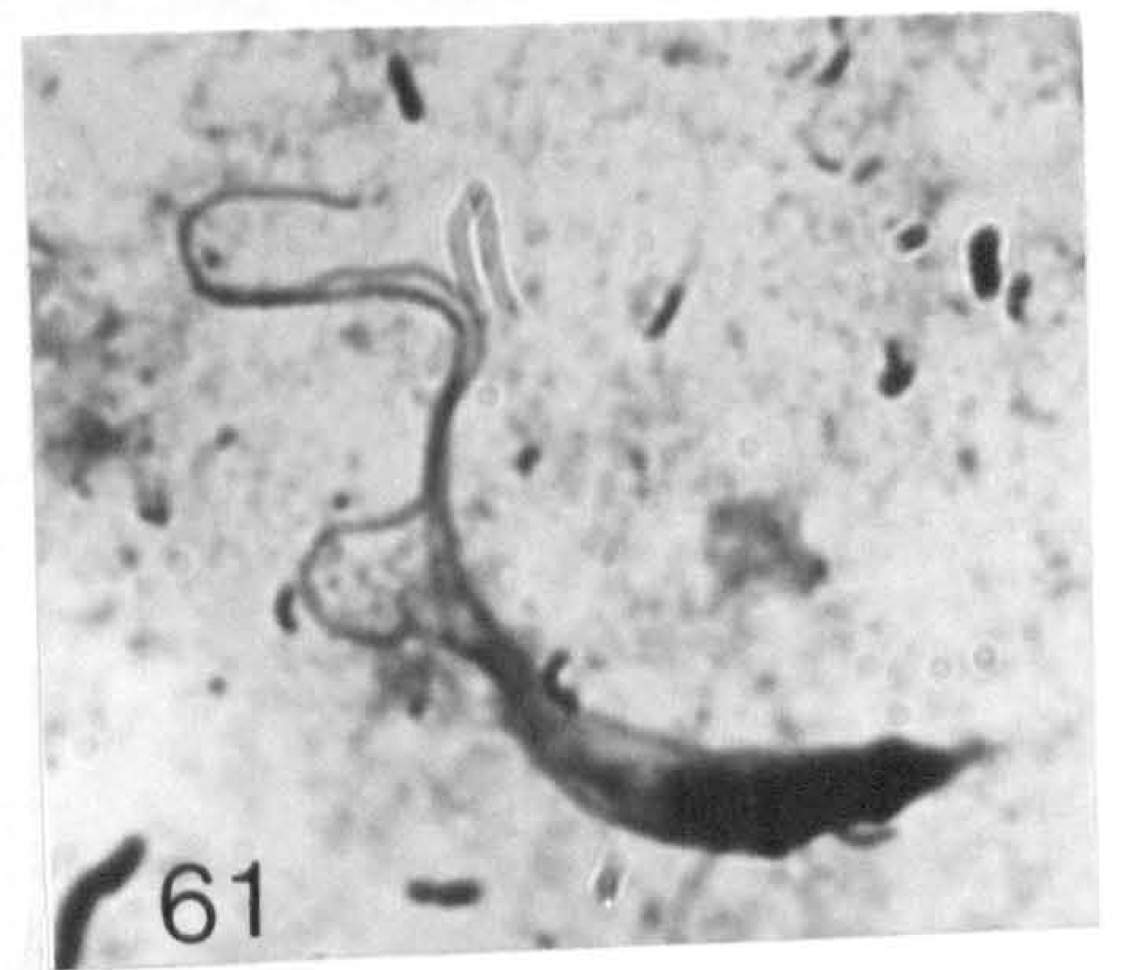
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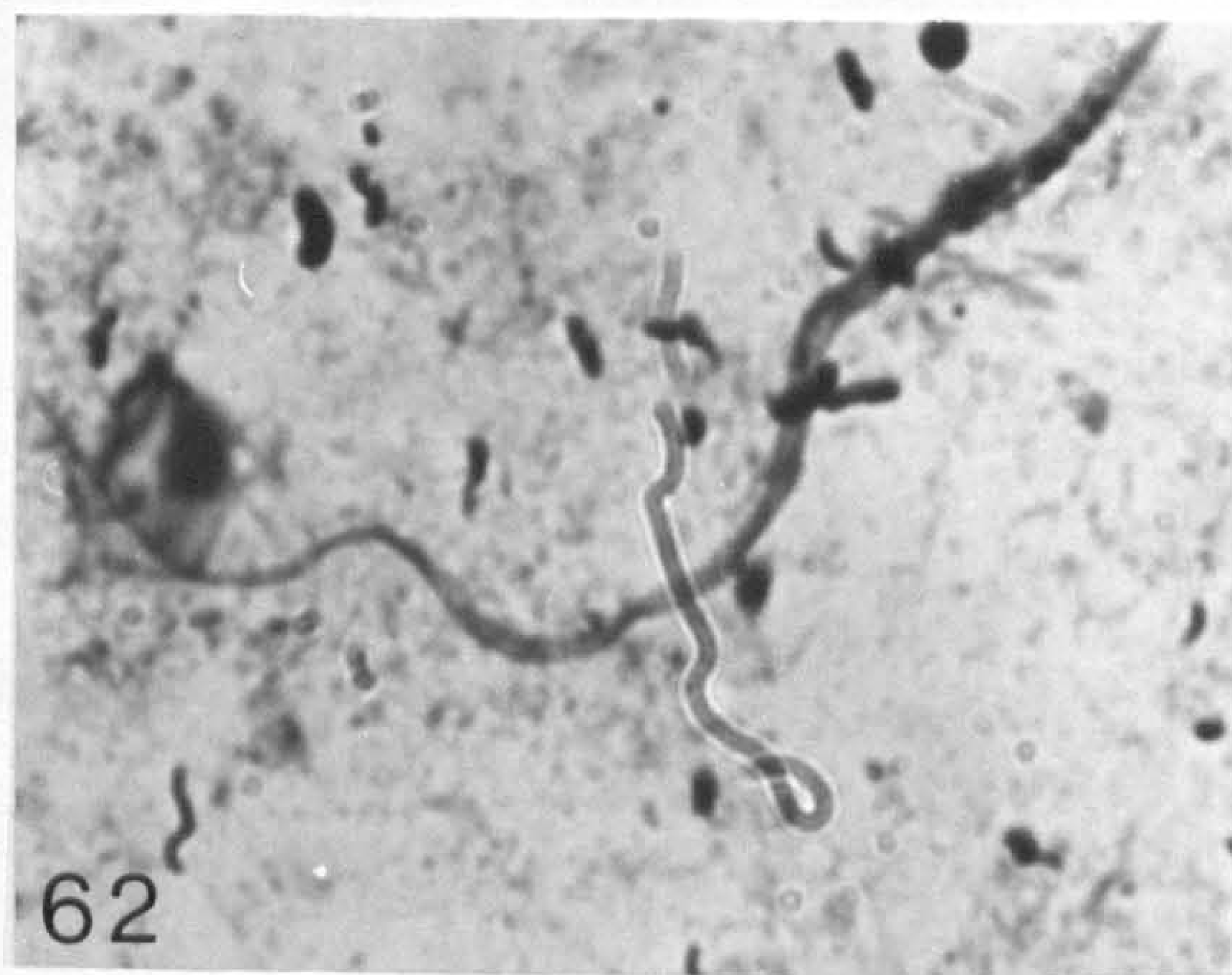
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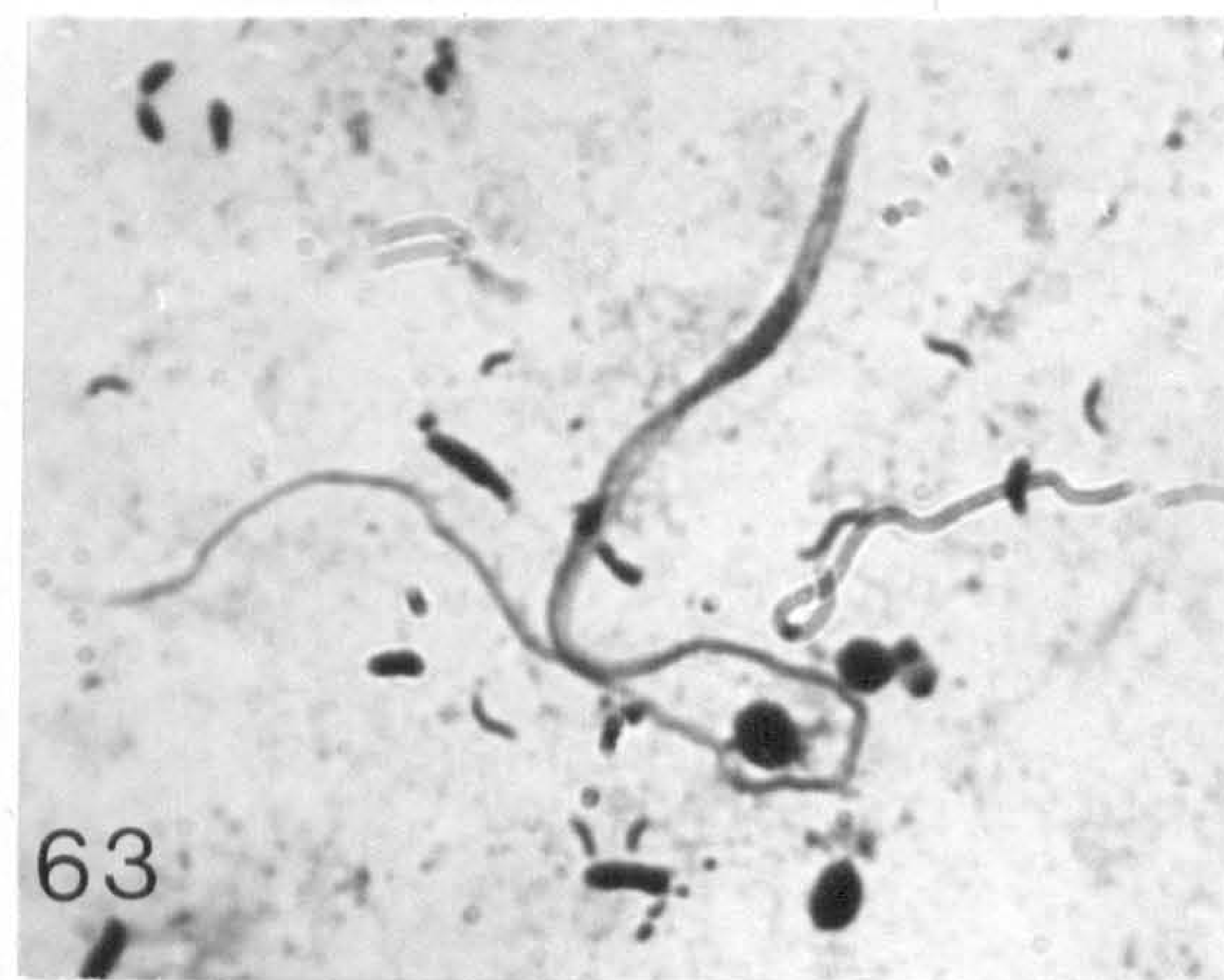
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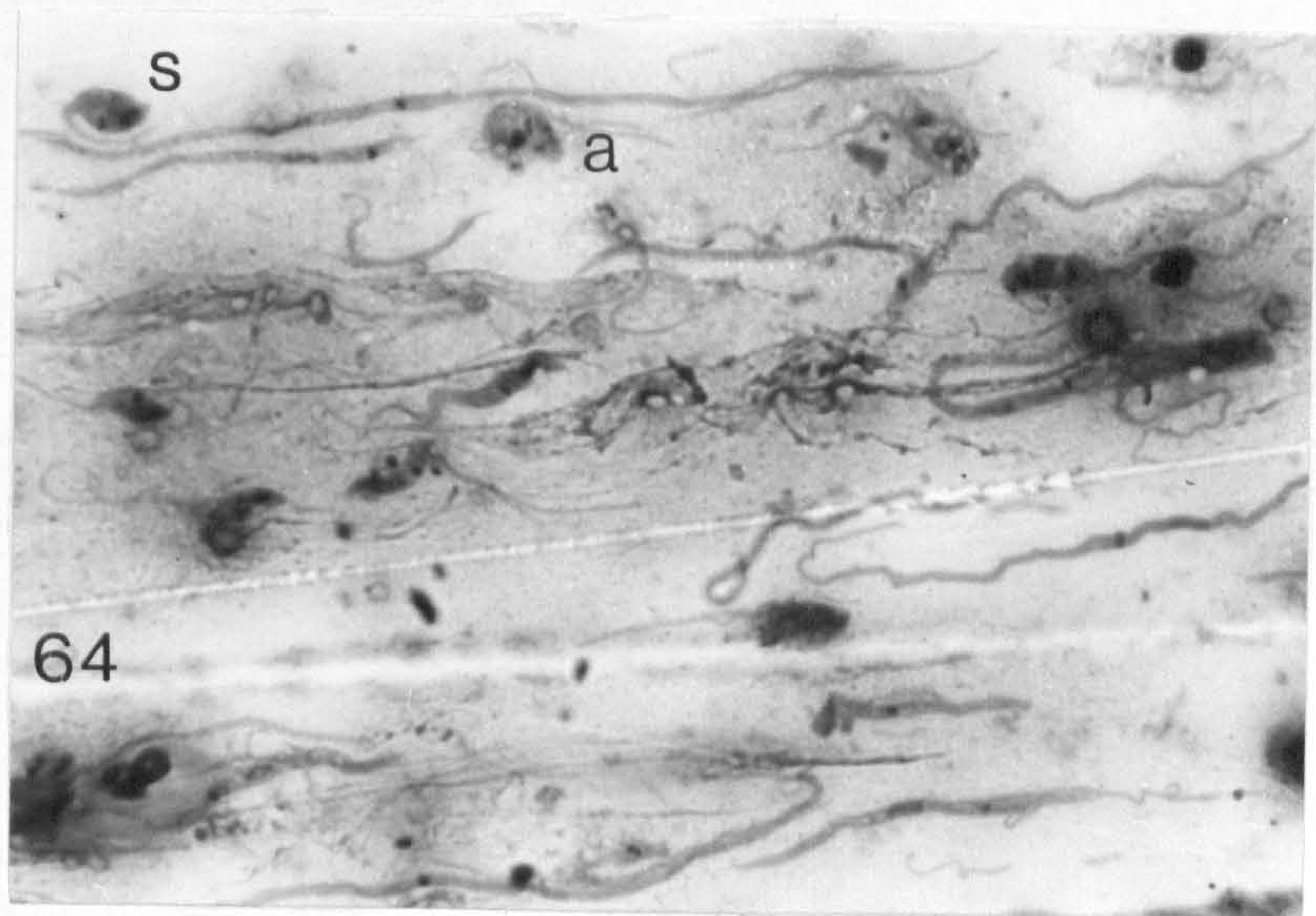
At about the middle period of digestion which is characterized by only a few intact erythrocytes from the fish host the intestine of an infected leech presents a most astonishing variety of forms. Most of these are pyriform,rounded or irregular shaped organisms with either short or stringy long flagella which were either tangled round the body of the parasite or had even broken loose (Fig.54,64,66). This form was considered to be the sphaeromastigote form of development and parasites measured 8.8 by 7.6 micron in average. After the flagella had been thrown off by the parasites and were observed free singly or in tight bundles (Fig.64), the trypanosomes appeared rounded or oval shaped and were considered to be amastigote forms (Fig.54,64). They had a small nucleus of 2.5 by 1.25 micron and a kinetoplast of very small dimensions,i.e. 0.6 by 0.6 micron, but no flagellum for locomotion.In the fine alveolar cytoplasm there were sometimes present a few vacuoles and fine dark staining granules. The size of the parasites ranged from 6.9-8.8 micron in length (average 7.5 micron) and 5.0-7.5 micron in width (average 5.7 micron). These forms were usually dividing very actively and rarely was an individual observed which did not undergo division. This was indicated by the large number of organisms with 2 nuclei and 2 kinetoplasts (Fig.53,55). Multiple division was also observed (Fig.55,65).

64. Smear from proboscis of leech showing numerous thread-like infective stages of trypanosomes in the trypomastigote form, but also amastigote and sphaeromastigote(s) forms and free flagella x 400

65. Smear from the crop(stomach) of leech showing multiple division(arrow), a number of intermediate stages and also free flagella x 1,000

66. Smear from the intestine of leech showing post-division stages, dividing stages and also the developing stage of a haemogregarine(arrow) x 1,000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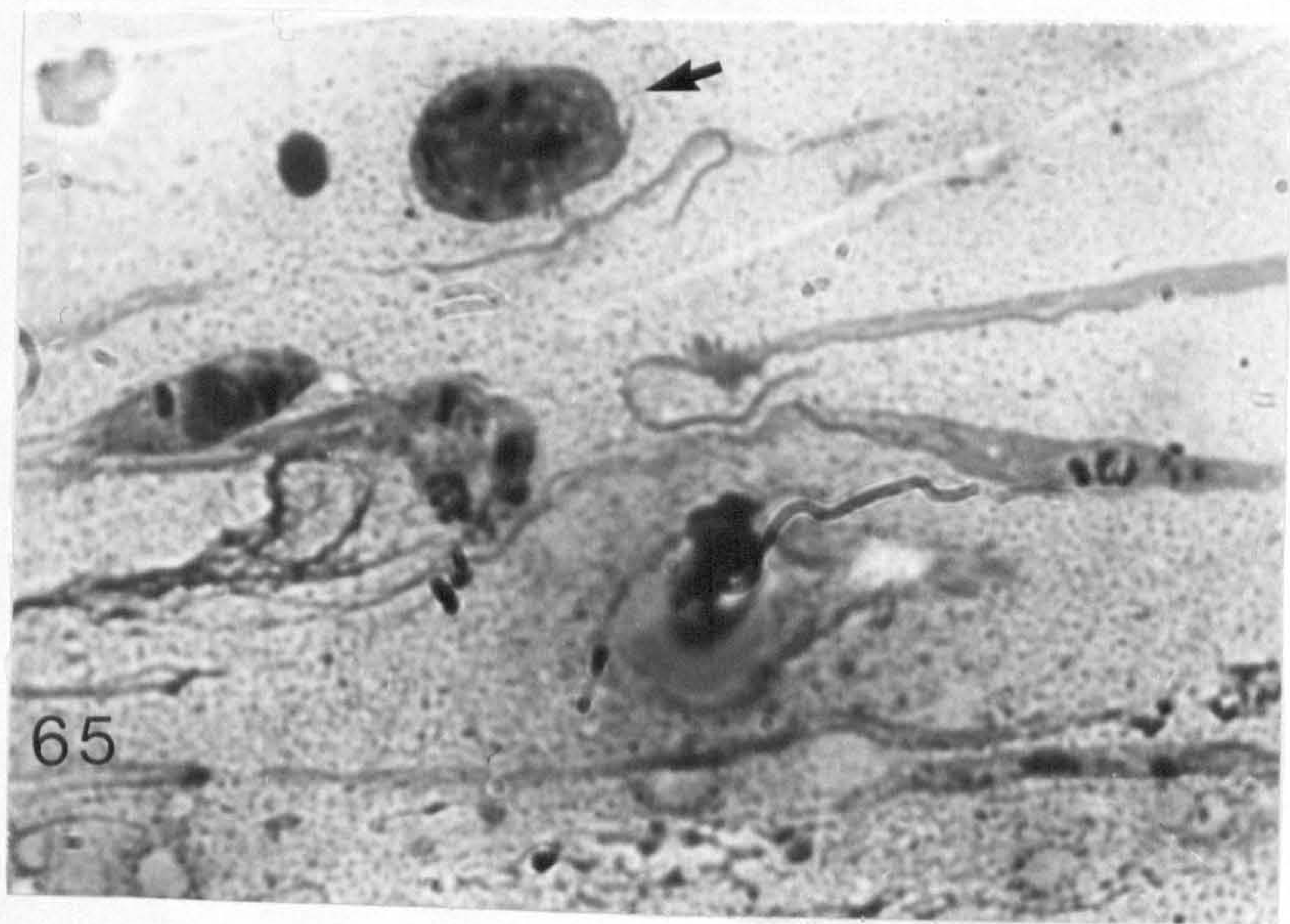
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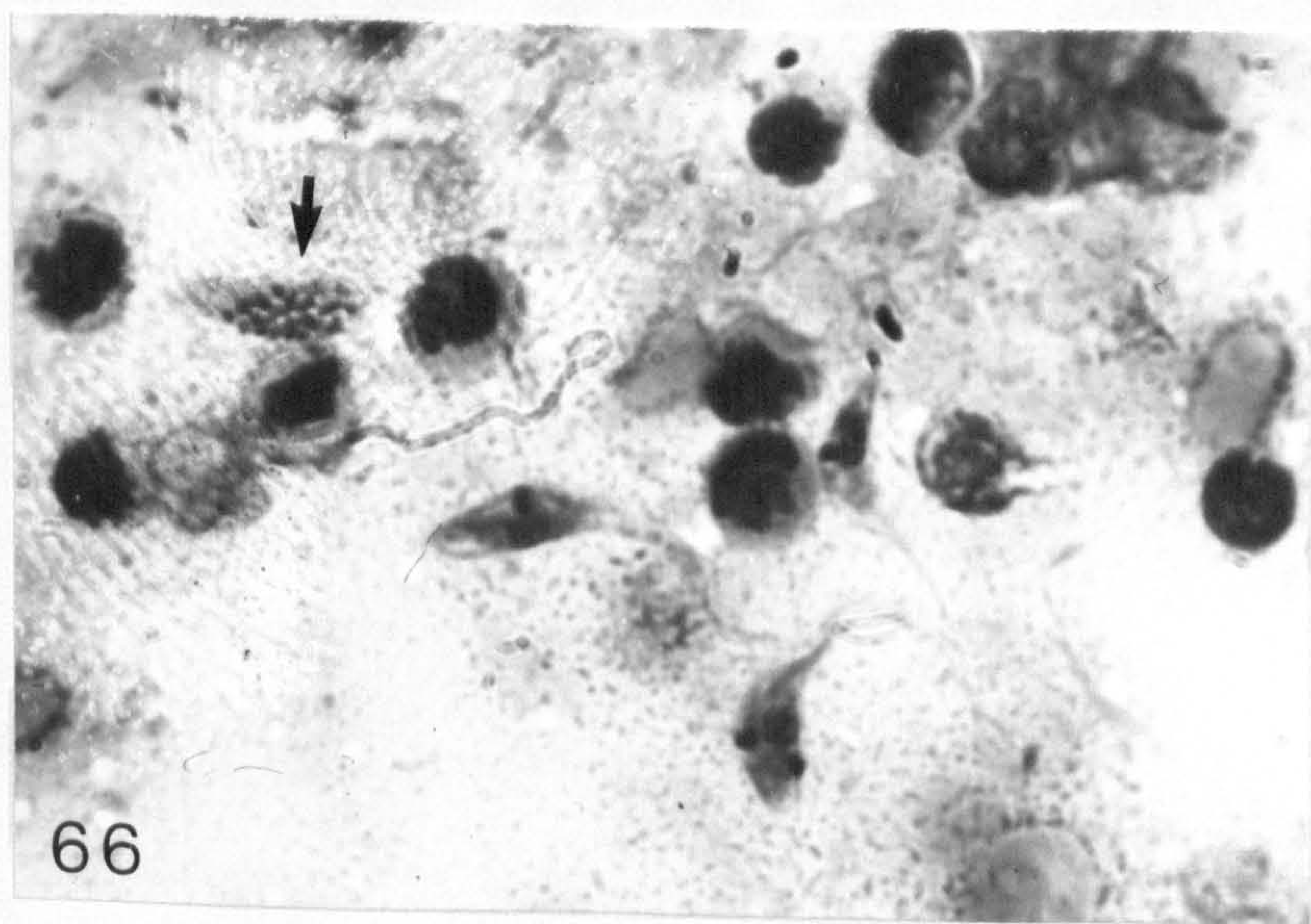

66

After division had taken place these rounded parasites tended to become more oval or irregularely shaped and developed a short flagellum (Fig.54-58). The kinetoplast became fairly large, very deeply stained and rod-shaped. The flagella extended as thick prolongations. These intermediate stages had a length of 10.2 (8.8-12.5) micron, a width of 4.9 (2.9-6.1) micron and a short stumpy flagellum of 5.3 (2.25-8.8) micron. In the dark blue staining cytoplasm there was a dark red staining nucleus of 3.2(2.5-5.0) by 2.9(2.5-3.8) micron and numerous large vacuoles in the posterior part of the parasite. The dark purple staining kinetoplast was located posterior to the nucleus and in iuxta position. It measured 1.3 by 0.8 micron in average. These stages developed with increasing length of the flagellum and elongation of the body into characteristic epimastigote forms. Their dark staining purple cytoplasm contained a well defined bright red staining large nucleus (average 3.5 by 3.1 micron) and an intensively dark blue staining and very distinctly elongate kinetoplast of 2.4(2.2-2.5) by 0.5 (0.4-0.7) micron situated now in anterior position to the nucleus Fig.57,58). The still rather straight flagellum measured 18.7 micron in average length (range 16.2-22.5) and the length of the body was 13.6 (12.5-15.1) micron, the width 3.9 (2.9-5.1) micron. The posterior end of these parasites exhibited a number of dark staining granules surrounded by brown staining pigment. In the

more elongate forms of this stage the nucleus became more elongate too and attained a length of 3.8 micron. This appeared to lead to the final stage of the parasite coinciding with the end of digestion when no fish host erythrocytes are found intact throughout the alimentary tract of the leech. Most of these forms were found in the proboscis of the leech but could also be observed in large numbers in the intestine together with other developing forms (Fig.65). This last stage seen and considered the infective stage consisted of slender trypanosomes of considerable length and of trypomastigote form (Fig.62). When these parasites have migrated up into the proboscis they were even becoming more slender until they were almost thread-like with hardly any cytoplasm (Fig.63,64). They had an average length of 34.1 micron (range 23.8-50.2) and an average width of 1.2 (range 1.0-1.5) micron. The free flagellum measured an average of 32.5 micron (range 22.5-60.1) so that the whole individual parasite came to an average size of 66.6 micron in total length. The nucleus was quite distinctly stained, very long and narrow and granular in appearance and measured 5.6 (2.2-8.8) by 0.9 (0.6-1.25) micron. The kinetoplast was comparatively small and unconspicuous, averaged 1.2 by 1.2 micron and was located at a distance of 7 micron (range 5.0-10.1) posteriorly from the nucleus. No vacuoles were present. These parasites moved actively and very rapidly through the field of observation.

4.3. Stages of development of *Haemogregarina sachai* n.sp. in the marine leech *Calliobdella nodulifera*

Interpretation of the various developmental stages of this haemogregarine in the alimentary tract of experimentally infected leeches proved extremely difficult. The earliest stage observed were *zygotes* (Fig.67,72,73) some of them with large nuclei and granular cytoplasm and perhaps undergoing further division. A very distinctly identified stage seemed to be an early *oocyst* (Fig.68) with the remainders of a large nucleus at the periphery and about 10 rounded to oval shaped bodies considered *sporoblasts* which in turn contained more than 16 individual nuclei each. The further stages observed were of irregular shape (Fig.69) and often lobed (Fig.70). They were surrounded by a fine cystic membrane and filled with numerous granules appearing more condensed at the periphery. These might have been mature oocysts (?) but no exact measurements were available. It is also not clear whether or not the following stage represents a *sporocyst* (?) in which the dark staining large nuclei (3 appear still intact at the periphery) undergo fragmentation into *sporozoites* (Fig.71). Questionable intermediate stages (Fig.72,73) were found frequently. They measured an average of 7.6 micron in length (range 6.2-9.4) and an average of 6.3 micron in width

FIGURES 67 - 77.

Development of HAEMOGREGARINA SACHAI N.SP.
in CALLIOBDELLA NODULIFERA

67. Two early zygotes(?)with distinct membranes and granular cytoplasm or enlarged tissue cells(?) x 1,000

68. Oocyst with approximately 10 sporoblasts containing 16 or more nuclei x 1,600

69. Oocyst(?) with granular cytoplasm more condensed at the periphery x 400

70. Large lobed oocyst(?) containing numerous granules x 400

71. Sporocyst(?) with 3 dark staining nuclei at the periphery x 400

72. Questionable stage of development (early zygote?) x 1,250

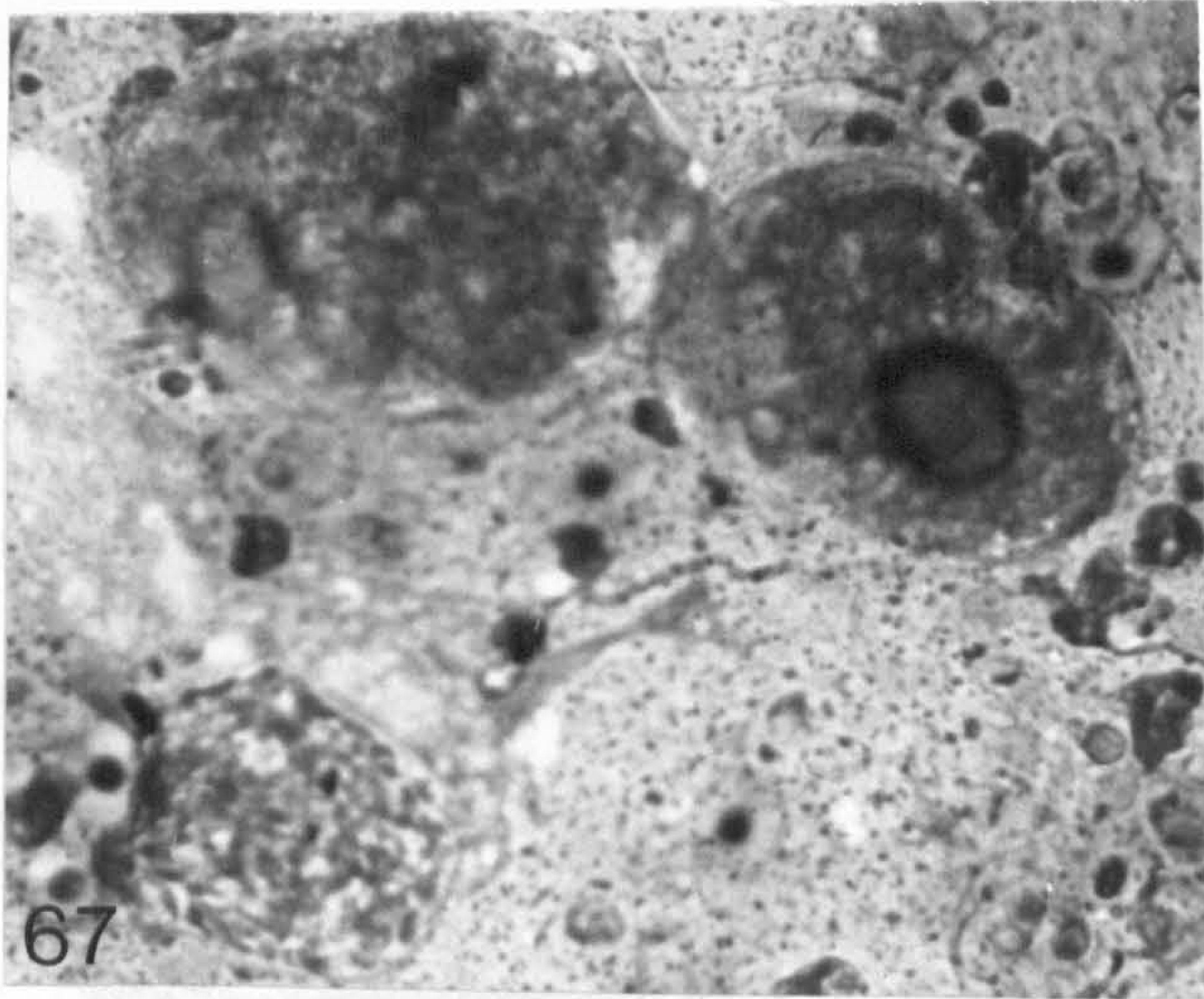
73. Questionable stage of development (early zygote?) x 1,250

74. Questionable stage of development with two granulated nuclei x 1,000

75. Immature sporozoite(?)with rounded poles and large granular nucleus.Also a questionable stage as seen Fig.72,73 x 1,000

76. Two immature sporozoites (?) x 1,000

77. Lancet-shaped sporozoite with large polar cap near pointed anterior end x 1,600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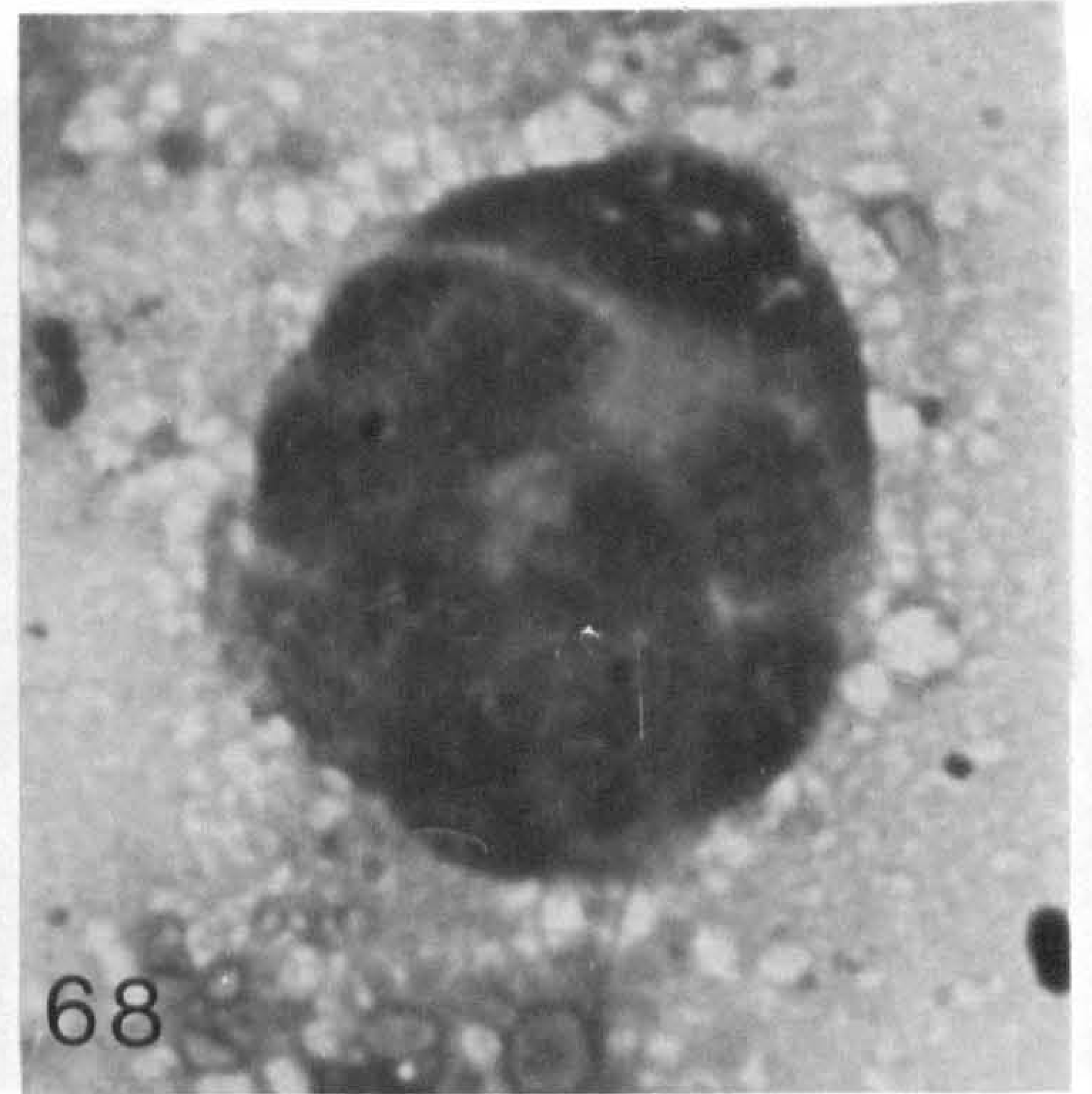
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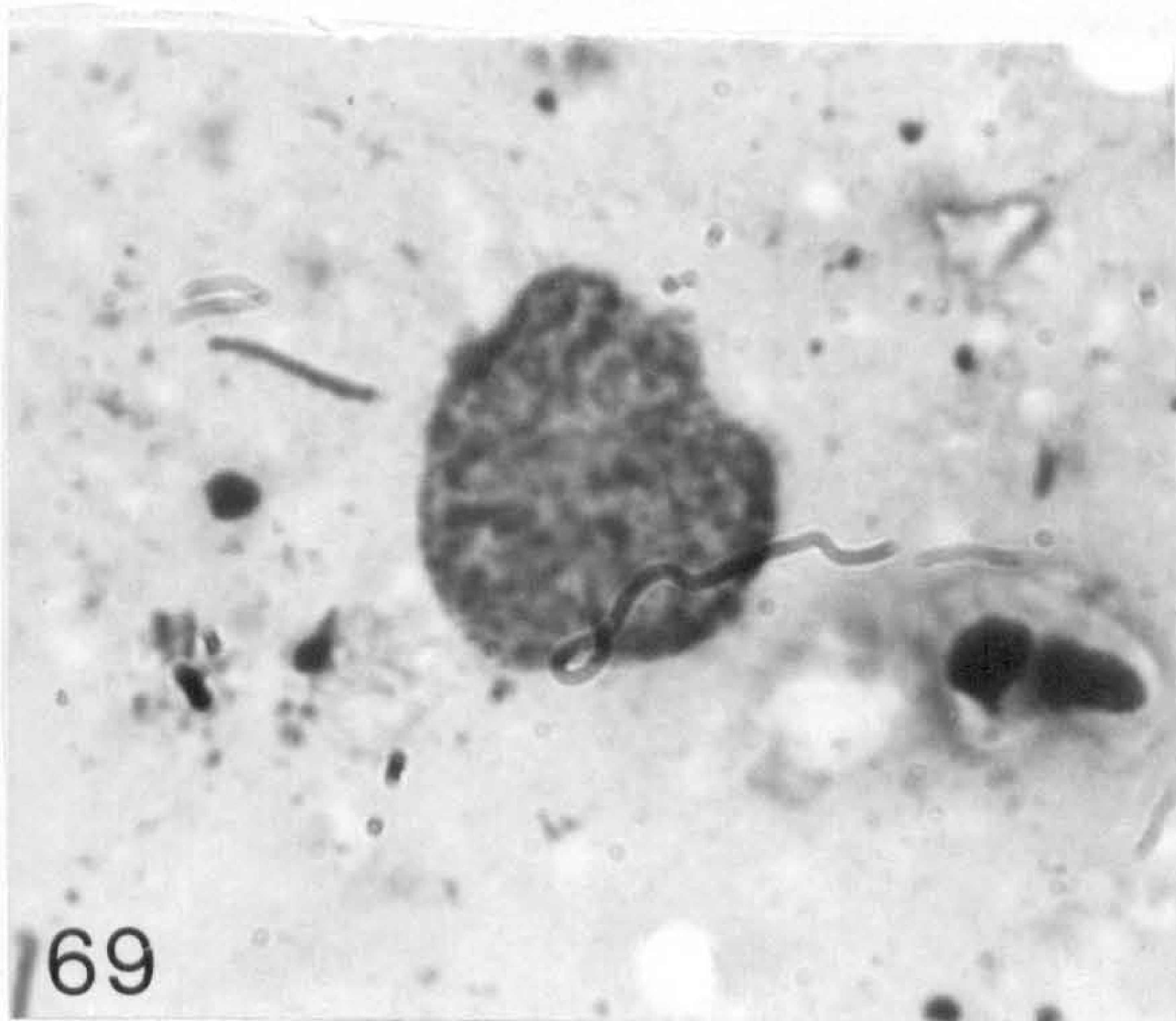
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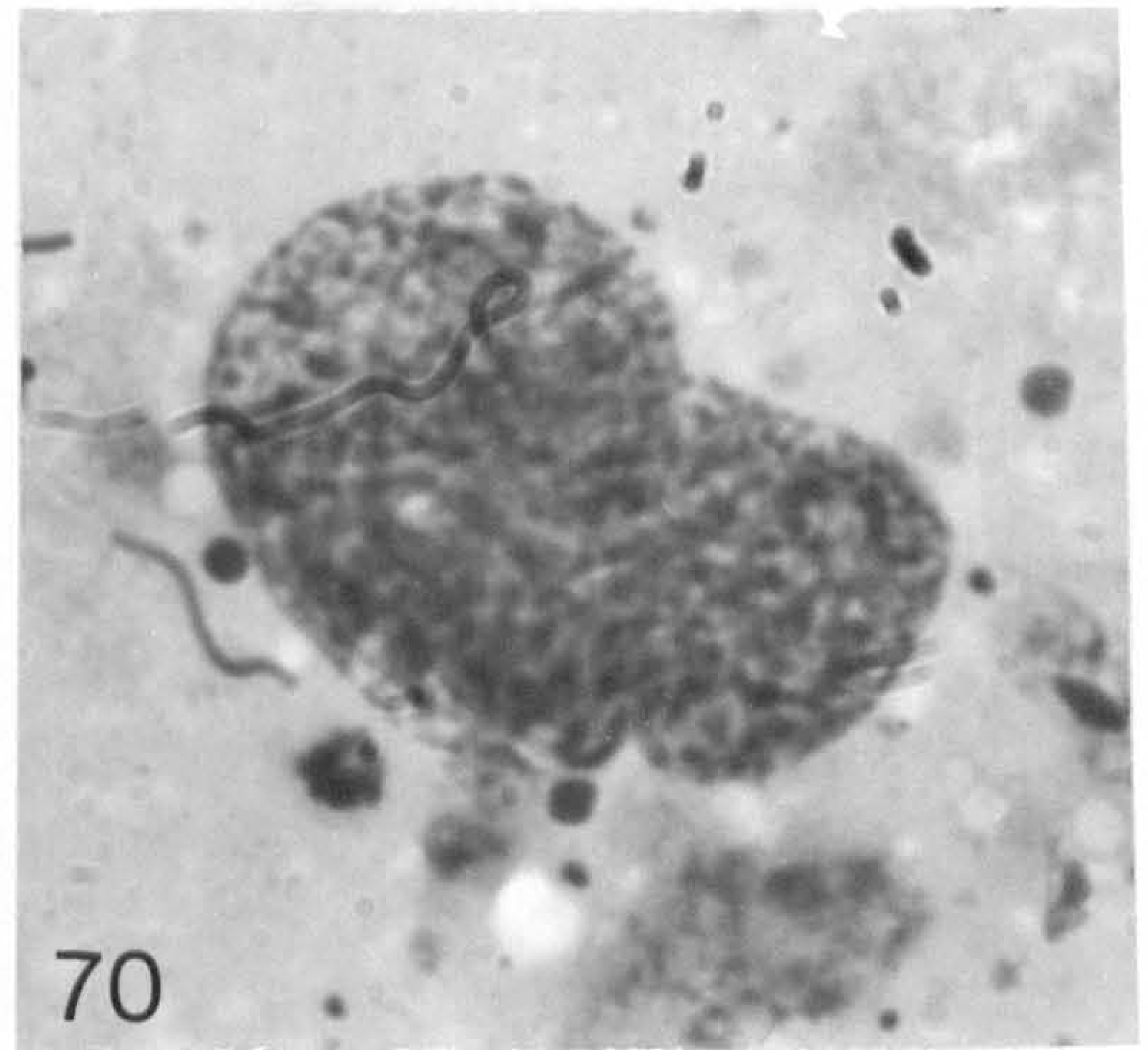
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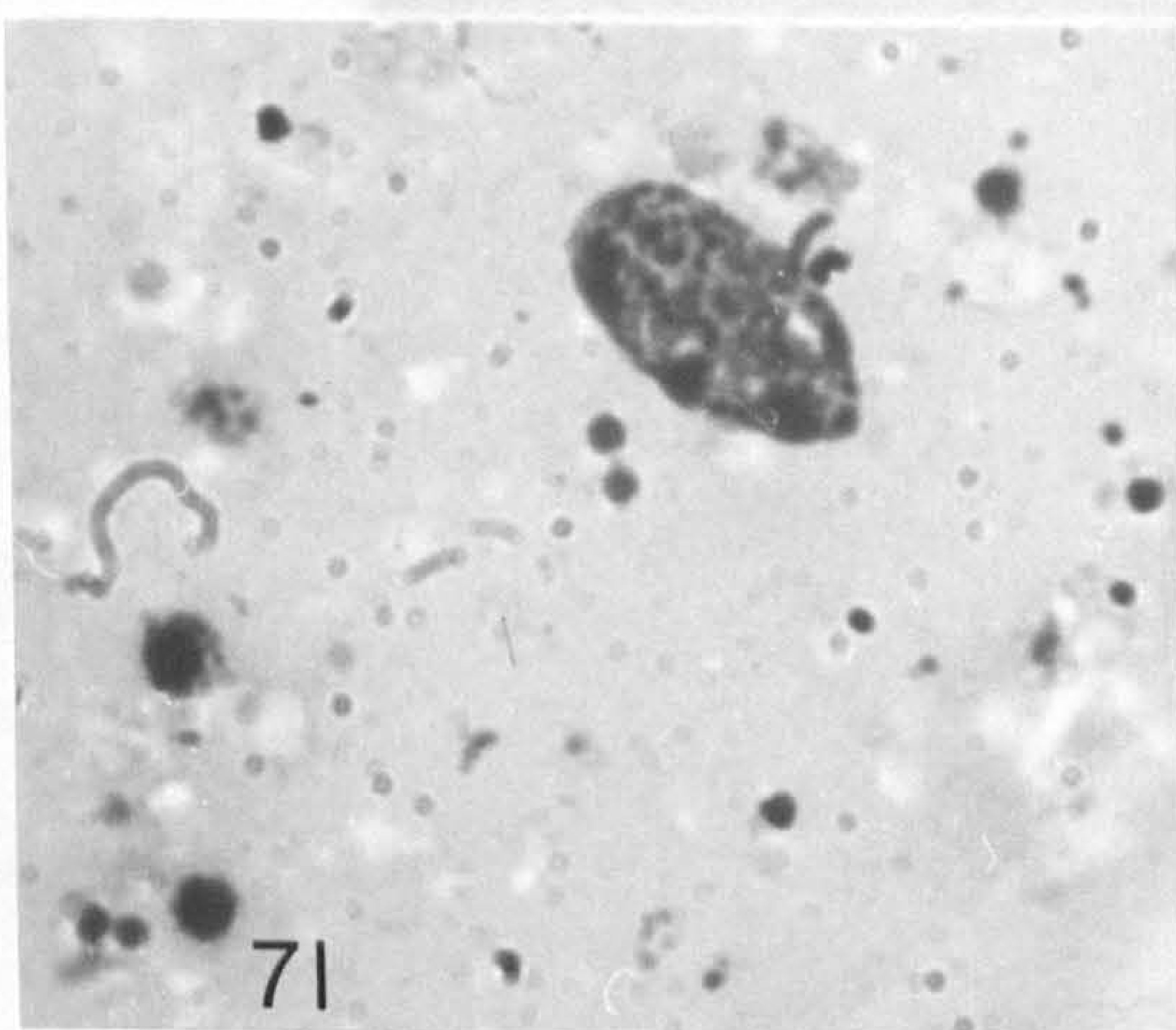
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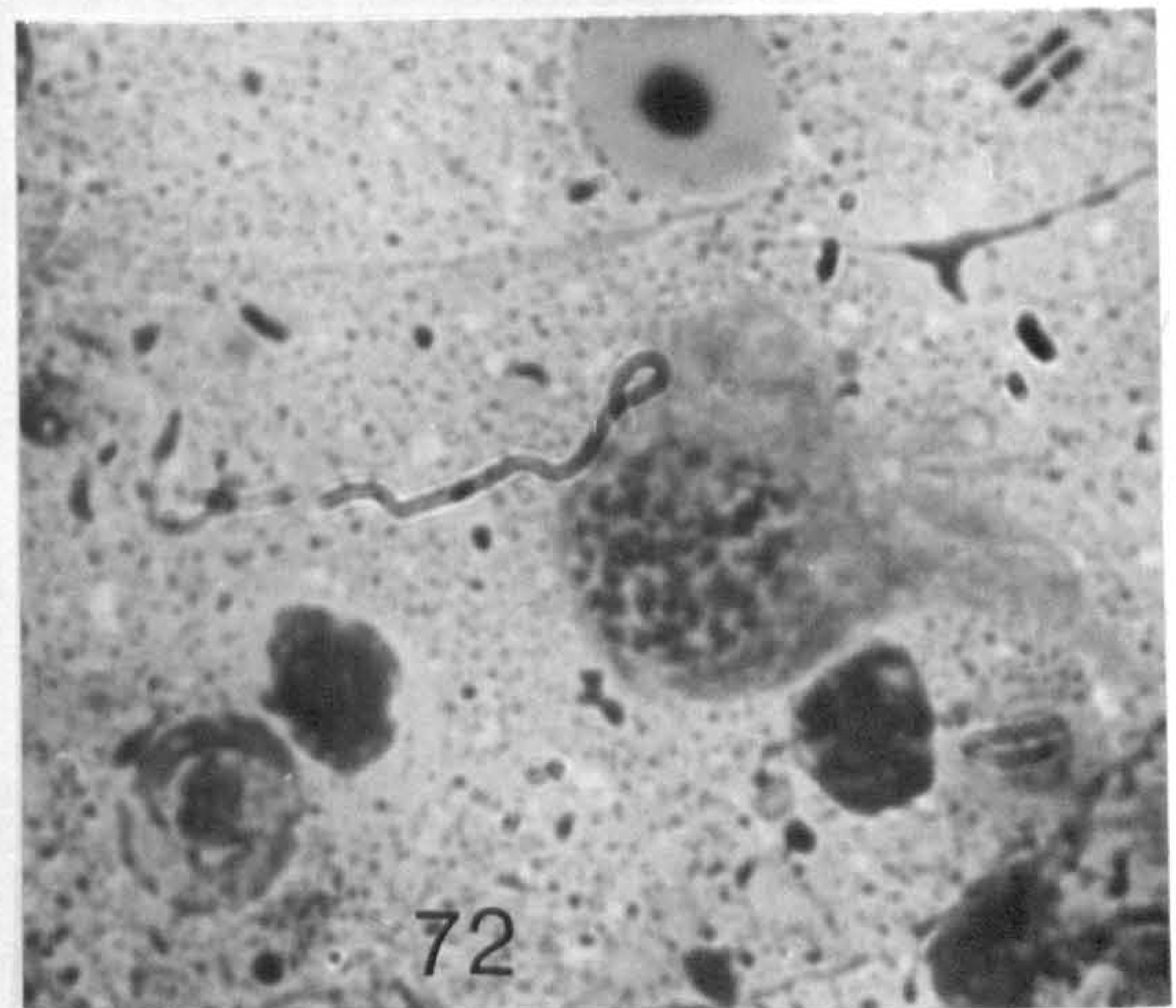
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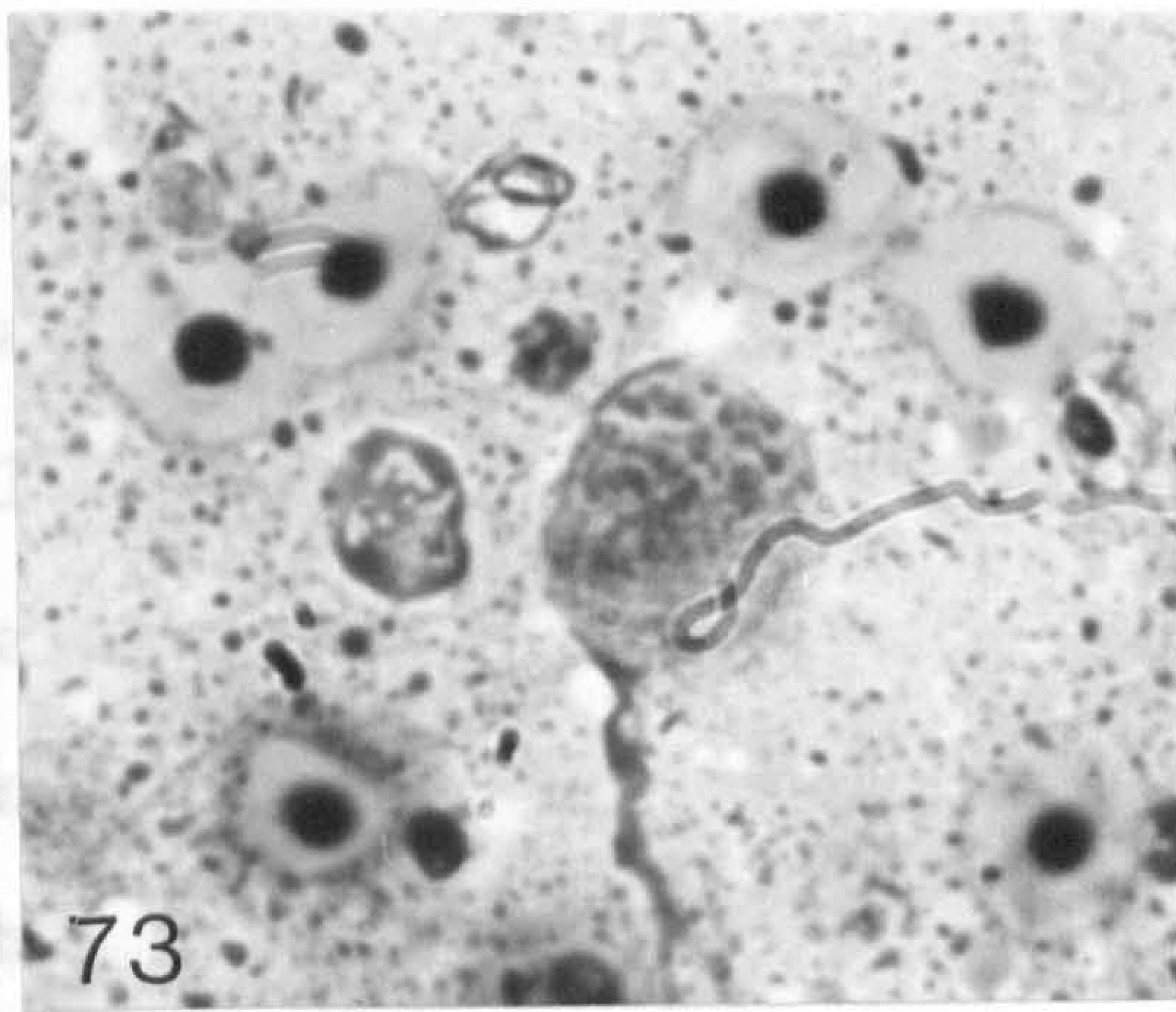
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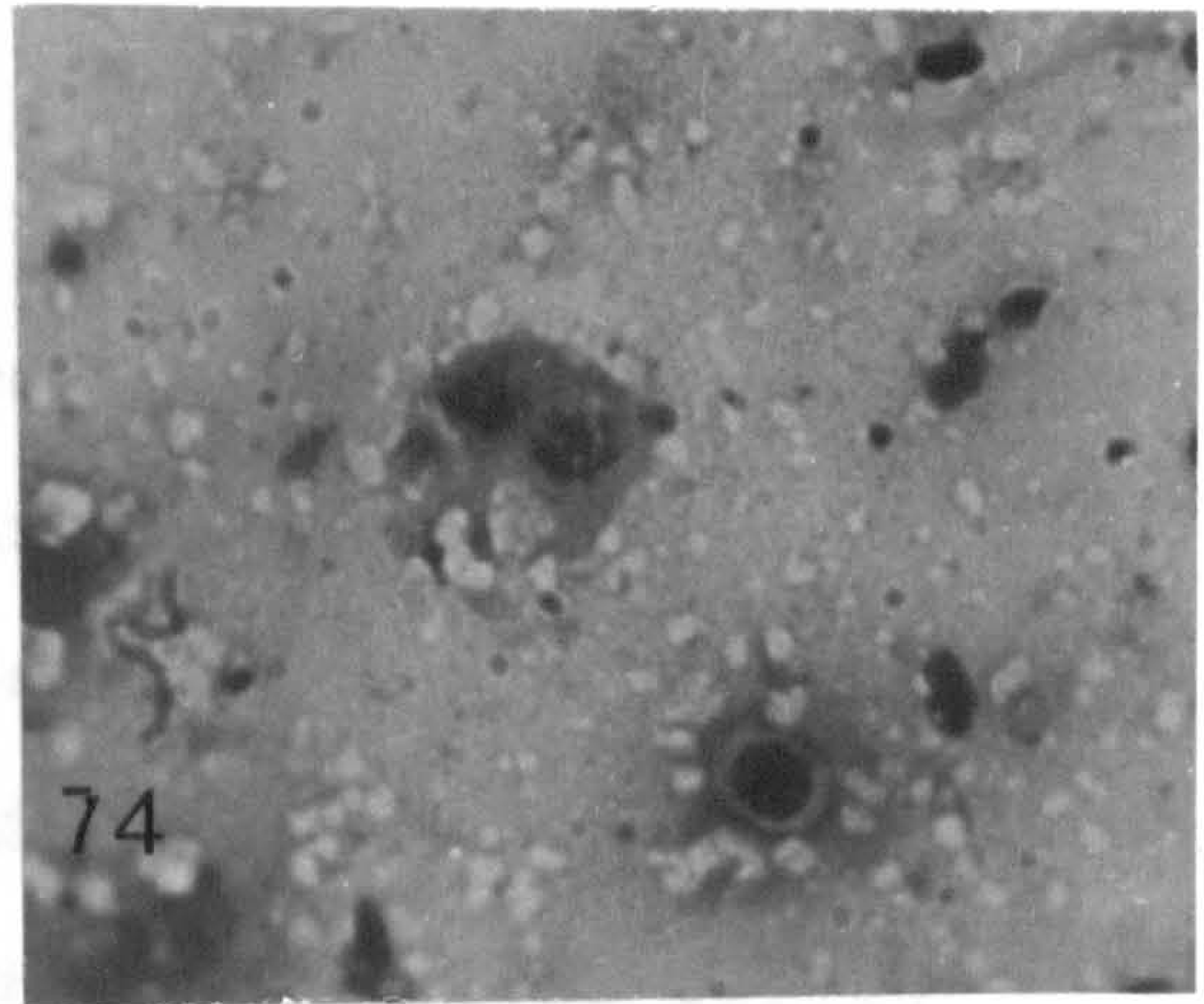
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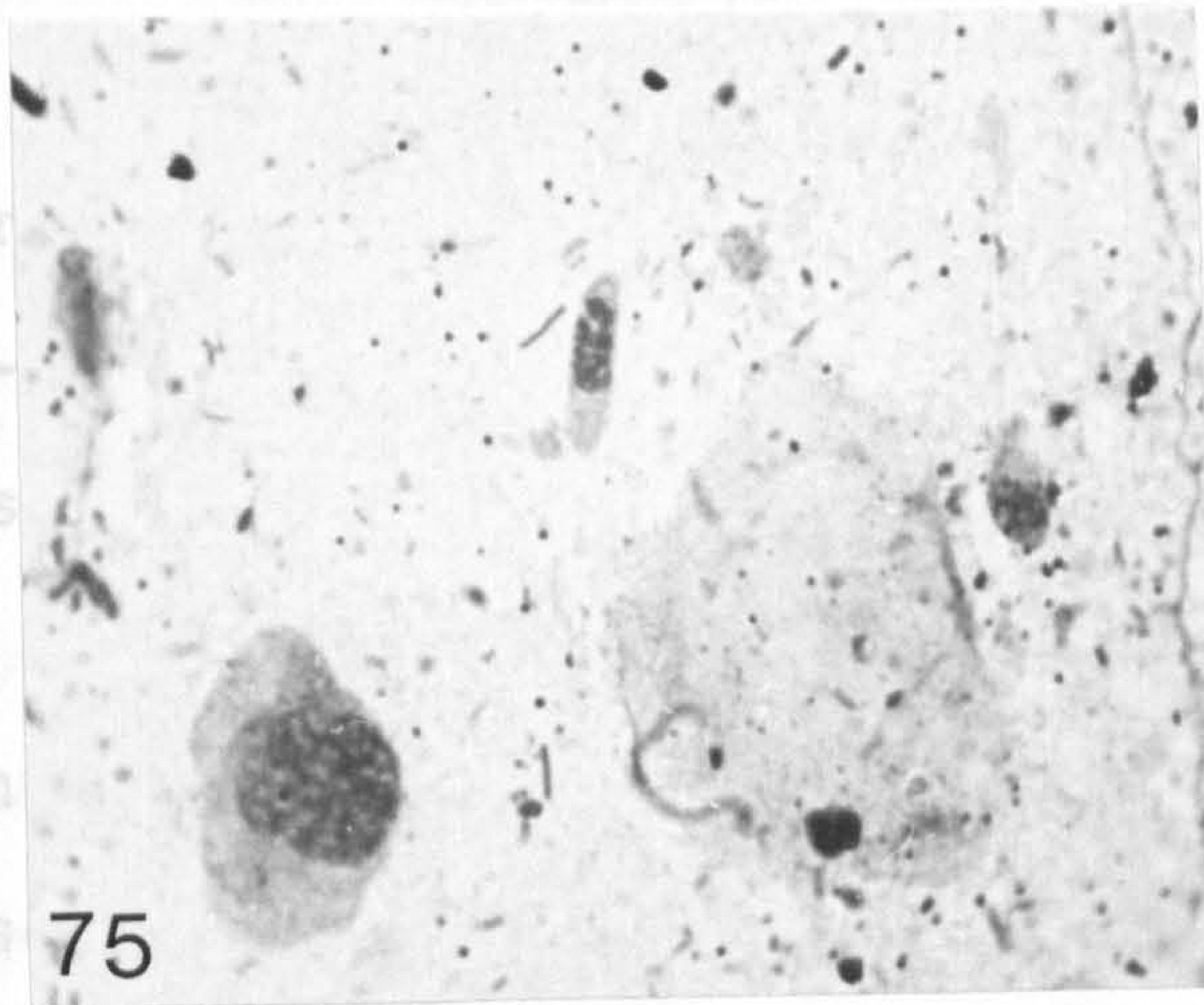
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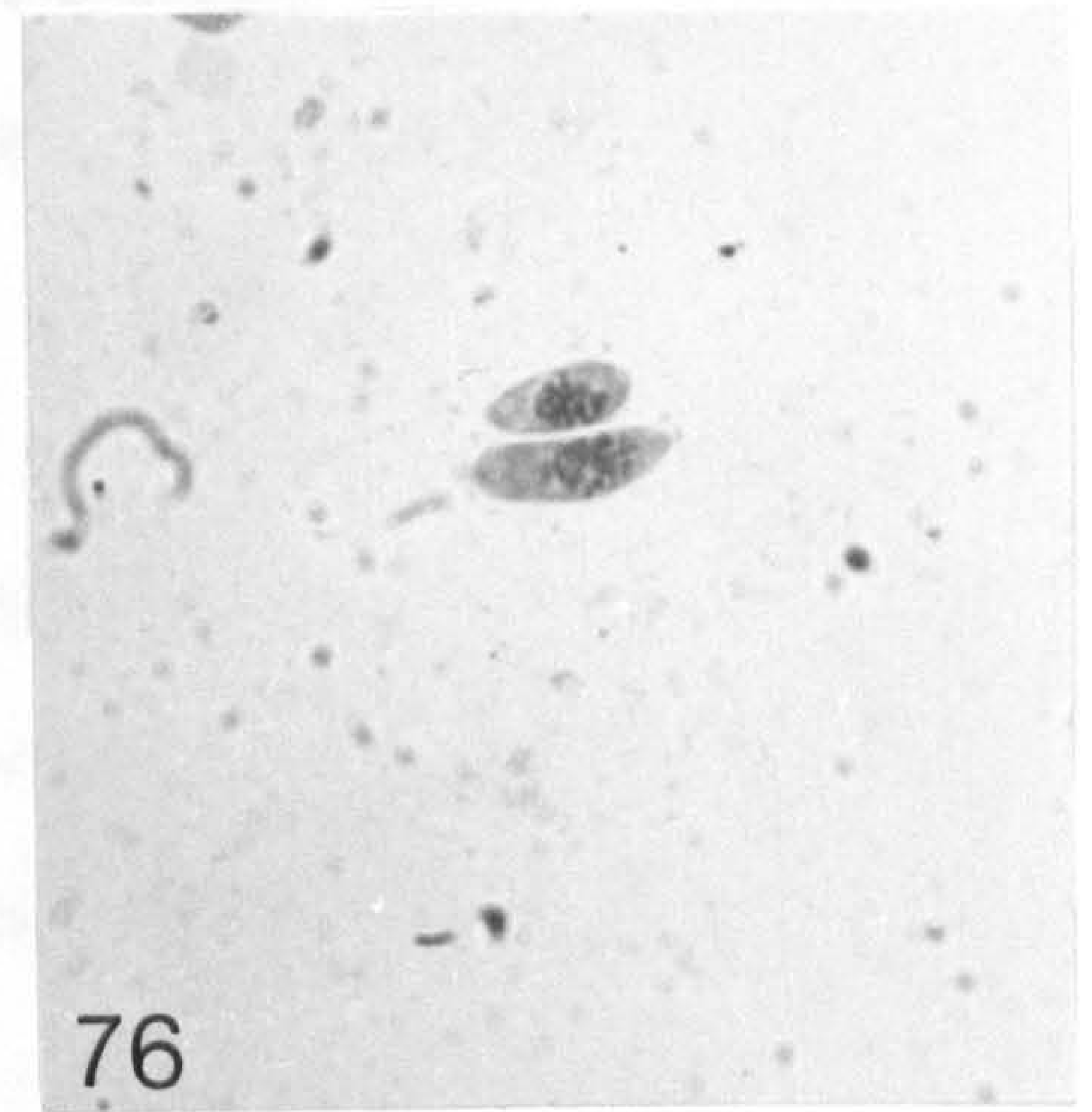
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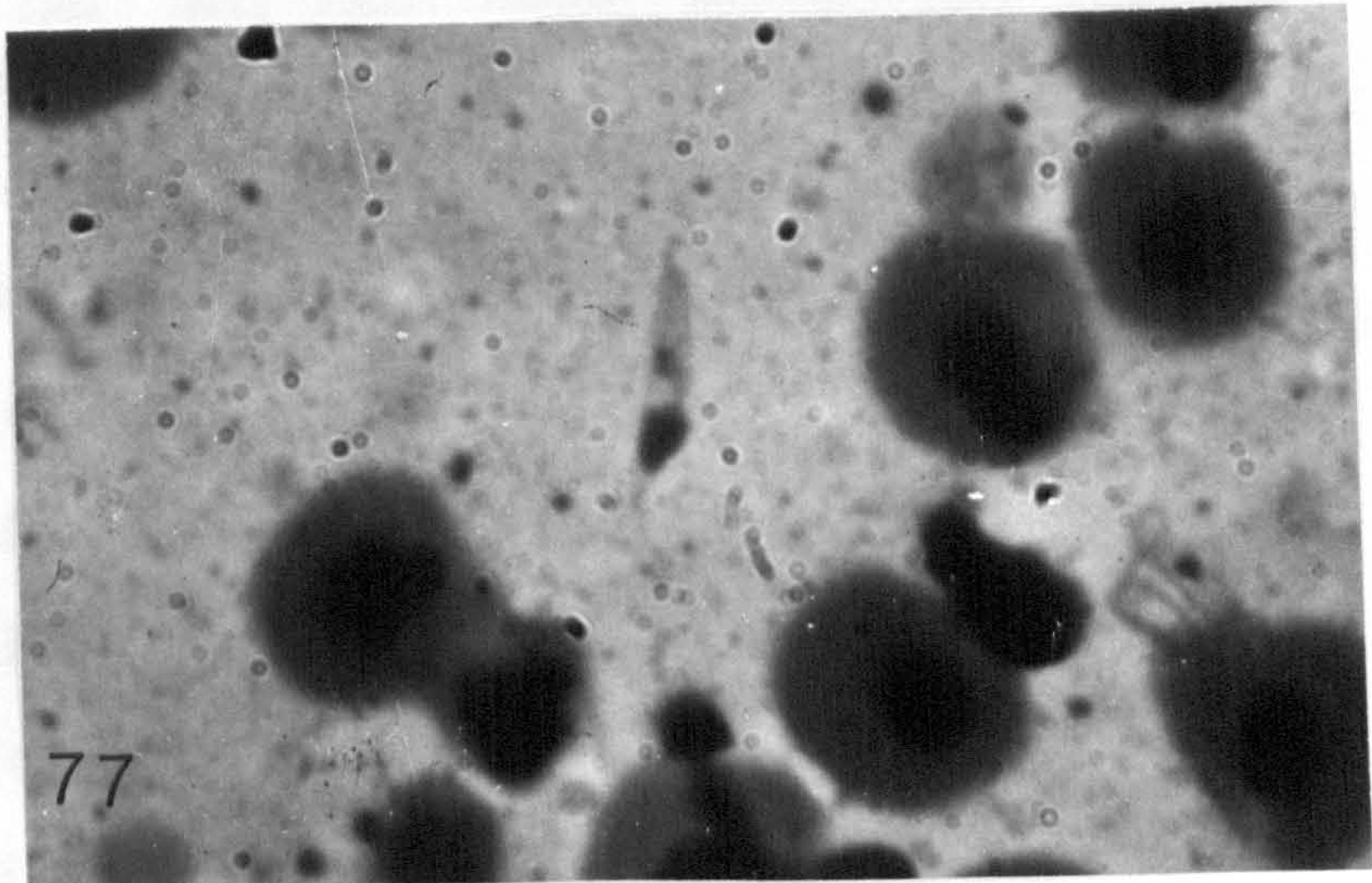
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(range 5.0-7.5).They might have been early zygotes or a stage towards sporozoite production. Two granular nuclei were also seen (Fig.74) and apparently separated into individual organisms with both poles rounded and a faintly blue staining cytoplasm (Fig.75). A characteristicly bright red staining nucleus filled 2/3 of these parasites and contained approximately 12 granules in parallel arrangement. The nucleus measured an average of 1.8 (range 1.4-2.1) by 1.4 (range 1.2-1.5) micron. The parasites had an average size of 3.6 (range 3.1-4.0) by 1.4 (range 1.2-1.5) micron and were considered immature sporozoites. These stages became more elongate with one pole pointed and one pole blunt (Fig.76). They eventually appeared as numerous free lancet-shaped organisms (Fig.77). These were characterized by a fine pointed anterior end with a dark staining large polar mass of an average size of 1.5 (range 1.3-1.6) by 1.3 (range 1.3-1.4) micron located very close to the anterior pole. The very small compact nucleus was in central location and the posterior end was tapering and somewhat rounded. The length of these parasites measured from 4.1-6.4 micron (average 5.0) and their width from 1.0-1.4 micron (average 1.1). They were considered mature sporozoites because of the sudden increase in their abundance from the 10th day after initial infection of the leeches and their morphology might have been designed to penetrate new host cells.

4.4. Stages of development of *Haemogregarina simondi* in the marine leech *Hemibdella soleae*

The developmental forms of *H.simondi* as encountered in the alimentary tract of the vector leech *Hemibdella soleae* can be described as follows. Although these stages could not be exactly located in the alimentary tract of these leeches and appeared to be randomly distributed some explanation seems possible from the morphology and size of the stages of development found and is suggestive for a life cycle of this haemogregarine in *Hemibdella soleae*.

Syngamy was never seen and one of the earliest stages observed appeared to be an oval-shaped structure measuring 35 by 40 micron and containing a large nucleus surrounded by a thin nuclear membrane. The chromosomal material inside this nucleus appeared to be breaking up into fragments. The cytoplasm stained deep blue and was of very granular appearance and no "reduction spindle" was observed (Fig.78). This stage was considered an early *zygote* with the nucleus preparing for division. Another zygote measured 36.4 by 32.5 micron and contained 2 nuclei in close proximity, presumably after division had taken place. The larger nucleus measured 11.0 by 9.1 micron, the smaller nucleus

FIGURES 78 - 88.

Development of HAEMOGREGARINA SIMONDI
in HEMIBDELLA SOLEAE

78. Early zygote with large nucleus undergoing division x 1,200

79. Zygote with 2 nuclei in close proximity x 1,000

80. Early oocyst with sporoblasts x 400

81. Large oocyst with numerous sporoblasts x 400

82. Mature oocyst with sporoblasts x 400

83. Liberated sporoblast,now sporocyst with 5-6 dark nuclei x 1,000

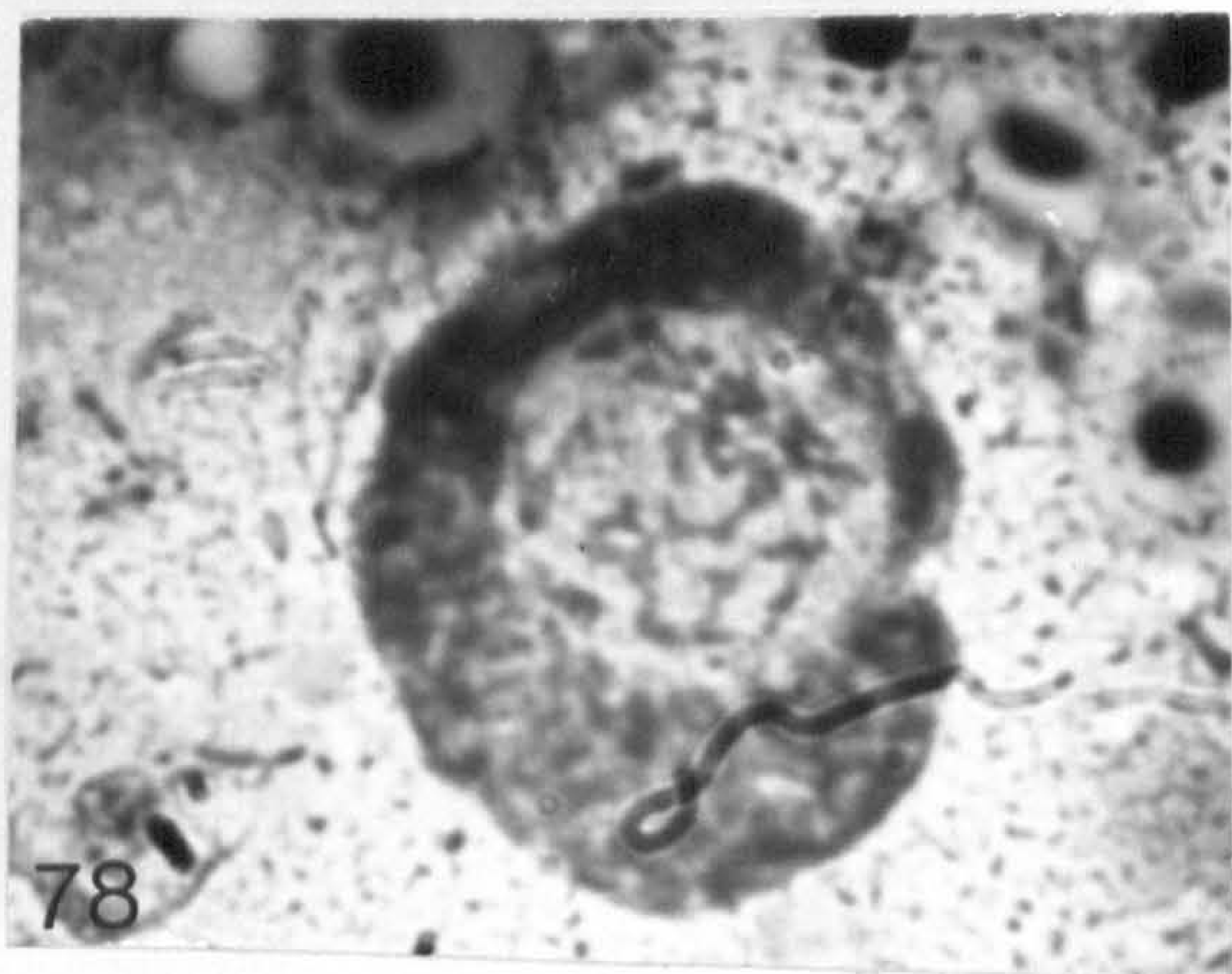
84. Sporocyst with 10 nuclei x 1,000

85. Sporocyst with 26 nuclei x 1,000

86. About 24-26 sporozoites after rupture of sporocyst x 1,200

87. Two individual sporozoites with bi-polar refractile bodies x 1,200

88. Enlargement of Fig.80 showing sporoblasts containing 6-8 distinctly staining granules(arrow) x 1,250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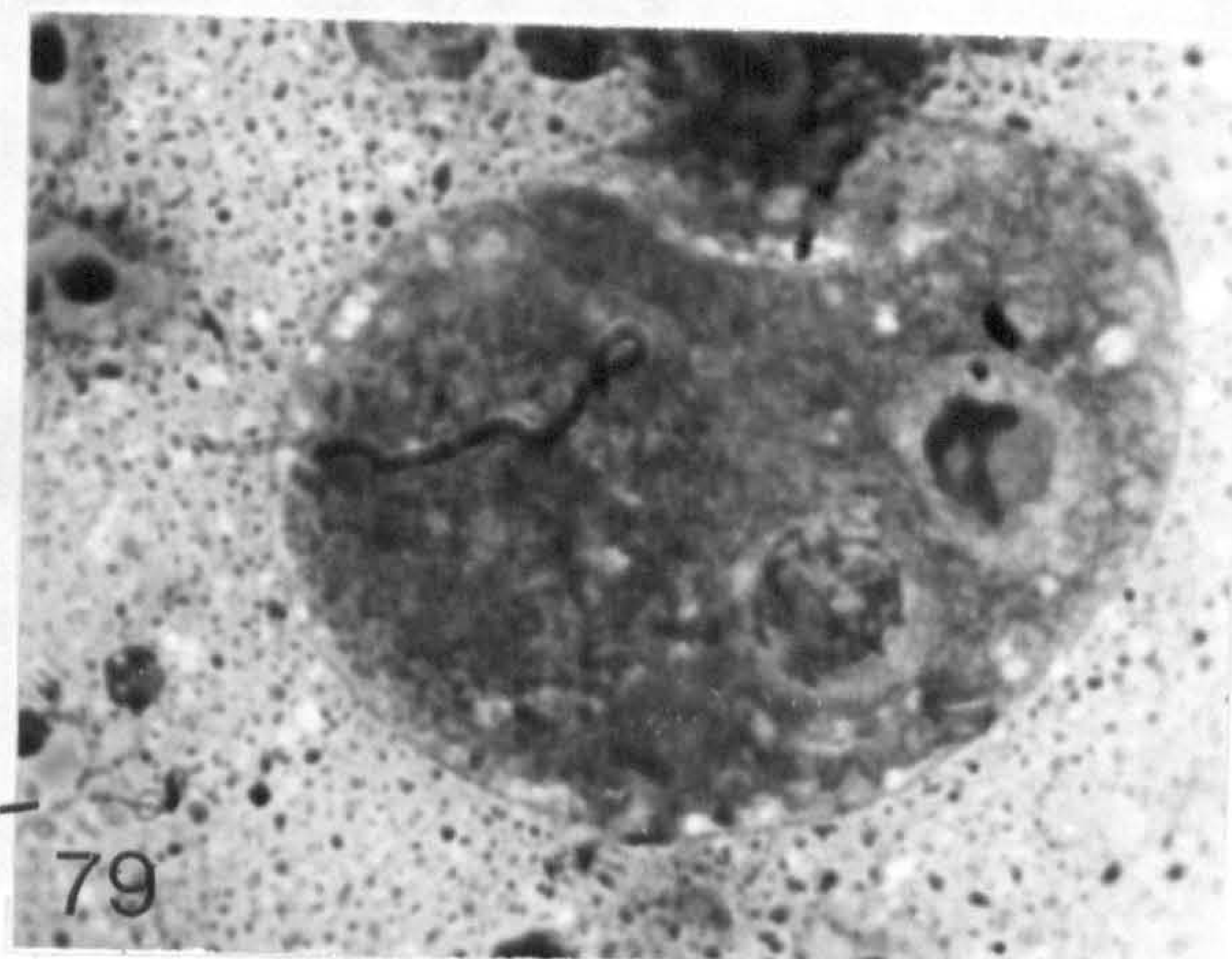
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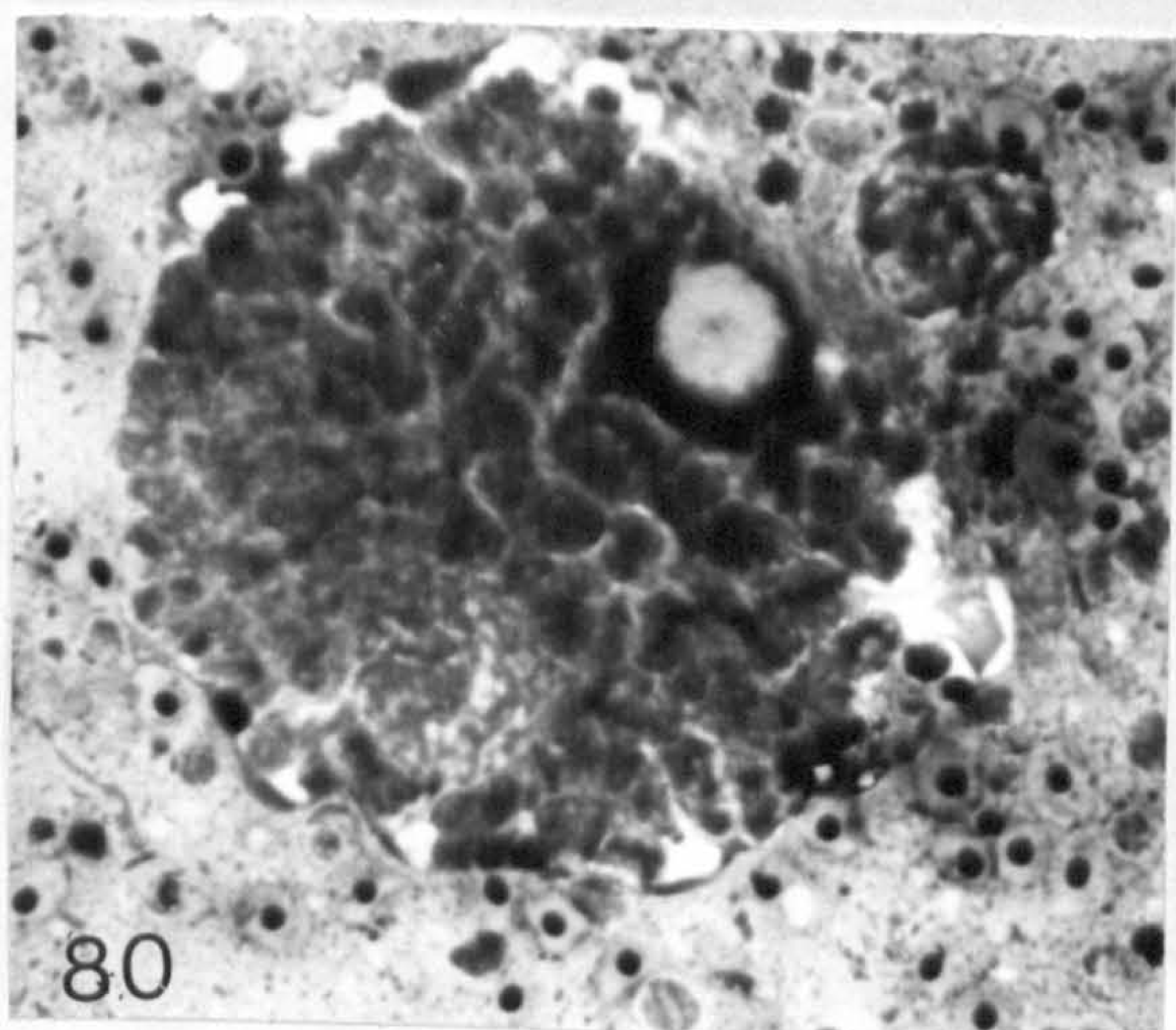
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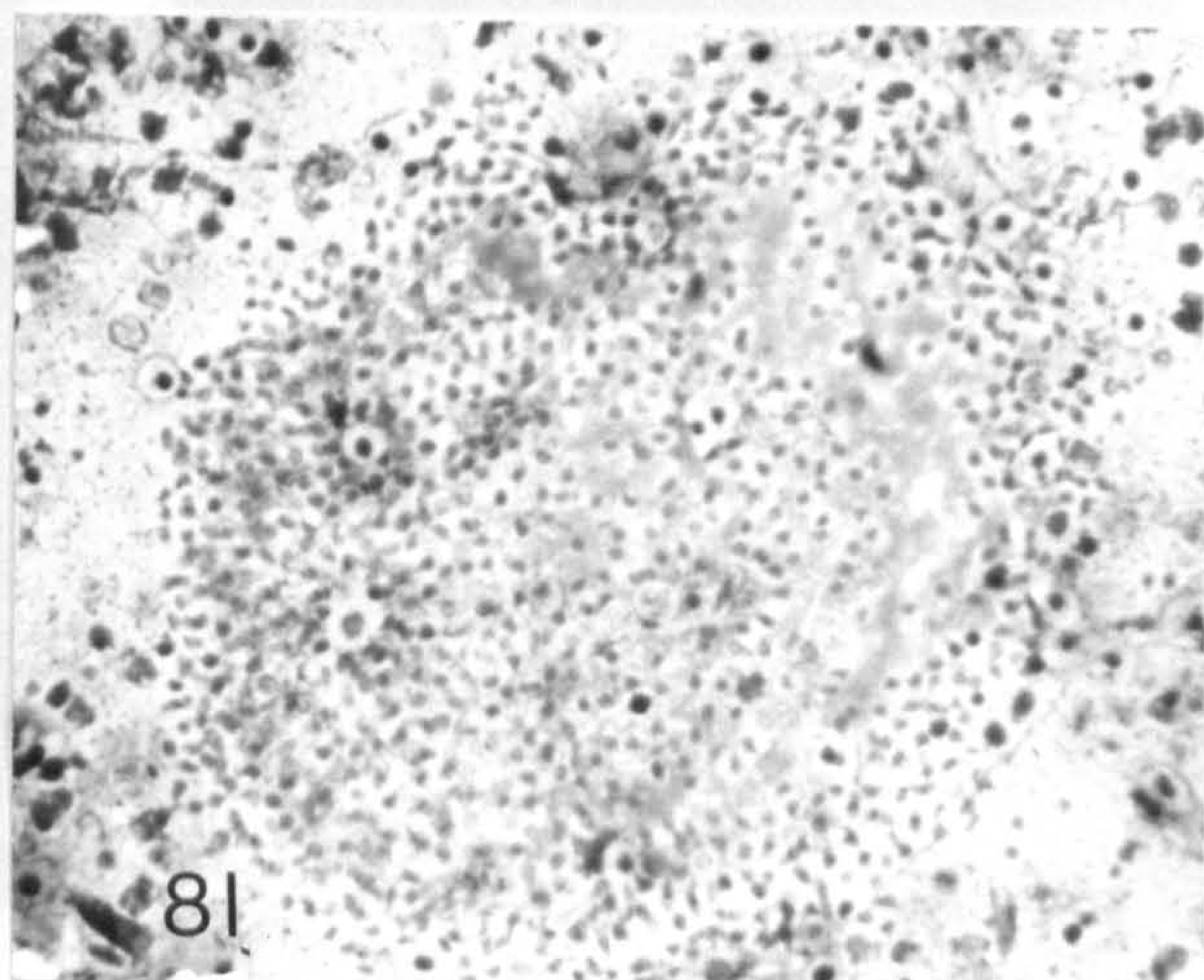
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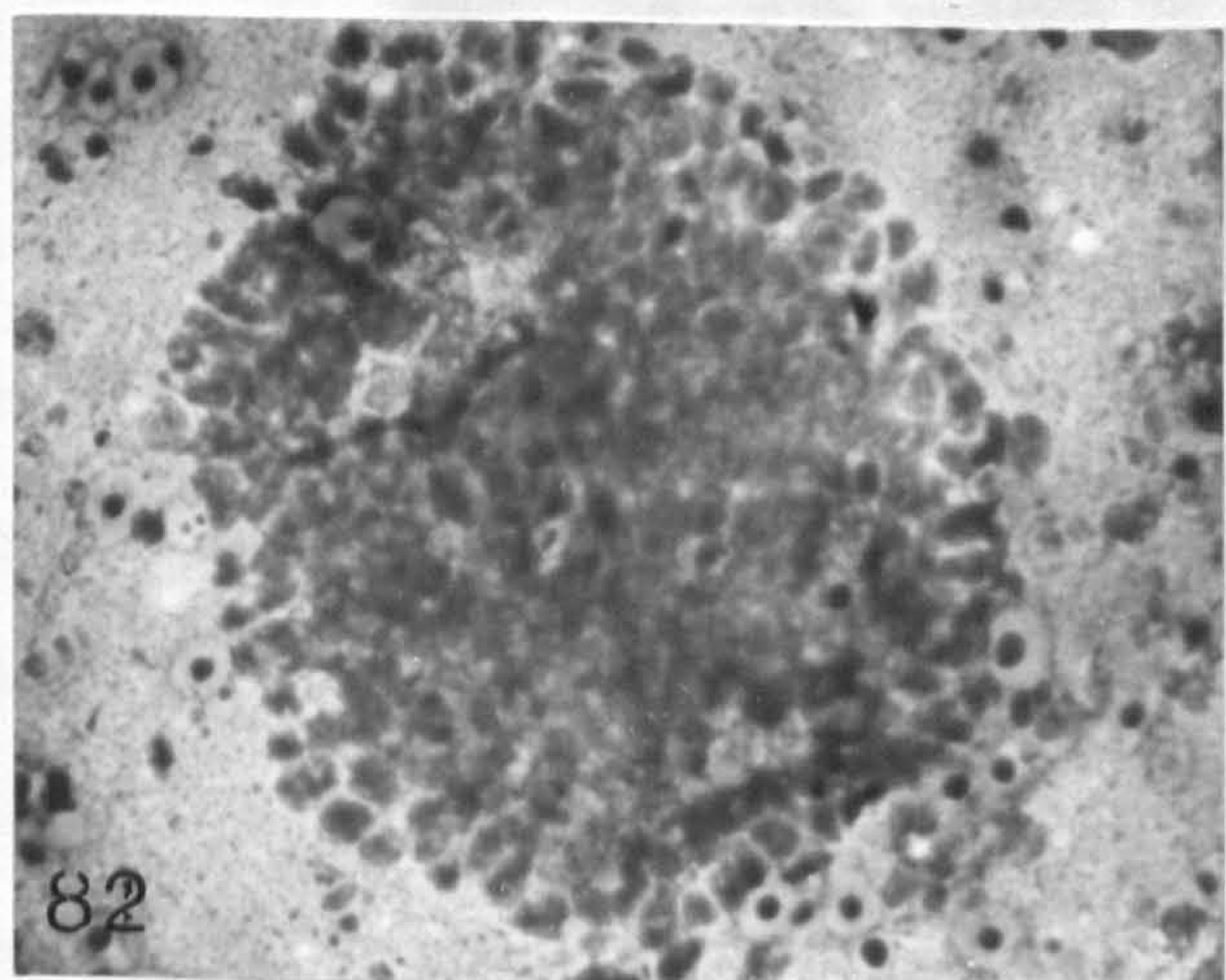
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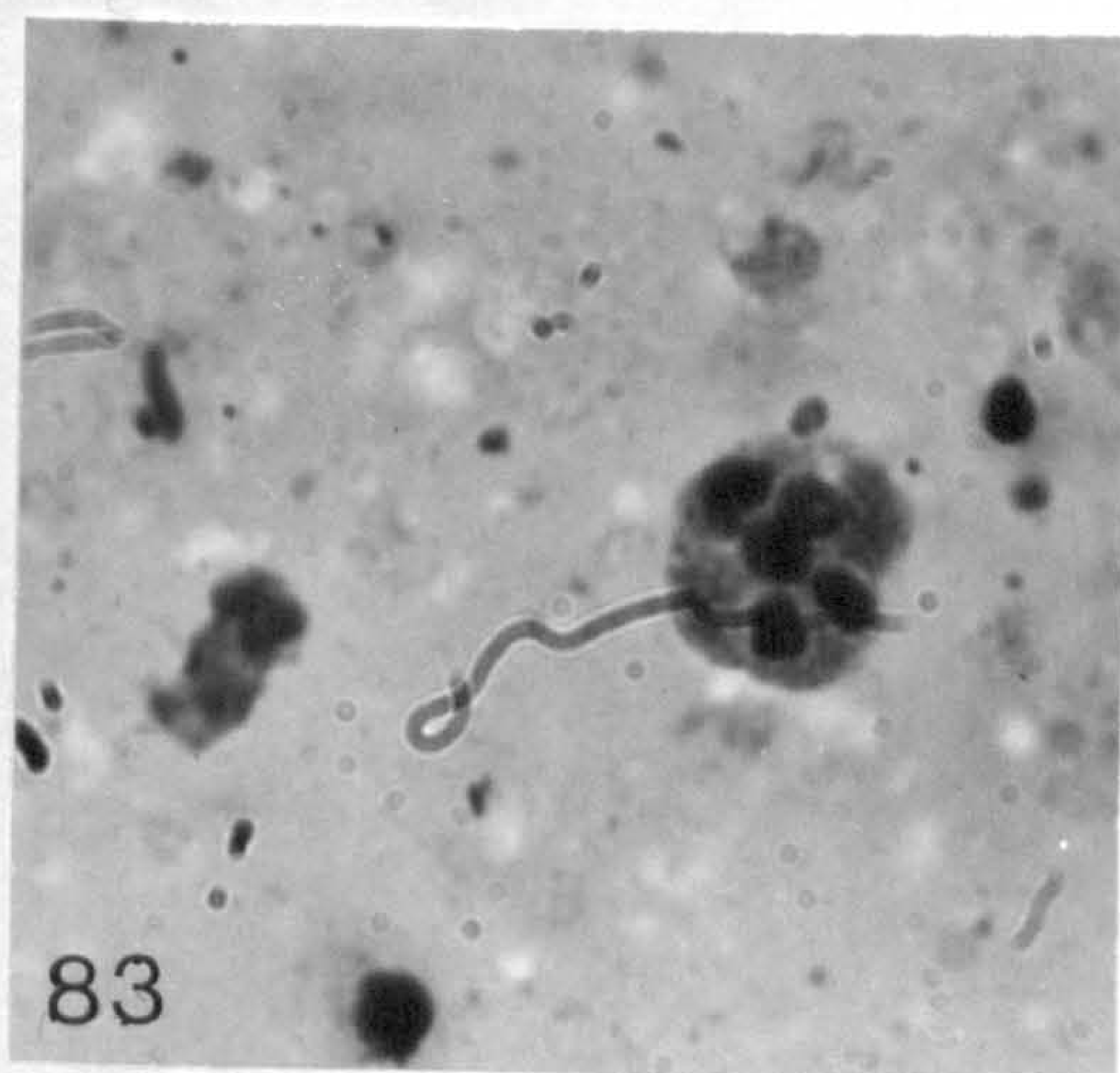
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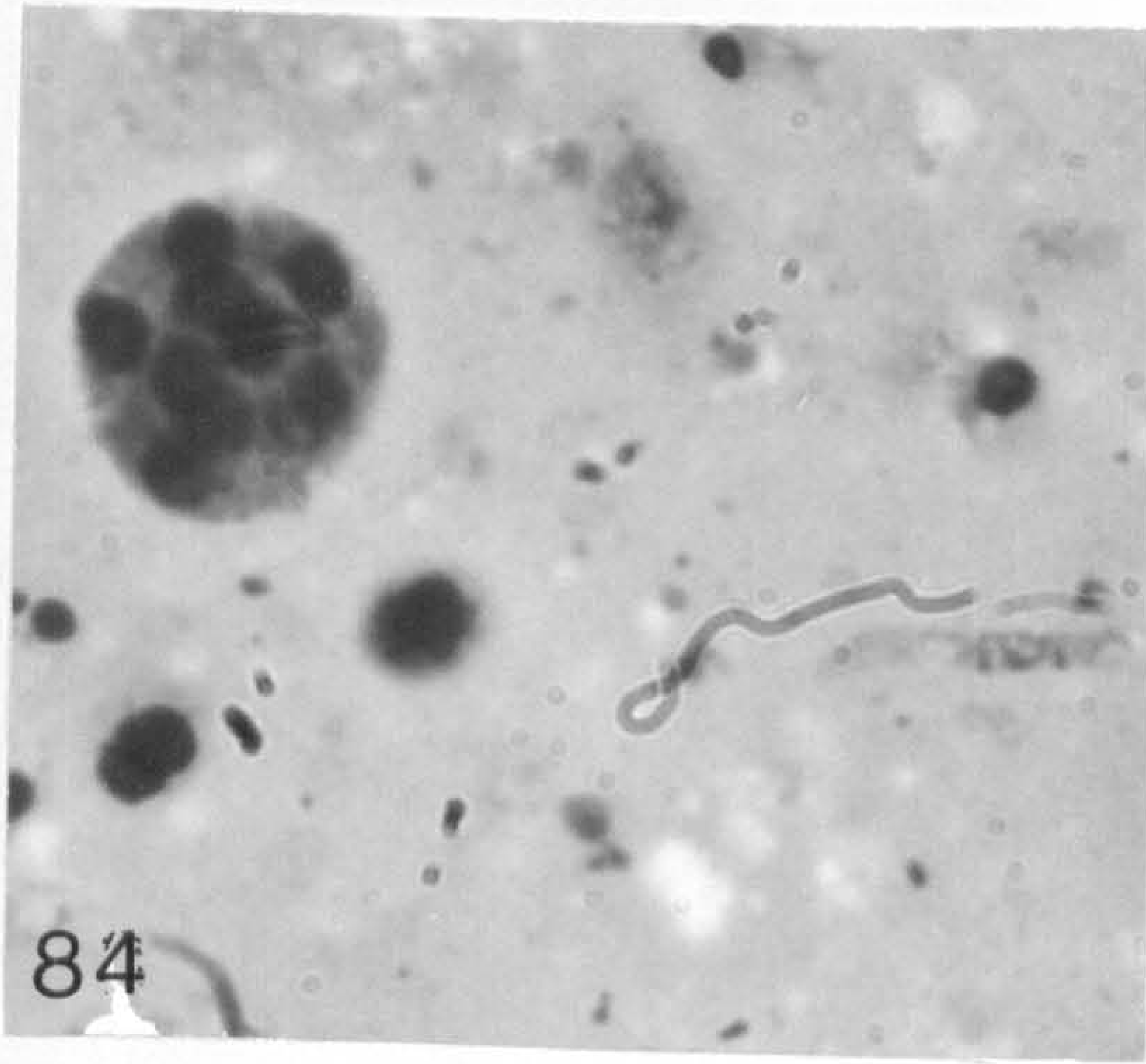
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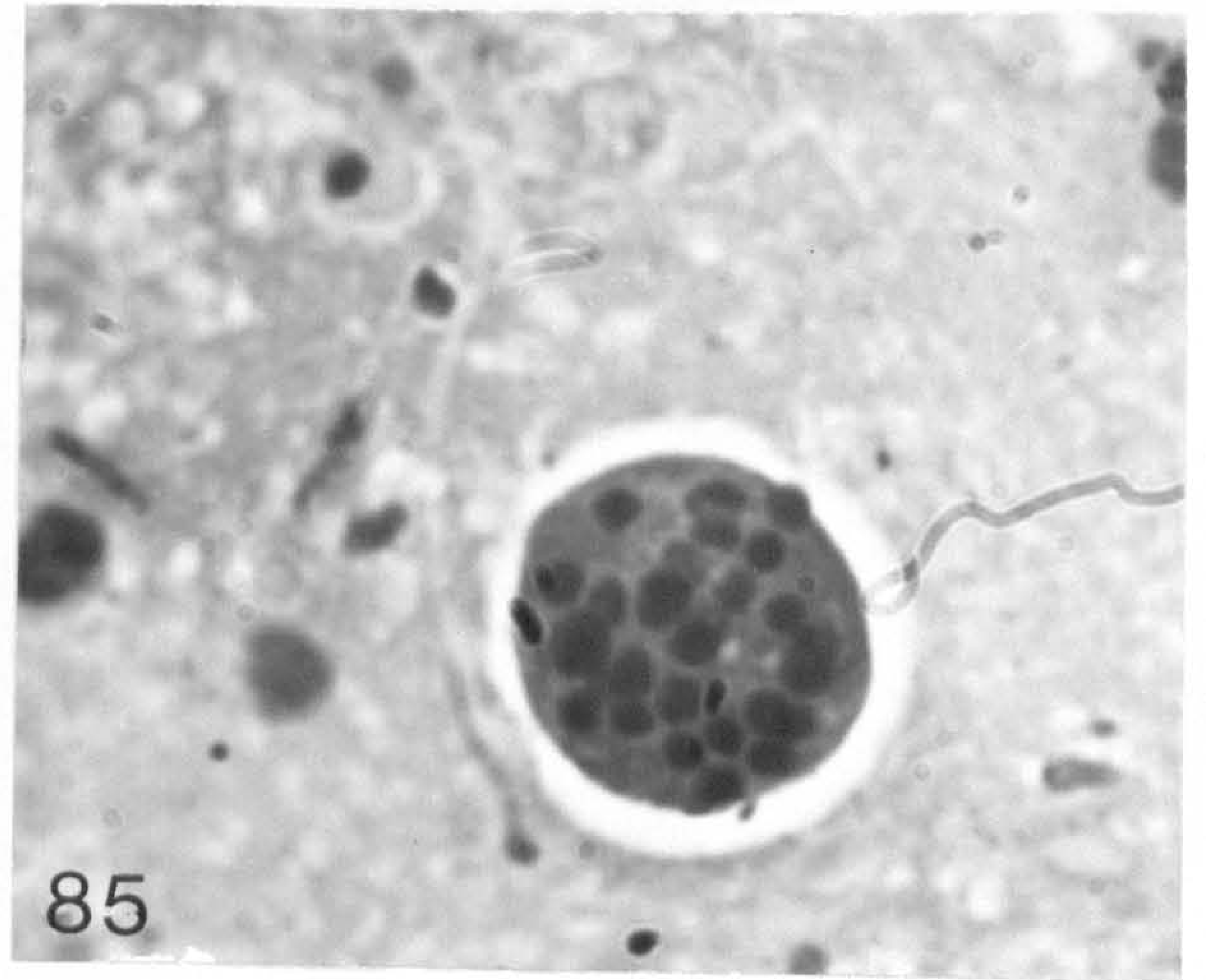
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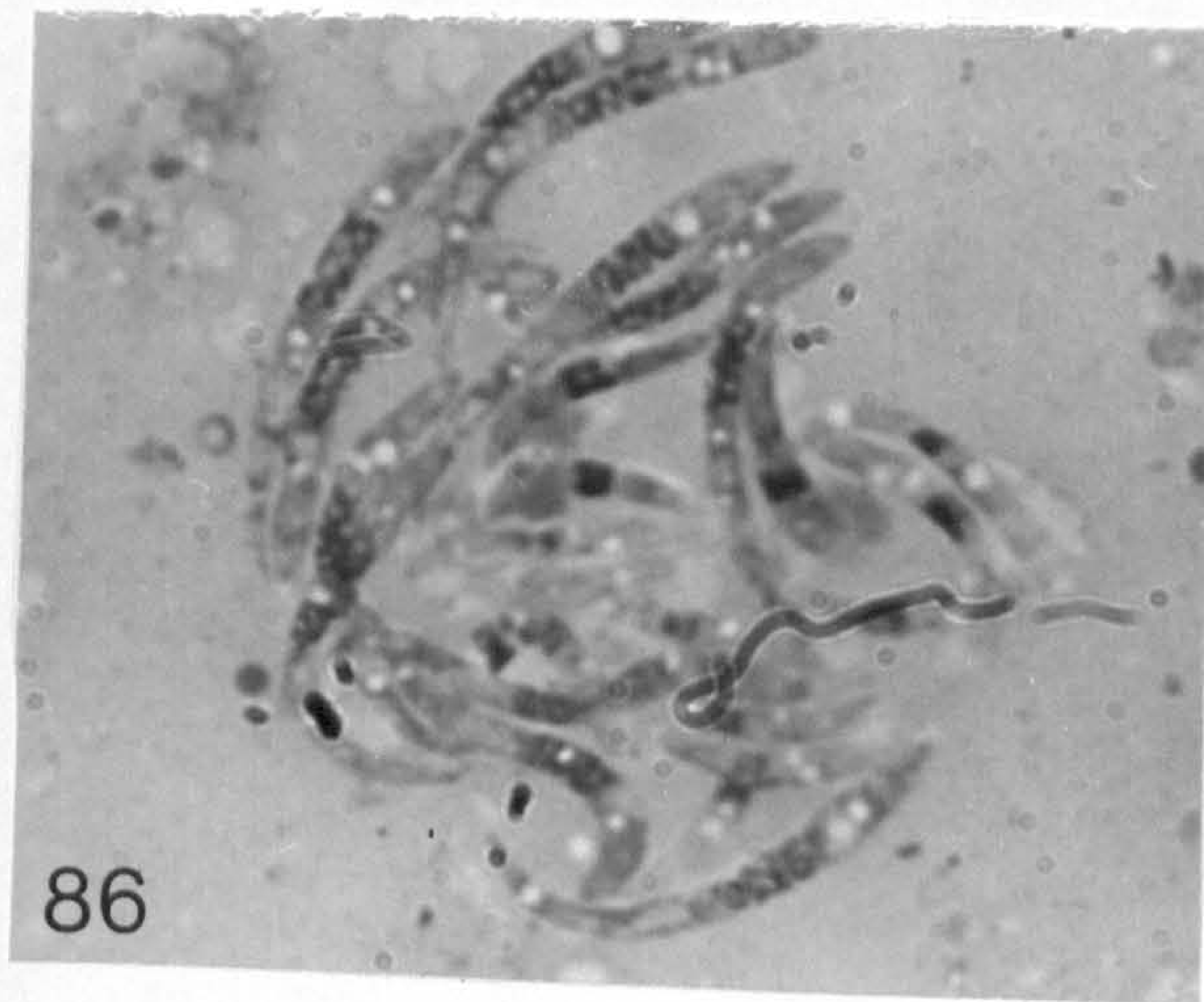
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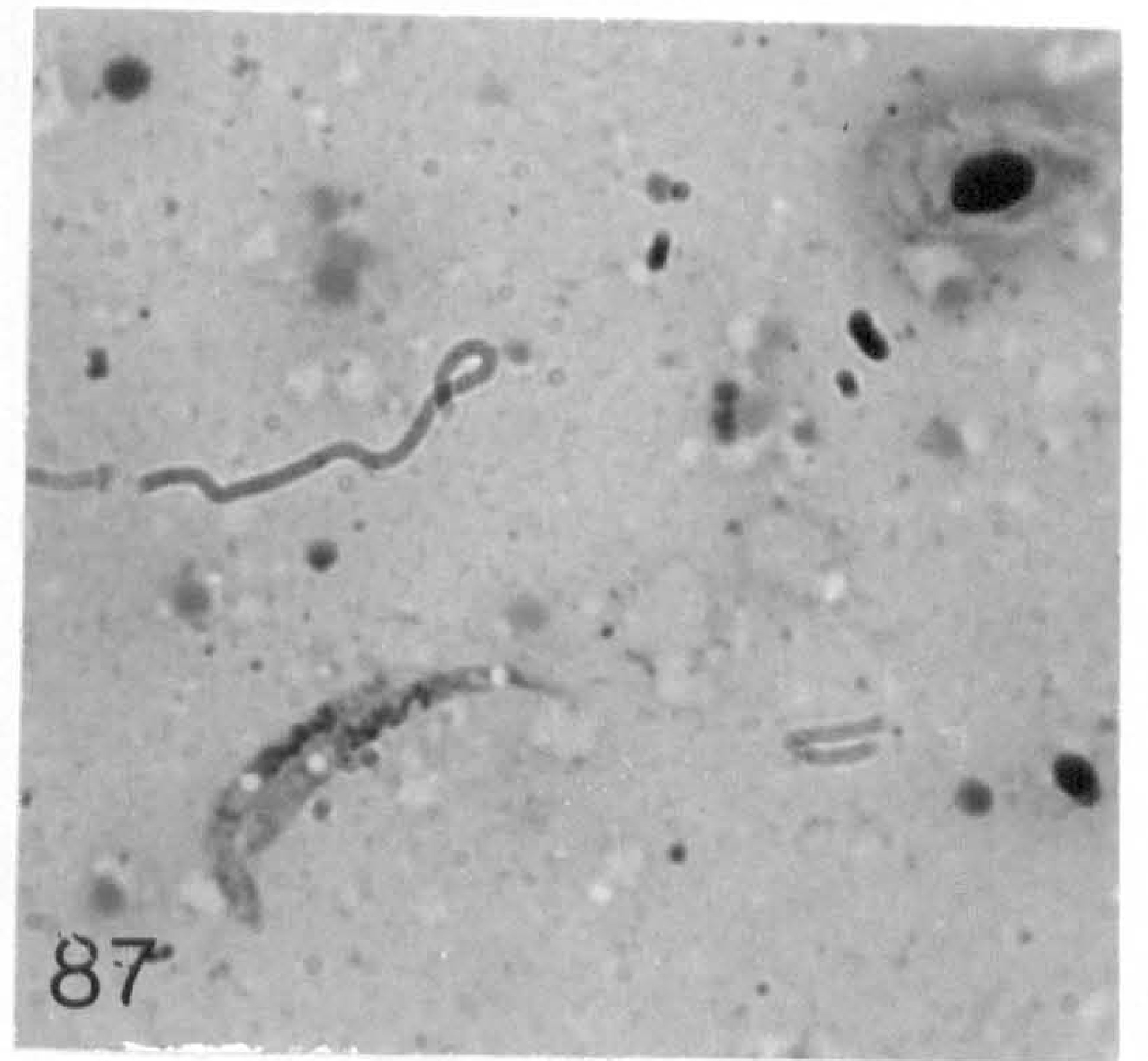
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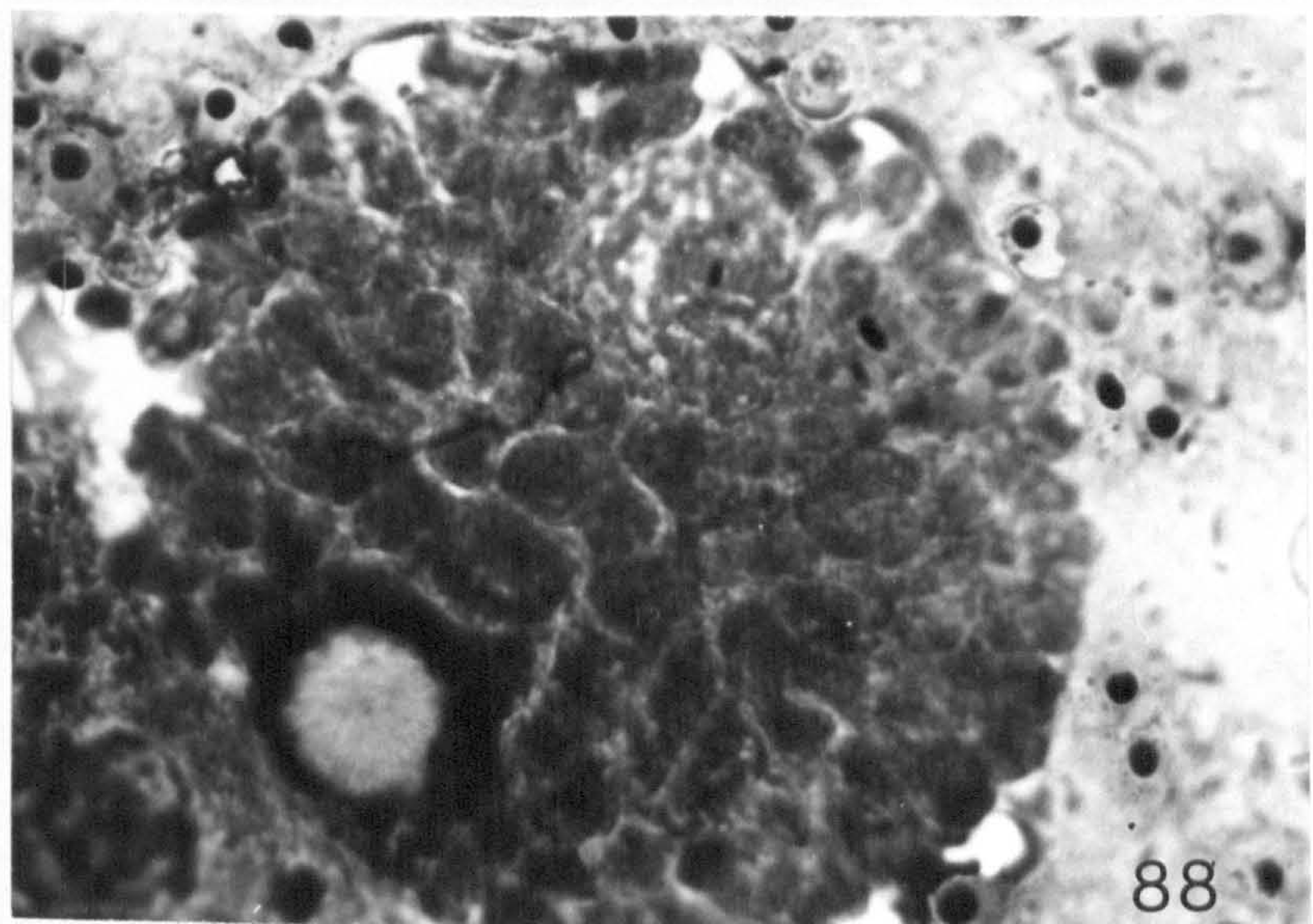
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measured 5.2 by 5.2 micron. The cytoplasm was of fine granular composition but appeared more vacuolized. Yet another zygote measured 45.3 by 29.3 micron and also appeared to have 2 nuclei in a fine granular cytoplasm interrupted by areas of more or less extensive vacuolization (Fig.79). In a later stage the two nuclei seemed to have moved apart. A differentiation had taken place into approximately 80-100 deeply stained nuclei (Fig.80,88). This stage measured 88 by 100 micron and was considered to be an early oocyst containing numerous sporoblasts. This oocyst stage seemed to enlarge further to 248 by 220 micron containing several hundred sporoblasts (Fig.81) and its size was later reduced to a more compact structure of 132,0 by 129.6 micron (Fig.82). It appeared then that these sporoblasts broke out of the oocyst and further nuclear division ensued. This stage was now regarded as sporocyst. These sporocysts measured about 7.6 by 7.8 micron and contained 5-10 spheric,dark staining nuclei (Fig.83,84) inside a granular cytoplasm. They further increased to about 16.7 by 14.7 micron containing now approximately 24 nuclei (Fig.85). These nuclei apparently differentiated into small individual organisms and were released after rupture of the sporocyst.They were considered sporozoites and numbered between 24-26 (Fig.86). These sporozoites were crescentic shaped organisms with a thin tapering posterior end and

a club-shaped and bluntly pointed anterior end (Fig.87). They measured between 9.1-10.4 micron in length (average 9.7) and an average of 0.6 micron in width. The elongate and bright red staining nucleus was composed of 10-12 intensively staining granules and measured an average of 2.8 by 0.6 micron taking up the central 1/3 of the sporozoite. Near both ends of the parasite two distinct refractile bodies smaller than the nucleus and often filling the total width of the parasite were observed. In addition smaller vacuoles or refractile bodies were also seen in the anterior club-shaped portion of the parasite. Sometimes there were found also small oval or round parasite stages of 3.9-6.5 micron in length (average 5.2) and 2.6-3.2 micron in width (average 2.9) with a comparatively large nucleus of 2.6 by 2.4 micron in average. It is assumed that these might have been developing sporozoites from a sporocyst which had been accidentally ruptured probably through mechanical pressure before these sporozoites had matured and actually reached their normal size as in Fig.86,87.

4.5. Transmission Experiments using marine piscicolid leeches as possible vectors of haemogregarines

4.5.1. Calliobdella nodulifera

Attempted transmission of Haemogregarina sachai n.sp.

to apparently uninfected *Scophthalmus maximus* by using leeches as either mechanical vectors or as true intermediate hosts was unsuccessful. Likewise the force-feeding of experimentally infected leeches to *Scophthalmus maximus* failed.

4.5.2. *Hemibdella soleae*

Attempts to induce an infection with *Haemogregarina simondi* in *Scophthalmus maximus* by force-feeding them haemogregarine infected leeches were not successful. However,experimental transmission of *H.simondi* to apparently uninfected hatchery *Solea solea* was achieved by having infected leeches feed on these fish. Also the two sentinel fish placed into one of the tanks at Hunterston containing haemogregarine infected leeches and their equally infected fish hosts showed a moderate infection with *H.simondi* when blood smears were examined approximately 2 months after the onset of this experiment. Haemogregarine infected leeches did not transmit these parasites to *Scophthalmus maximus* when feeding on these fish.

4.6. Transmission experiments with *Lernaeocera* sp.

All attempts to transmit *H.simondi* to *Scophthalmus maximus* by force-feeding this copepod to the fish failed.

5. DISCUSSION

5.1. Marine leeches as vectors of haematoprotozoan parasites of marine fishes

5.1.1. *Calliobdella nodulifera* (Malm, 1863)

5.1.1.1. Host specificity

This leech is not confined to one host, having a wide host spectrum. *Calliobdella nodulifera* was originally recorded from *Gadus morhua* (Malm,1863). Later Johansson (1896)(cited by Leigh-Sharpe) found it in addition on *Gadus aeglefinus*, *Gadus merlangus*, *Merluccius merluccius*, *Molva vulgaris*, *Raja batis*, *Hippoglossus vulgaris*, *Anarrhichas lupus* and *Trigla gurnardus*. Olsson (1893) cited by Leigh-Sharpe) also collected it from *Gadus virens*, *Sebastes norvegicus*, *Raja fullonica*, *Acanthias vulgaris* and *Chimaera monstrosa*. The leeches used by Leigh-Sharpe (1916/17) for his studies had been collected from the Clyde by the Scottish Marine Biological Association, Millport. They were obtained from head and gill covers of *Gadus carbonarius*. The material used during the present investigations was

collected in the same area of the Clyde but from *Gadus morhua* only. The bulk of the evidence suggests that the family Gadidae is the preferred host for *Calliobdella nodulifera*, see also Table 9.

This wide host spectrum of *C. nodulifera* makes it very suitable as an intermediate host for a variety of blood parasites for many marine fishes. In the present study it was found to be harbouring developmental stages of *Trypanosoma* sp. and *Haemogregarina* sp. It was for these reasons of availability, wide host spectrum and apparent susceptibility to haematoprotozoan parasites that *C. nodulifera* was selected as a possible host for vector studies of *Haemogregarina sachai* n.sp. of *Scophthalmus maximus*.

5.1.1.2. The possible role as vector of trypanosomes

Both, trypanosomes and haemogregarines; have been described from *Gadus morhua*, the principle host of *C. nodulifera* in the Clyde area. Nikitin (1927) described *Trypanosoma murmanensis* from *Gadus morhua* (syn. *callarias*) in Russia, although he did not describe its frequency. So (1972) examined 180 *Gadus morhua* from 2 different locations in Newfoundland, Canada, but he found only one fish infected with trypanosomes. The flagellate he described differed from *T. murmanensis* only in its

TABLE 9

MARINE PISCICOLID LEECHES AND THEIR FISH HOSTS

Order: HIRUDINEA: Suborder: RHYNCHOBDELLEIDEI:

Family: PISCICOLIDAE (ICHTHYOBDELLIDAE)(after Herter,1935; Mann,1962)

Scientific Names	Marine Fish Hosts
Branchellion ravenelii(Girard)	Dasyatis hastatus
Branchellion torpedinis (Savigny)	Raja clavata[++] Torpedo marmorata[+] Labrus sp. Trygon pastinaca Scophthalmus maximus[+] Torpedo torpedo
Branchellion parkeri	Raja nasuta[++]
Pontobdella muricata (Linnaeus)	Raja clavata[++] Raja batis[++] Torpedo marmorata[+] Torpedo torpedo Torpedo ocellata[+] Pleuronectes sp.[+,++]
Oxytonostoma typica (Malm)	Raja radiata[+,++]
Ganymedebdella cratere Leigh-Sharpe	Callionymus lyra[+,++]
Calliobdella nodulifera (Malm)	Gadus morhua[+,++] Gadus aeglefinus[+] Gadus merlangus Gadus virens Gadus carbonarius Merluccius merluccius Molva vulgaris Sebastes norvegicus Raja batis[++] Raja fullonica Acanthias vulgaris[+] Chimaera monstrosa Anarrhichas lupus[+] Trigla gurnardus Hippoglossus vulgaris
Calliobdella punctata Van Beneden and Hesse	Blennius pholis[+,++] Cottus bubalis[+,++]
Calliobdella lophii (Van Beneden & Hesse)	Lophius piscatorius

TABLE 9

Scientific Names	Marine Fish Hosts
Scorpaenobdella lubrica (Grube)	Umbrina cirrhosa Corvina umbrina Sargus annularis Labrus sp. Caranx trachurus Solea solea[+,++] Gobius niger[+,++] Scorpaena scrofa Scorpaena porcus Cottus bubalis[+,++] Uranoscopus scaber Blennius pholis[+,++] Lophius piscatorius Coris giofredi
Piscicola rectangulata Levinsen	Gadus macrocephalus Myoxocephalus polyacanthocephalus
Piscicola geometra (Linnaeus)	Cottus scorpius[+,++] Platichthys flesus[+,++]
Platybdella buccalis Nigrelli	Macrozoarces americanus[+]
Platybdella anarrhichae (Diesing)	Anarrhichas lupus[+] Lycodes pallidus
Platybdella olriki Malm	Cottus scorpius[+,++]
Platybdella fabricii Malm	Cottus scorpius[+,++]
Ottonia brunnea (Johansson)	Cottus scorpius[+,++]
Ottonia scorpii (Malm)	Cottus scorpius[+,++]
Abranchus microstomus Johansson	Cottus scorpius[+,++]
Abranchus blennii Jones	Blennius pholis[+,++]
Abranchus sexoculatus (Malm)	Gadus morhua[+,++] Cyclopterus lumpus Zoarces viviparus[+]
Hemibdella soleae (Van Beneden & Hesse)	Solea solea[+,++] Solea impar Solea monochir[++] Solea hispida
Malmiana sp. (M. nuda Richardson)	Gadus morhua[+,++]
Sanguinothus pinnarum De Silva and Burden-Jones	Myoxocephalus scorpius Cottus bubalis[+,++]

TABLE 9

Scientific Names	Marine Fish Hosts
Oceanobdella sexoculata (Malm)	Cyclopterus lumpus Gadus morhua[+,++] Zoarces viviparus[+,++]
Oceanobdella microstoma (Johansson)	Cottus scorpius[+,++]
Oceanobdella blennii (Knight-Jones)	Blennius pholis[+,++]
Pterobdellina jenseni Bennike and Bruun	Raja lintea[+] Raja batis[++]
Trachelobdella lubrica, Grube	Blennius pholis[+,++] Cottus bubalis[+,++] Anarrhichas lupus[+] Gobius niger[+,++] Scorpaena porcus Scorpaena scrofa Solea solea[+,++]
Ichthyobdella semicoeca Blainville	Trigla sp.[+,++]
Johanssiona abditovesiculata Selesnsky	Tetraodon hispidus[+]
Johanssiona kolaensis Selensky	Anarrhichas lupus[+]
Ichthyobdella sp. (after Ronald, 1959)	Hippoglossus vulgaris Scophthalmus rhombus

(+) fish species reported with haemogregarines

(++) fish species reported with trypanosomes

greater length and the location of its nucleus in the tapering anterior half of its body. Since piscine trypanosomes are often highly polymorphic the author suggested that his trypanosome might well be identical with *T.murmanensis*. This hypothesis received support from Khan (1974) who found *T.murmanensis* in *Gadus morhua* from the same location as So (1972) and was able to transmit the trypanosome by way of the marine leech *Myzobdella* sp. to apparently healthy cod. It appears that *Gadus morhua* is also parasitized by the leech *Abranchus sexoculatus* (Herter,1935) and therefore it would be interesting to speculate if *T.murmanensis* can also develop in a different species of marine leech. That several species of trypanosomes can undergo development in the same piscine leech was first shown by Brumpt(1906).

In the present study only 7 *Gadus morhua* were available from the Clyde near Millport,in addition to 7 juvenile cod from Oban,but none of them were found parasitized on examination of blood smears. Since no mature trypanosomes were detected in *C.nodulifera* here apart from trypomastigote forms with pronounced undulating membranes but of much smaller dimensions, no comparison of structure and measurements could be made with trypanosomes described from *Gadus morhua* elsewhere. However, since *T.murmanensis*

is the only trypanosome described from Gadus morhua and the family Gadidae in general it is most likely that the stages of trypanosomes found during these investigations in the alimentary tract of C.nodulifera are part of the life cycle of the cod trypanosome T.murmanensis first described by Nikitin (1927).

C.nodulifera is also reported from the family Rajiidae. Members of this family have been regularly observed to be parasitized by trypanosomes (Table 1). Trypanosoma rajae was first described from Raja asterias (syn.punctata), Raja undulata (syn.mosaica), Raja macrorhynchus and Raja clavata by Laveran and Mesnil (1902) and it has also been reported by Coles (1914) from Raja batis, known to be parasitized by C.nodulifera and thus its possible vector.

5.1.1.3. Possible role as vector for haemogregarines

Haemogregarines have also been encountered in Gadus morhua (Fantham et al.1942).The parasite they found was considered to be identical with Haemogregarina aeglefini which had been earlier described from Gadus aeglefinus by Henry (1913). Since both fish species are parasitized by C.nodulifera it is conceivable that H.aeglefini is also not host specific and might even

have a wider range. The developmental stages of the haemogregarine found in the alimentary tract of C.nodulifera in the present study might therefore have belonged to H.aeglefini also.

Haemogregarines have also been reported from other Gadidae viz. Haemogregarina sp. from Urophycis chuss (Mavor,1915); Haemogregarina urophycis from Urophycis tenuis (Fantham et al.1942); H.gadi pollachii from Gadus pollachius (Henry,1910) and Haemogregarina sp. from Gadus virens (Henry,1912).

A few other marine fish species parasitized by C.nodulifera have also been reported infected with haemogregarines. H.anarrhichadis was described from Anarrhichas lupus by Henry (1912) who suggested that another leech tentatively identified as Trachelobdella lubrica might be the vector. The leech host Squalus acanthias(syn.Acanthias vulgaris) has been found infected with H.bigemina (Wenyon,1926).

Among the Rajidae, H.delagei was described from 5 different species, strongly supporting the possibility of a common vector for this parasite. The species involved were Raja undulata(syn.mosaica) and Raja asterias(syn.punctata) reported by Laveran and Mesnil (1902) from France; Raja microocellata by

Robertson (1906) from England and *Raja radiata* and *Raja senta* by So (1972) from Canada. Although no haemogregarines have been described as yet from *Trigla gurnardus*, other *Triglidae* have been reported with either haemogregarines,i.e. *H.rovignensis* in *Trigla lineata* (Minchin and Woodcock,1910) or trypanosomes,i.e.*Trypanosoma triglae* in *Trigla* corax (Neumann,1909), both records from Italy. Neumann found *H.scorpaenae* in *Scorpaena ustulata* and another member of the family Scorpaenidae,i.e. *Sebastes norvegicus* has been found parasitized by *C.nodulifera*.

Among the Pleuronectiformes infections with either haemogregarines or trypanosomes or both appear to be quite common (Table 1). *C.nodulifera* is also found on marine pleuronectids,e.g.*Hippoglossus vulgaris*. Thus it was not at all surprising to find developmental stages of *Haemogregarina sachai* n.sp. in the alimentary of *C. nodulifera* when the latter were experimentally fed on highly parasitized *Scophthalmus maximus*. Both the fact that this leech readily took turbot as its host and also that the haemogregarines were observed in the digestive tract of experimentally infected leeches over more than 20 days underlines the likelyhood of *C. nodulifera* as a potential vector for *H.sachai* n.sp.

That *Calliobdella* sp.leeches can serve as vectors for haematoprotozoan parasites has been shown by Brumpt (1904) who successfully infected *Cottus bubalis* with trypanosomes via *Calliobdella punctata*.

5.1.2. *Hemibdella soleae* (Van Beneden and Hesse,1863)

5.1.2.1. Host specificity

The genus *Hemibdella* was created by Van Beneden and Hesse(1863) for a small leech parasitizing the common sole (*Solea solea*). It had been described earlier by Van Kröyer (1843) as *Piscicola soleae* and by Malm(1863) as *Platybdella soleae*. Selensky(1931),however,examining the original specimens from Van Kröyer and Malm decided on morphological grounds that all were specimens of *Hemibdella soleae*.

An outstanding feature of this leech is a peripheral modification of the posterior sucker so that it can adhere to the ctenoid scale of *Solea solea* so well that it is only with difficulty that the leech can be removed without removing the whole scale. This mode of attachment appears to be specific for *Hemibdella soleae* and the scales of *Solea solea* are exactly suited for the posterior sucker of this leech. The spines of other *Soleidae* appear either too thin (Selensky,1931)

or they are much larger and their ends lie flatter as in Solea lascaris (Llewellyn,1965) and therefore appear less suitable for attachment.

According to Selensky(1931) Hemibdella soleae is the only leech parasitizing Solea solea and as such is strictly host specific. This was underlined in a study by Llewellyn,1965) who failed to feed Hemibdella soleae on Solea lascaris. From 1457 marine flatfishes examined by Selensky, only the genus Solea,i.e. Solea impar and Solea monochir from the Mediterranean were infested. Hemibdella soleae had been found before only on Solea solea in Denmark,France,Northwest Ireland,Greenland and North America (Herter,1935). No leeches were found by Llewellyn(1965) in British waters on Solea lascaris and Buglossidium luteum and he concluded that British Hemibdella soleae seem to be confined to Solea solea. However, one other piscicolid leech with a wide host range,i.e.Scorpaenobdella lubrica has also been reported from Solea solea (Herter,1935)(Table 9.).

The mechanism of host specificity of Hemibdella soleae was very obvious during the present study when attempts were made to attach this leech to other flatfish as Scophthalmus maximus and Pleuronectes platessa for transmission experiments. In Pleuronectes platessa these attempts failed and in Scophthalmus maximus only ver few leeches appeared to attach and feed on the lateral fins.

5.1.2.2. The possible role as vector of trypanosomes

In the present study only one specimen of *Trypanosoma soleae* was discovered in the blood of the wild *Solea solea* examined. This trypanosome of the sole had been last reported from France around the turn of the century (Laveran and Mesnil,1901; Lebailly,1906). In addition Yakimoff (1912,cited by Laveran and Mesnil) described the new species *Trypanosoma dorhni* from *Solea monochir* in the Mediterranean. The length was given as 41 micron and this would fit well into the size range for *T.soleae* of 40-47 micron (Laveran and Mesnil,1901). *T.dorhni* might well be synonymous with *T.soleae* since it was common practice to describe a new species of parasite from each new host where it was found. In the present study *T.soleae* was characterized by a length of 63.8 micron which would agree with the 65 micron length given by Lebailly (1906) for the same parasite, indicating the polymorphic structure of this marine trypanosome. *Hemibdella soleae* also parasitizes *Solea monochir* (Selensky,1931) and thus might have been the vector for *T.dorhni*.

5.1.2.3. The possible role as vector for haemogregarines

Haemogregarina simondi encountered in wild *Solea solea* in the present study was very similar to the parasite first described by Laveran and Mesnil (1901),and later

by Lebailly (1904) and Henry (1910). Hemibdella soleae was the only leech encountered on Solea solea in this study and it was also heavily parasitized by developing stages of Haemogregarina simondi which had been occasionally observed by others before (Brumpt,1904; Lebailly,1906 and Neumann,1909).

The only other record of a haemogregarine from a member of the Soleidae is that of Haemogregarina clavata first described by Neumann (1909) in Solea lutea (syn.Buglossidium luteum) from the Mediterranean and later also observed by Kohl-Yakimoff and Yakimoff (1915) at the same location. Marine leeches were never described from this species of sole and also Llewellyn (1965) never found Hemibdella soleae on it in British waters. If this leech should infest Solea lutea only in the Mediterranean then it would be the vector for two distinct haemogregarines. This appears to be rather doubtful since Selensky(1931) and Llewellyn (1965) have observed that Hemibdella soleae had difficulty in attaching even to the ctenoid spines of related species of Soleidae, and one has probably to look for an other vector for H.clavata of Solea lutea.

5.2. Development of haematoprotozoan parasites of marine fish within marine leeches

5.2.1. Trypanosomes

In the present study it has been shown that a trypanosome from *Gadus morhua*, presumably *Trypanosoma murmanensis* developed in the marine leech *Calliobdella nodulifera* suggesting it to be the the intermediate host in the life cycle of this trypanosome. The different developmental stages observed in the alimentary tract of *C.nodulifera* when compared with the similar findings of Brumpt(1906), Robertson (1906,1909) and Neumann (1909) support this hypothesis although no transmission experiments could be performed to prove that such a cyclical development takes place with *C.nodulifera* as the intermediate host and *Gadus morhua* as the final host. The only measurements given by other authors for the developing stages of trypanosomes from the alimentary tract of the vector leech were those by Neumann (1909) of *T.variabile* of *Raja punctata* in the gut of the intermediate host *Pontobdella muricata*. The first forms seen by Neumann and characterized by a distinct undulating membrane measured from 10-25 micron in length and from 2-3 micron in width with a free flagellum of 3-5 micron and can only be compared

with the rarely seen forms of trypomastigote stages in the present study which had approximately the same measurements for length and width but a much longer free flagellum (23.8 micron).Further described is the amastigote form of the parasite which measured from 3.0-7.0 micron in length and 4.0 micron in width and thus compared well with the slightly larger amastigote form found in C.nodulifera measuring from 6.9-8.8 micron in length and from 5.0-7.5 micron in width. In addition, the infective trypomastigote stage,such as observed by Neumann in Pontobdella muricata,showed a strikingly similar morphology as in the present case. The spirochaete-like slender parasites measured from 13.0-45.0 micron in length and from 0.5-1.0 micron in width and had a free flagellum of 5.0-10.0 micron. The corresponding stage in Calliobdella nodulifera measured from 23.8-50.2 micron in length and from 1.0-1.5 micron in width with a free flagellum averaging 32.5 micron and thus giving this stage a considerable overall length. However, it is felt that from size differences of developing stages of trypanosomes in the intermediate host no deductions can be made regarding the species of trypanosome from the final host because of the polymorphy of the organisms and the possibility of mixed infections.Similar observations were also made by Robertson (1911) in freshwater leeches.

5.2.2. Haemogregarines

Although haemogregarines had been observed in marine leeches by other authors (Robertson,1907; So,1972) and especially in the leech *Hemibdella soleae*,ectoparasite of *Solea solea* (Brumpt,1904; Lebailly,1906 and Neumann,1909) no evidence for a cyclical development was found and no transmission experiments were pursued. Brumpt (1904) only briefly mentioned having observed stages of *Haemogregarina simondi* in *Hemibdella soleae*. Lebailly(1906),however, found typical forms of *H.simondi* (probably gametocytes) in the same leech and also noticed another stage measuring 22.0 by 2.0 micron with 2 nuclei (perhaps an early zygote). Also Neumann (1909) detected only free stages resembling those of *H.simondi* in *Solea solea* in the vector leech. In the present study it was possible to transmit *H.simondi* from already infected *Hemibdella soleae* to apparently uninfected hatchery *Solea solea* via the leech. However, it was not possible to show a stage to stage development of this haemogregarine in an individual leech because all the leeches derived from *Solea solea* were already infected with *H.simondi* and the hatching of young uninfected leeches from cocoons was not accomplished. The developmental stages of *H.simondi* found in *Hemibdella soleae* in the present study varied from

leech to leech but when they were considered together they supported the theory of a haemogregarine life cycle, similar to *H.stepanowi* of *Emys orbicularis* described in the intermediate host the freshwater leech *Placobdella catenigera* by Reichenow (1910) or to *H.nicoriae* of *Geoemyda* (syn.*Nicoria*) *trijuga* described in the intermediate host the freshwater leech *Ozobranchus shipleyi* by Robertson (1910).

Some confusion still exists regarding the sequence of developing stages of the Haemogregarinidae in their intermediate hosts.

In the genus *Karyolysus* according to Reichenow (1921) the zygote develops into an oocyst containing 35-60 sporoblasts.The latter transform into mobile stages (*sporokinets*) of 28.3 by 4.8 micron which actively invade the egg cells of the female intermediate host, the mite *Liponyssus saurarum* where they differentiate further into numerous sporocysts of 20.0 by 10.0 micron containing 14-16 (*Karyolysus bicapsulatus*) or 30 (*K.lacazei*) sporozoites of 9.0 by 1.8 micron. Such a transovarian transmission was not seen by Bergle(1971) who found the complete development of *Karyolysus* sp. taking place in the haemolymph of the vector mites *Ophionyssus lacertinus* until the sporocyst containing 10 sporozoites was taking up orally by the final host *Lacerta sicula* when ingesting the mites and only then

in the gut of the final host would these sporocysts rupture and the sporozoites be liberated.

In the genus Hepatozoon according to Garnham (1954) the zygotes of 15-40 micron in diameter develop into large oocysts of 80-120 micron in diameter containing several hundred sporoblasts of 20 micron in diameter. After rupture of the oocyst these sporoblasts become sporocysts which enlarge to 30 by 25 micron in average containing from 12-40 sporozoites. These measure 20-27 micron in length and 4 micron in width,have a nucleus composed of approximately 10 fine distinct granules and in addition 1-4 oval or round hyaline bodies smaller than the nucleus in the cytoplasm. But other authors (Ball,Chao and Telford,1967) believe that the development up to the sporozoites takes place within an unruptured oocyst.

In the genus Haemogregarina according to Reichenow (1910) the total development of the parasite up to the final infective stage,i.e.the sporozoites takes place entirely within the oocyst without the formation of sporocysts. The sporozoites are wormlike with one end pointed and the other end blunt,measure from 12.0-14.0 micron in length by 1.5 micron in width and number only 8. This sequence of development could not be confirmed in the

present study for *H.simondi* in *Hemibdella soleae*. It rather appeared as if sporozoite development took place within a sporocyst which had derived from a sporoblast after rupture of the oocyst. In this respect the development resembled that of Garnham(1954) for *Hepatozoon argantis* in *Argas brumpti*.

However, further studies are urgently needed to support this view with the aid of uninfected cocoon-hatched leeches since these were not available in the present study for experiments showing a chronological development of *H.simondi* in the intermediate host *Hemibdella soleae*.

5.3. Possible vectors other than marine leeches for haematoprotozoan parasites of marine fish

5.3.1. Isopods

As early as 1902 when studies on haematoprotozoan parasites of marine fishes had just begun, Laveran and Mesnil suggested that an alternative intermediate host for such parasites might be an ectoparasite feeding on the gills. Although they did not find any ectoparasites except for Trichodina sp. on the numerous Blennius pholis parasitized by Haemogregarina bigemina, they suggested that perhaps the crustacean Praniza, the 2nd larva of the isopod Gnathia sp., might be a possible vector because it was usually found in great numbers on the skin of the same fish species.

Although fully aware of the suggestion of Laveran and Mesnil(1902) it was nevertheless impossible to study bloodsmears of Praniza in the present study because no larval Gnathia sp. were available for blood examination and they were also never found on any of the wild and farmed marine fish examined.

According to Monod (1926) Praniza sucks blood and lymph and can evolve on a large number of fish of

any species(Table 10) and is also independent of their environment,as in the case of the genus Paragnathia where this can occur in freshwater,brackish water and at sea. These larvae fix in a place on the fish host where it is easiest to reach a blood vessel,i.e. on the gills,interior of the mouth and within the nasal cavities but in young fish preferable on the fins. The feeding of blood engorges the Praniza considerably and they thus resemble ticks. The intensity of the infection appeared not of great effect on the hosts and Monod found 171 specimens on a Box salpa and 227 on a Labrus turdus with no apparent influence on the health of these fishes. The only pathogenic action observed was a local "tumorous" reaction with hyperplasia of epithelium but never a sign of mitosis so that a neoplastic condition could be excluded. A resorbtion of these lesions occurred after the disappearance of the external irritation.

Also Kearn (1976 pers.comm.) and Slinn (1977,pers.comm.) suggested that the larvae of Gnathia sp. might be possible candidates for vectors of the haematoprotozoan parasites of marine fish. Slinn had found this isopod on a number of teleost fishes from the Irish Sea including on one occasion Scophthalmus maximus. He even obtained one specimen from a very small hatchery reared Scophthalmus maximus. This would indicate also

TABLE 10

MARINE FISH HOST RANGE FOR THE BLOOD FEEDING LARVA PRANIZA OF THE ECTOPARASITIC ISOPODS OF THE FAMILY GNATHIIDAE (after Monod,1926)

Scientific Names	Locality
Squalidae	Brest/France
Rajidae	South Africa
Torpedinidae	Sri Lanka(Ceylon)
Gerres aprion	French Antilles
Epinephelus gigas	Mediterranean Sea
Serranidae	Brasil,Mediterranean Sea
Serranus hexagonatus	Red Sea
Box salpa	Mediterranean Sea
Chrysophrys aurata	Mediterranean Sea
Pagellus erythrinus	Brest/France
Mullus surmuletus	Brest/France
Trigla gurnardus	Scotland,Ireland
Cottus scorpius	England,Scotland
Cottus bubalis	England,Scotland
Cottidae	East Coast USA
Scorpaenidae	Mediterranean Sea
Mugil capito	Brest/France
Sphyraenidae	Mediterranean Sea
Labrus turdus	Mediterranean Sea
Labridae	Mediterranean Sea
Scaridae	French Antilles
Scomber scomber	Brest/France
Zeus faber	Roscoff/France
Gobius niger	Brest/France
Clinidae	Cape of Good Hope
Gadidae	North Sea,Med.Sea,Atlantic,USA
Urophycis sp.	Mediterranean Sea
Urophycis tenuis	USA
Gadus aeglefinus	USA
Soleidae	Adriatic Sea
Clupea sprattus	France
Salmo trutta	Ireland
Gobius minutus	France
Anguilla anguilla	France
Mugilidae	France
Pleuronectes platessa	Brest/France
Pleuronectes americanus	USA
Pleuronectes microcephalus	Scotland

that *Praniza* may be a possible vector for *Haemogregarina sachai* n.sp. and it could even belong to the diet of *Scophthalmus maximus*. Some of the smaller "tumorous" lesions observed in this fish during the present investigations might have in fact been the result of the isopods feeding activity rather than the product of the haemogregarine infection.

Monod (1926) suggested that there was probably no fish host which was not attacked by *Praniza* because he succeeded to infest experimentally a number of species including *Anguilla anguilla*,*Trigla lucerna*, *Cottus bubalis*, *Gobius minutus*,*Blennius pholis*, *Pleuronectes platessa* and *Callionymus lyra*, all of which had been described as being parasitized by various haematoprotozoan parasites (Table 1). *Praniza* also appears to be quite ubiquitous in its distribution and may also be actively or passively transported by a fish host over large distances similar to ticks being disseminated by migrant birds.

Since the juvenile stage of *Gnathia* sp.,i.e.the larva *Praniza* actively preys on a variety of marine fishes, feeds on blood and lymph only,and leaves its host from time to time, it might well be a very suitable alternative vector to marine leeches and thus would certainly prove worth to be further investigated.

5.3.2. Copepods

In the present study no haematoprotozoan parasites were detected in any of the copepods collected from wild marine fish but the sample of these ectoparasites was too small to draw any conclusions. Several authors have earlier hypothesized that copepods might serve as possible vectors for the haematoprotozoan parasites of marine fish because of the large number of individual fish species infected all over the world regardless of any specific habitat.

Keysselitz (1906) was unsuccessful when he looked for flagellates in ectoparasitic crustaceans of marine fish such as *Argulus foliaceus* and *Argulus coregoni*. Also Minchin (1909) suggested *Argulus* sp. as a possible intermediate host because it was found in abundance on the freshwater fishes examined by him. He assumed that these ectoparasites were blood feeders and considered them ideally suited for vector studies because of their habits of changing hosts and thus being able to disseminate blood parasites either mechanically or cyclical and also because of their transparency to study the development and the mode of transmission of haemoflagellates. Although he starved *Argulus* sp. and had them feed on trypanosome and trypanoplasm infected fish of various species he never was able to observe any haemoflagellates in live and dissected *Argulus* nor did he encounter any blood

corpuscles in these ectoparasites which made it doubtful for him that they were indeed blood feeders. This was later confirmed by Kabata(1970) who found that fish erythrocytes were too large in diameter to be sucked up by *Argulus* sp. although he observed that the haemolymph could be taken up without difficulty. There exists thus still the possibility that exoerythrocytic stages of haemogregarines because of their smaller dimensions could be transmitted by *Argulus* sp.

Neumann(1909) examined a number of *Anilocra mediterranea* on the skin of *Sargus annularis* and *Smaris vulgaris* but with negative results.

Henry(1913) found the copepod *Caligus scombri* on *Scomber scombrus* and suspected these ectoparasites to be possible vectors of haematoprotozoan parasites of this fish. Llewllyn(1965) found *Caligus rapax* on *Solea solea* and considered it as a possible alternative intermediate host for *Haemogregarina simondi*. *Caligus elongatus* (syn.*rapax*) is a parasitic copepod which has been recorded from a variety of teleost hosts including *Scophthalmus maximus* (Parker,1969) and *Scophthalmus rhombus* (Ronald,1959). It could well serve as a vector as it is often found free living in the plankton and may thus readily transfer from host to host.

Noble (1957) found a few copepods (no scientific names given) attached to the gills of *Callionymus lyra* heavily parasitized by *Haemogregarina quadrigemina* but they were negative for haemogregarines when examined. Saunders (1966) found small red copepods (unidentified) on *Euthynnus alletteratus* but both the parasites and the fish host were free of any haematoprotozoan parasites.

The blood feeding copepods of the genus *Lernaeocera* found in the present study on *Solea solea* were also suspected as vectors. Various of these ectoparasites contained free merozoites, micro-and macro-gametocytes and other developing stages of *Haemogregarina simondi* as revealed in smears. These could have been taken up by the ectoparasite with the blood meal but might have been also an indication that some development of the haemogregarine took place in *Lernaeocera* sp.(sporozoites(?) of *H.simondi*). These engorged female copepods stay for considerable times on the fish host so that a cyclical development of the haemogregarine appears quite possible. Although it is not possible for the blood feeding female stage of *Lernaeocera* sp.to leave its final host, because the head of the parasite is buried within the host and the antlers ramify through its tissues, the haemogregarines could possibly be transmitted via the eggs to the pelagic naupliar stages and thus may contribute to the transmission

of the haemogregarines to other *Solea solea* since also the whole life cycle of this ectoparasite on farmed fish is apparently speeded up by the warmer water temperatures used in aquaculture systems connected to the coolant discharge route of a power station (Slinn,1967).It may be of interest to note that among the other flatfish serving as intermediate hosts for *Lernaeocera* sp.also *Scophthalmus maximus* has been found infested with larval stages (Gouillart,1937). Since British marine fish farms are still stocked to a large extent with wild *Scophthalmus maximus* caught from the shallow beaches of North Wales, and *Lernaeocera* sp.is essentially a coastal parasite it might be possible that the larval stages of this copepod are introduced with young fish into the fish farms and then undergo a similar aberrant development to the final blood sucking stage as seen in *Solea solea* by Slinn (1970) and in the present study. However, none of the farmed *Scophthalmus maximus* examined during this investigation has yielded any ectoparasitic copepods of the genus *Lernaeocera*. Also Slinn (1977,pers. comm.)did not believe it to be likely that this parasite could serve as a vector for marine haematoprotozoan parasites. Although host changing juvenile stages of *Lernaeocera* sp. abrade the gill filaments or gill cavity wall by their feeding activities, it has not been shown if they feed on epithelial cells or blood. The only

means by which *Scophthalmus maximus* could get infected with the haemogregarine stages found in adult female *Lernaeocera* sp. would be by ingesting them when they are attached already to another fish but this seems rather unlikely.

The copepod *Lepeophtheirus thompsoni* is found in the gill cavity of *Scophthalmus maximus* in the Irish Sea (Slinn,1977;Leppington-Clark,1977;both pers.comm.). Leppington-Clark found that when *Scophthalmus maximus* were held in captivity the levels of infestation with *L. thompsoni* rose steeply and regular mechanical removal became essential. Juvenile fish less than 10 cm long did not support adult *L.thompsoni* but the juvenile stages of this ectoparasite were frequently encountered on the skin of young fish. It is also not known if these stages are blood feeders, but if this was the case then these copepods might also come into consideration as possible vectors for haematoprotozoan parasites.

5.3.3. Trematodes

Llewellyn (1954) studied several ectoparasitic monogenean trematodes of the *Polyopisthocotylea* from the gills of marine fishes and examined their food and gut pigment. He found blood of the host

fish in the intestine of the following trematodes: Hexabothrium appendiculata from the fish host Scylliorhinus canicula; Kuhnia scombri from Scomber scombrus; Anthocotyle merlucci from Merluccius merluccius; Axine belones from Belone belone; Diclidophora merlangi from Gadus merlangus and Diclidophora luscae from Gadus luscus. He concluded that blood comprised the major part of the diet of these trematodes in contrast to the Monopisthocotylea which were not blood feeders and appeared merely to feed on eroded tissue or mucus. A large number of different species of monogenean trematodes has been recorded from Scophthalmus maximus by Ronald(1959) but it is not known if they feed on blood.

The only monogenean trematode found on Solea solea during this study was Entobdella soleae. However, Kearn (1963) gave conclusive evidence that E.soleae feeds exclusively on the epidermis of its teleost host and on mucus and mucus cells. However,there appears to be some evidence that Amphibdella torpedinis,an ectoparasitic trematode of Torpedo marmorata, feeds on blood and thus perhaps could be a vector for Haemogregarina lobianci which was described from this fish by Kohl-Yakimoff and Yakimoff (1915). More studies are indicated to investigate if trematodes could serve as vectors of the haematoprotozoan parasites of marine fishes.

5.4. The diet and feeding behaviour of marine flatfish of aquaculture importance with regard to possible oral transmission of haematoprotozoan parasites

Marine flatfish appear to be rather commonly infected with haematoprotozoan parasites in the wild (Table 1). Some fishes of the Pleuronectiformes such as Scophthalmus maximus, Solea solea and Pleuronectes platessa have become of considerable importance in the development of techniques for marine fish farming (Shelbourne,1964, 1967; Purdom,1973). More recently two of these (Solea solea and Scophthalmus maximus) have shown clinical signs in connection with infections of haematoprotozoan parasites in aquaculture (Ferguson and Roberts,1975; Kirmse,1975; Kirmse and Ferguson,1976) as observed in the present study. Since an oral transmission of these parasites had been postulated frequently by several authors (Neumann,1909; Saunders,1959;Kirmse,1975) it was felt of value to give some consideration here to the food and feeding habits of Solea solea and Scophthalmus maximus.

5.4.1. Solea solea

According to Muus(1964) Solea solea feeds mainly on thin-shelled bivalves, bristle worms, crustaceans

and some small fishes such as *Ammodytes* sp. and *Pomatoschistus* sp. This was confirmed by Grzimek (1970) who added echinoderms and very small fish such as *Scophthalmus maximus* and *Limanda limanda* to their diet.

These marine fish species belonging to the diet of adult *Solea solea* could well serve as potential intermediate hosts if an oral route of transmission of the haematoprotozoan parasites of marine fish is considered.

Limanda limanda has been found infected in nature by *Trypanosoma limandae* (Brumpt and Lebailly,1904). *Scophthalmus maximus* was found infected with *Haemogregarina sachai* n.sp. in the present study and during earlier investigations (Ferguson and Roberts,1975;Kirmse,1975) but in farmed fish only. However,the infection was seen only in older fish and neither *Scophthalmus maximus* or *Limanda limanda* of this size are likely to be hunted for by *Solea solea*. Nothing is known about the haematoprotozoan parasites of *Ammodytes* sp. and unfortunately no such fish were available in the present study for examination. Among the genus *Pomatoschistus* (family *Gobiidae*) *Pomatoschistus* (syn.*Gobius*) *minutus* has been reported to be infected with *Haemogregarina minuta* by Neumann(1909).

If an oral route of infection by ingestion of an infected intermediate host were possible, Ammodytes sp. and Pomatoschistus sp. would be the most likely vectors for the marine haematoprotozoan parasites of Solea solea because of their small sizes.

5.4.2. Scophthalmus maximus

It has been shown conclusively that Scophthalmus maximus are visual feeders and actively hunt for their food, frequently at night (Müller,1968; De Groot,1969; Jones,1970). Already juvenile fish of 33 mm were intensive hunters and fed mainly on amphipods which made up 78.8% of the diet(Müller,1968).Mysids and isopods were secondary food items, but also small Ammodytes lancea of 30-36 mm length and Pomatoschistus minutus belonged to the diet. De Groot(1971) found that Scophthalmus maximus of up to 10 cm length fed primarily on polychaetes and molluscs but as they increased in size their diet changed to Crangon vulgaris and above 20 cm they became fish feeders taking mainly Ammodytes sp. With further increase in size the diet changed gradually to gadoid fish. Similar observations on the diet of juvenile Scophthalmus maximus were also made by Jones(1973) who found that the change to the adult diet began at the start of the second year of life when the fish were in a length range of 10-15 cm.

Adult fish live on a diet composed almost exclusively of other fish and Jones (1970) found the composition of 44 stomachs to be composed mainly of gadoid and pleuronectoid fish and Ammodytes sp. apparently making up 38.6% of the diet.

An active predatory behaviour predisposes to heavy parasitism. This can be seen in Scophthalmus maximus where almost 100% of the wild fish population is infested by the cestode Bothriocephalus scorpii (De Groot,1971). An active host such as this fish may acquire therefore numerous different kinds of parasites because of a taste for a wide variety of foods. Thus wild Scophthalmus maximus might get infected with haematoprotozoan parasites of the marine environment through ingestion of a variety of marine animals belonging to its normal diet (Table 11).

Not much is known about the haematoprotozoan parasites of amphipods,isopods,mysids and polychaetes which appear to be the staple diet of juvenile Scophthalmus maximus and the amphipods and copepods examined in the present study were all negative. Only polychaetes were found to carry a number of Sporozoa in the gut (Ray,1930). It is also not known whether Tellina tellina comprises part of the mollusc diet of juvenile Scophthalmus maximus. Two sporozoan parasites had been described

TABLE 11

THE DIET[x] OF SCOPHTHALMUS MAXIMUS WITH REGARD TO POSSIBLE ORAL TRANSMISSION OF HAEMOGREGARINES

Polychaeta:	Nephthys caeca(Müller) Pectinaria koreni(Malmgren) Spio filicornis(Fabricius)
Cumacea:	Pseudocuma longicornis(Bate)
Isopoda:	Idotea viridis(Pallas) Idotea granulosa(Rathke) Eurydice pulchra(Leach)
Amphipoda:	Gammarus pulex(L.) Gammarus locusta(L.) Gammarus zaddachi(L.) Corophium volutator(Pallas) Haustorius arenarius(Slabber) Bathyporeia guilliamsoniana(Bate) Bathyporeia pelagica(Bate) Pontocrates arenarius(Bate) Niphargus sp.
Mysidacea:	Neomysis vulgaris (Sars) Schistomysis parkeri(Norman) Schistomysis spiritus(Sars) Schistomysis ornata(Sars) Schistomysis kervillei(Sars) Paramysis arenosa(Sars) Gastrosaccus spinifer(Göes) Praunus sp. Siriella spp.
Malacostraca:	Crangon crangon(Fabricius) Portumnus latipes(Pennant)
Insecta:	Dipteran larvae
Mollusca:	Donax vittatus(da Costa)
Pisces:	Gadus morhua(L.)+ Gadus merlangus (L.)+ Gadus aeglefinus(L.)+ Callionymus lyra (L.)+ Trigla gurnardus(L.) Trachinus vipera(Cuv.& Val.) Ammodytes lancea(Lesauv.) Pomatoschistus minutus(Pallas)+ Gobius sp.+ Pleuronectes platessa(L.)+ Limanda limanda(L.)+ Solea solea(L.)+ Solea lutea(Bonaparte)+

(+) fish species reported with haemogregarines
(x) after Todd(1905,1907);Burd(1949,cit.Jones,1970); Müller(1968);De Groot(1969,1971);Jones(1970,1972,1973).

recently from this bivalve (Buchanan,1977) but these did not belong to the haematoprotozoans.

However, among the fish diet of Scophthalmus maximus almost all species have been reported infected with haematoprotozoan parasites (Table 1,11). In addition the examination of the tanks at Hunterston (W.F.A.) revealed a large number of marine animals which could be considered a welcome addition to the monotonous pelleted diet of farmed Scophthalmus maximus (Table 12). Any of these species could serve as an intermediate host for Haemogregarina sachai n.sp. if one considers an oral route of infection as a possibility here.

5.4.3. General Considerations

So far the genus Haemogregarina has been transmitted usually through the invasion of infective stages from the bloodsucking intermediate host during a blood meal. This appeared to have been the case also in the present study where Haemogregarina simondi was probably being transmitted by the bloodsucking leech Hemibdella soleae to Solea solea. However,it was not possible to infect any fish by the oral route using vector leeches as a diet and force-feeding them to these fish. In nature it appears not to be uncommon that fishes feed on leeches (Robertson,1911) and also Reichenow (1921) observed that the turtle

TABLE 12

MARINE ANIMALS FOUND IN THE HOLDING TANKS OF THE WHITE FISH AUTHORITY FISH FARM AT HUNTERSTON

Common Names	Scientific Names
Cornish sucker	Lepadogaster lepadogaster (Bonaterre) (Fam.Gobiesocidae)
Goldsinny wrasse	Ctenolabrus suillus (L.) (Fam.Labridae)
Corkwing wrasse	Crenilabrus(=Symphodus) melops(L.) (Fam.Labridae)
Sea-snail	Liparis liparis (L.) (Fam.Cyclopteridae)
Lump-sucker	Cyclopterus lumpus (L.) (Fam.Cyclopteridae)
Butterfish	Pholis gunellus (L.) (Fam.Pholidae)
Common eel	Anguilla anguilla (L.) (Fam.Anguillidae)
Common shanny	Blennius pholis (L.) (Fam.Blenniidae)
Goby	Pomatoschistus minutus (L.) (Fam.Gobiidae)
Plaice	Pleuronectes platessa (L.) (Fam.Pleuronectidae)
15-spine-stickleback	Spinachia spinachia (L.) (Fam.Gasterosteidae)
Common starfish	Asterias rubens (L.) (Class Asteroidea:Order Forcipulata)
Squat lobster	Galathea strigosa (L.) (Class Malacostraca:Suborder:Anomura)
Spiny squat lobster	Galathea squamifera (L.) (Class Malacostraca:Suborder Anomura)
Hermit crab	Eupagurus bernhardus (L.) (Class Malacostraca:Suborder Anomura)
Shrimp	Crangon crangon (L.) (Class Malacostraca)
Sea slug	Aeolidia(=Aeolis) papillosa (L.) (Class Gastropoda:Fam.Aeolididae)
Tortoiseshell limpet	Acmaea virginea (Müller) (Class Gastropoda:Fam.Acmaeidae)
Common periwinkle	Littorina littorea (L.) (Class Gastropoda:Fam.Littorinacae)
Rag worm	Nereis diversicolor(Müller) (Class Polychaeta)
Lug worm	Arenicola marina (L.) (Class Polychaeta)
Gammarus	Gammarus locusta (L.) (Order Amphipoda)
Sea squirts	Class Ascidiacea

Emys orbicularis would occasionally feed on the intermediate host, the leech *Placobdella catenigera*, which is the vector for *Haemogregarina stepanowi* of these turtles. It was not investigated further whether the haemogregarines could be transmitted in such a way.

In the genus *Karyolysus* and the genus *Hepatozoon* most authors agree on oral transmission but do not exclude the possibility that accidental contamination with faeces or transmission during the blood meal could take place also. However, in *Hepatozoon* it was only by feeding invertebrate intermediate hosts containing mature sporozoites that experimental infection was successful (Mackerras,1962; Ball,Chao and Telford,1967,1969). Also Bergle (1971) observed that the vector mites *Ophionyssus lacertinus* of *Karyolysus* sp. were often eaten by the final host *Lacerta sicula* and he concluded that this was the only way that transmission was achieved.

That blood parasites which are normally transmitted by bloodsucking invertebrates can also be transmitted orally has been shown frequently in trypanosomiasis (Moloo,Losos and Kutuzu,1973; Soltys,Thompson and Woo,1973) Also Kirmse and Taylor-Lewis (1976) fed trypanosome-infected ticks (ixodids and argasids) to laboratory

rats and mice and achieved oral transmission.

It is not impossible that an oral route of infection might also exist regarding marine haemogregarines, especially in fish with such distinct and characteristic predatory feeding behaviour as Scophthalmus maximus. That transmission experiments have failed so far does not necessarily argue against this hypothesis because the invertebrates fed to Scophthalmus maximus might not have contained the necessary infective stages (sporozoites) of the haemogregarine. Also it could be that the fish used in experiments had earlier acquired a certain immunity and were thus refractive to the parasite. Other factors which might interfere with experimental transmission include different temperature and pH of the water,antigenicity of the blood of the host as compared with the possible vector and the influence of gastric juices and digestive enzymes on the parasites in case of oral transmission. The fact that only fish raised under elevated temperatures (Hunterston) showed the infection with Haemogregarina sachai n.sp. indicates that temperature probably plays a major role in the transmission.

The problem remains that under the same conditions of aquaculture only Scophthalmus maximus derived from the wild as juvenile fish and not the hatchery fish became

infected. Were the juvenile fish already infected at an age of 4-6 months and measuring approximately 5-7 cm in length or is it, because hatchery fish actually never learned how to hunt for a prey because they were supplied with ample food since hatching from the egg? Wild *Scophthalmus maximus* introduced to a fish farming situation could be expected to have retained some of their predatory instincts and thus might feed on small wild fish occasionally entering the tanks. This might also explain the at random distribution of infected fish in different tanks (Kirmse,1975).

The possibility that frozen and thawed sprats (*Sprattus sprattus*) might introduce the infection was excluded here because both wild and hatchery *Scophthalmus maximus* were fed with these. In addition the freezing might have killed the haematoprotozoan parasites although this has not been proven here.

5.5. Transmission experiments using marine leeches as possible vectors for haemogregarines

Most of the transmission experiments using leeches as possible vectors for haematoprotozoan parasites were performed on various species of trypanosomes and trypanoplasms, both from marine and freshwater fishes (see review of the literature). Considering the large number of haemogregarines which have been described from marine fishes (Table 4,5) it is surprising how little work has been carried out on vector studies and especially the role of piscicolid leeches in their transmission.

No complete life cycle of haemogregarines has ever been described in fish but developing stages of haemogregarines were observed in a number of leeches parasitizing marine fish hosts (Brumpt,1904; Lebailly, 1906; Robertson,1907; Neumann,1909 and So,1972).

In the present study using *Calliobdella nodulifera* and *Hemibdella soleae* as possible vectors for haemogregarines, results differed widely. *C.nodulifera* was employed to transmit *Haemogregarina sachai* n.sp. from infected *Scophthalmus maximus* to apparently uninfected ones. Transmission experiments failed but up to the 21st days after previous feeding of

the leeches developmental stages of the haemogregarine were nevertheless observed within the leeches.

Hemibdella soleae infected with *Haemogregarina simondi* in nature were however used successfully to transmit this haemogregarine to apparently uninfected hatchery *Solea solea* both in the laboratory and under natural conditions in the aquaculture system at Hunterston. It was not possible to transmit *H.simondi* via the leech to *Scophthalmus maximus* probably because the leeches had difficulty in attaching themselves to this host. Although a small number was observed feeding the possibility exists also that the infective stages of the parasite might not have reached the proboscis of these leeches.

Difficulties in interpretation of the results of transmission experiments have already been described by the author in a previous investigation on the then unidentified haematoprotozoan parasite of farmed *Scophthalmus maximus*. A periodicity of parasitaemia as observed in the circulating blood even of fish with extensive clinical manifestations of infection provided rather inconsistent results. Thus fish or leeches used in these experiments may not have been free of haematoprotozoan parasites despite extensive screening. In addition there may have been differing levels of host susceptibility or immunity in the test fish.

There is always an interpretive problem also as to whether or not the stages of haematoprotozoan parasites encountered in the leeches are in fact developing stages of parasites from other hosts or parasites of the leeches themselves. Also,proof has to be established, that leeches when feeding on fish, are indeed transmitting these parasites and do not just stimulate the reproduction of already present, but hitherto undetected,dormant, blood parasites or their tissue forms.Also leeches used as experimental vectors may not have infectious stages in their proboscis at the time of feeding on a given fish host. That not every leech is suitable as an intermediate host for haematoprotozoan parasites was also observed by Brumpt (1906 a,1906 b) who found that some trypanosomes, although developing in the stomach of the leech,never reached the proboscis,and presumably were digested without being transmitted. This was observed also in argasid ticks when fed on higher animals parasitized with various strains of trypanosomes (Kirmse and Taylor-Lewis,1976).

Another difficulty for interpretation of transmission results is the hostspecificity. Under natural conditions not all leeches are as host specific for a particular fish species as would appear to be the case with Hemibdella soleae from Solea solea in British waters (Llewellyn,1965).Calliobdella nodulifera is able to

parasitize a large variety of marine fish. This lack of host specificity appears to be the rule in marine leeches (Table 9) and compares well with the situation among the freshwater leeches of the genus *Piscicola*. According to Keysselitz (1906) the very variable species *Piscicola geometra* was found on *Cyprinus carpio*, *Tinca tinca*, *Rutilus rutilus*, *Esox lucius*, *Abramis brama*, *Barbus fluviatilis*, *Cobitis barbatula* and others.

This wide host range makes interpretation of leech vector studies complicated because it creates the possibility of encountering in one leech the developing stages of a number of different blood parasites from such diverse groups as trypanosomes,trypanoplasms and haemogregarines. Likewise it is well recognized that a particular fish host can support multiple infections (Table 1). Even more confusing is the possibility of detecting within a single fish host developing stages of several different species of haemogregarines as has been reported for example in the case of *Callionymus lyra* where three haemogregarines were described: *H.callionymi* (Brumpt and Lebailly,1904;Henry,1910); *H.quadrigemina* (Brumpt and Lebailly,1904;Noble,1957); and *H.binucleata* (Henry,1910). Two different haemogregarines have also been described from *Gobius niger*, i.e. *H.blanchardi* and *H.gobi* (Brumpt and Lebailly,1904).

It is necessary for certainty of interpretation to have uninfected leeches feed on infected fish and to follow the development of the acquired blood parasite in that leech.This has been possible in similar studies on the development of haematoprotozoan parasites of domesticated animals (Schein,Warnecke and Kirmse,1977). Uninfected parasite free leeches can only be obtained by breeding them from cocoons collected in the wild as suggested by Keysselitz (1906). This was achieved by Robertson (1906) using cocoons of Piscicola sp. She also collected cocoons of Pontobdella muricata from the Clyde,hatched these in the laboratory and used them successfully to describe the developmental stages of Trypanosoma rajae from Raja sp.(Robertson,1909).

In the present study the Calliobdella nodulifera leeches did produce cocoons and also the Hemibdella soleae leeches deposited their cocoons immediately after removal from their fish hosts but no young leeches hatched in either case.

There remains still the possibility that haematoprotozoan parasites might even be transmitted transovarially via the eggs of the vector as occurs with Babesia bigemina in Boophilus microplus vector ticks (Muangayai,1974).Siegel (1903) when describing the life cycle of Haemogregarina stepanowi of turtles in the vector leech Placobdella catenigera claimed

that he found parasites in many undeveloped embryonic leeches as well as in recently hatched leeches. Brumpt (1904) denied that blood parasites of fishes could be transmitted in the vector leech via the egg and Keysselitz (1906) never encountered infected eggs from infected Piscicola geometra.He suggested that transovarian infection if it ever took place would play only a minor role in transmission.

Not only is it necessary to breed parasite-free vector leeches but also the experimental fish hosts to be infected ought to be parasite-free. This has never been possible to prove with certainty in all of the previous transmission experiments. Even Robertson(1909) transmitting Trypanosoma rajae via Pontobdella muricata leeches to apparently uninfected Raja sp. had to admit that the non-infection status of these wild test fish was very difficult to prove.

The optimum approach would be to use hatchery reared fish but even then it is essential to have proof that the haematoprotozoan parasites of fish cannot be passed on to future generations of fish via the egg.

In the present study hatchery reared Solea solea were used successfully as sentinels and in transmission experiments, but although these fish were apparently

free of blood parasites according to the bloodsmears examined this still does not exclude the possibility that *Hemibdella soleae* infected with *H.simondi* had entered the hatchery tanks via the seawater pipeline and thus infected also the hatchery fish. Slinn(1967) does not believe that this could happen on the grounds that only few leeches would reach the tanks and might not be enough to maintain an infection.

So until these haematoprotozoan parasites become of such paramount economic importance that fishfarming establishments are prepared to provide sufficient uninfected hatchery fish for transmission studies it will not be possible to investigate further the life cycle and transmissability of these haematoprotozoan parasites such as the haemogregarines *H.sachai* n.sp. and *H.simondi* affecting farmed *Scophthalmus maximus* and *Solea solea*,respectively, via the vector leeches or other possible vectors.

5.6. Migration of marine fish in relation to vectors of haematoprotozoan parasites

5.6.1. *Scophthalmus maximus*

According to Harden-Jones (1977,pers.comm.) little is known about the migration pattern of *Scophthalmus maximus* . The only major work was carried out by Jones (1970). He found a seasonal pattern in abundance judging from the results of commercial catches with peaks in early summer (May-June) and in autumn coinciding with the fish aggregating on the spawning grounds.These areas are the Long Forties off Aberdeen, the Dogger Bank and the South-East of the German Bight for the North Sea and the fish actively migrate between these sites. This spawning migration brings the fish from deeper waters to the flatter coasts to spawn in 10-40 m depth. The pelagic eggs and the larvae in metamorphosis drift in the upper layers of the water and the young fish prefer flat sand beaches as the ones in North Wales from where all the young *Scophthalmus maximus* in the fish farms at Hunterston derived. In case of an oral route of transmission of haematoprotozoan parasites to these fish the sandy beaches might be quite suitable because they are also the habitat for small fish as *Pholis gunellus*,*Pomatoschistus minutus* and *Pleuronectes platessa* which have been found to harbour

haematoprotozoan parasites frequently and also belong to the diet of Scophthalmus maximus. Also an ectoparasitic crustacean might be successful here as a vector. However, marine leeches are most likely found in somewhat greater depth and might thus infect older fish when they are gathering for spawning.

5.6.2. Solea solea

This fish is found in summer on flat mud grounds and moves in winter to deeper waters. The spawning grounds appear to be the Southern North Sea on a small stretch in front of the German-Dutch coast at depth of 10-30 m where the fish move from deeper waters in May. As a result of pelagic drift and migration from the spawning grounds (the main areas are still unknown) young fish up to 2 cm are distributed in shallow waters with fine sand until they reach at least a length of 15 cm and the age of 1 year (Harden-Jones,1968).

Llewellyn (1965) investigated Solea solea from Plymouth, the Menai Straits and the coastal waters of Swansea/Wales during summer and found a high incidence of infestation with Hemibdella soleae in adult fish. Since the young fish he examined were almost all free from leeches he concluded that the fish had not yet reached the environment were large numbers of cocoons are deposited.

Hemibdella soleae cocoons are deposited on shell fragments and large gravel particles and cannot be attached to fine sand (Llewellyn,1965). That may be the reason also why small Solea solea seine-netted at Oban in the present study were apparently free of haemato-protozoan parasites. It is also unlikely that infection through an intermediate host takes place in the intertidal zone because of the damaging effects the tidal waves may have on cocoons and leeches. Patches of shell gravel suitable for cocoon laying and rocky bottoms typically occur off shore. Solea solea more than 18 cm in length and approaching maturity seem to frequent deeper waters and this is probably where the infestation with leeches takes place. It is with these fragmented shells that the fish camouflage themselves and thus by their behaviour contribute to their own infestation with leeches. The infected farmed Solea solea examined at Hunterston had derived from the English Channel in April and showed ripe gonads in June. It is assumed that they were either caught at their feeding grounds or on the way to the spawning grounds but it is not clear where the fish had their first contact with the leech vector for H.simondi.

Nothing is known about a possible migration of the marine leeches although it seems obvious that they are disseminated passively being attached to a migrant fish host. Some fresh-water leeches migrate actively against currents(Richardson, 1953)

S E C T I O N C

PATHOGENICITY OF MARINE FISH HAEMATOPROTOZOAN PARASITES WITH SPECIAL REFERENCE TO AQUACULTURE

1. INTRODUCTION

Haematoprotozoan parasites in general appear of a commensal nature in fishes but little work has been done on the pathogenicity of these parasites and on their effects on growth and development of their hosts. Judging from surveys of wild fish populations, haemogregarines are the most common blood parasites found in marine fishes but the effect they may have upon the health of fishes and whether or not they may be a factor in the periodic disappearance of commercially important species from a given area are virtually unknown. The same applies to the less common trypanosomes, a group of such great significance as parasites of higher vertebrates especially of domestic lifestock and man.

The only observations available on the pathogenicity of haematoprotozoan parasites of fishes concern the trypanoplasms. Here disease and death of the hosts were not only seen in the wild but also in various aquacultural projects, where a considerable economic impact, especially in freshwater fish farming, was noted. Most of the trypanoplasms found to date have

been described from freshwater fishes. There are only three records from marine fishes but a pathogenic activity was only observed in one species. Nothing is known about the pathogenicity of Haemohormidium sp. and Dactylosoma sp. which have been encountered occasionally in the blood of fishes.

However, with increasing intensification of aquaculture, which has latterly extended to marine fish farming of various species of highly valued flatfish, it was to be expected that haematoprotozoan parasites might become of increasing importance, taking advantage of a new and ecologically favourable environment.

Thus in 1971 small wild turbot (Scophthalmus maximus), a marine flatfish species of economical importance, were introduced into a fishfarming project on the Westcoast of Scotland. In October 1973 a proliferative condition of the haemopoietic tissue was observed in approximately 1% of these fishes, the cause being a haematoprotozoan parasite of hitherto unknown identity. While the pathogenicity of this organism was under investigation, the disease reappeared during summer and autumn 1976 in two other fishfarms raising turbot at an adjacent site to the first outbreak. In addition an anaemic condition with limited mortality was encountered in adult sole (Solea solea) in the early summer of 1977 on the fishfarm where the disease in turbot had

originated. It was found to be associated with the concerted action of two blood-sucking ectoparasites (a copepod and a marine leech) and a haematoprotozoan parasite, namely a haemogregarine.

Further studies on the pathogenicity of these parasites of marine fishes under aquaculture conditions and their effects on other related fish species were carried out and the economic importance, the possible influence of infection on growth and development and any possible means of therapeutic control were also investigated.

2. REVIEW OF THE LITERATURE

2.1. Trypanosomes

In contrast to the extensive corpus of knowledge on the pathogenic action of trypanosomes in mammals, little is known about the pathogenicity of trypanosomes of fishes. Laveran and Mesnil (1902) when describing _Trypanosoma rajae_ in different species of the Rajidae remarked that these parasites were rarely found in the blood and that they appeared to have no pathogenic influence on the fishes involved. Hofer (1904) described considerable losses in carp culture during 1900-1902. These fishes were very weak during winter and shortly afterwards and were lying for several weeks on one side with their heads and tails bent downwards. If they were put in a normal position, they would swim around for a while but then fall back into the previous position. Together with complete exhaustion there was a considerable loss in weight but no other symptoms were observed and no bacteria or parasites were present. The author called the condition "sleeping sickness" of carp, though only a few fishes had trypanoplasms in the blood, since he found the symptoms quite suggestive

for trypanosomiasis. However, he concluded that despite the fact that trypanosomes are frequently found in large numbers in carp no disease of epizootic proportions had been found to date which could definitely be linked with the parasites. Lebailly (1905) never observed any disease symptoms in marine teleost fishes in which trypanosomes could be incriminated but he did suggest that the report by Doeflein (1901) on a disease of tench (*Tinca tinca*) with symptoms of somnolence and high mortality might have been due to the presence of trypanosomes in the blood of these fishes.

2.2. Trypanoplasms

The trypanoplasms of fish appear to be of more importance than the trypanosomes especially with regard to their pathogenicity under aquaculture conditions. Of all the haematoprotozoan parasites of fish the pathogenicity of trypanoplasms has been best substantiated.

As early as 1902, Laveran and Mesnil, creating the new genus *Trypanoplasma* with the type-species *Trypanoplasma borreli* mentioned briefly that it caused disease and death in fish in captivity. Plehn (1903) found extreme anaemia in carp (*Cyprinus carpio*) heavily infected with *Tr.cyprini*. The fish were lethargic, the gills and internal organs

pale and the blood rather watery and light pink in colour. Experimental transmission was achieved from carp to carp but not to salmonids. Léger (1904) described an acute infection in minnows (Phoxinus laevis) exhibiting a considerable anaemia, decolourization and immobility and the fish refused to feed and died. Keysselitz (1906) described the pathological conditions seen in carp infected with Tr. borreli. He found a high degree of anaemia with pallor of the gills and internal organs such as the liver and kidney, a decrease in the number of erythrocytes linked with a considerable increase in the number of leucocytes and, most striking, infective emboli produced by the flagellates in the capillaries. Death resulted from the anaemia. However, the author suggested that the nutritional condition of the fish host played a major role in the pathogenicity of these parasites and that the pathogenic action of the trypanoplasms per se ought not be exaggerated. Under the influence of good feeding levels he observed that the fish recovered completely. Mavor (1915) described an infection of Tr. borreli in a single Catostomus commersonii, a fish closely related to carp, which had been readily caught with a hand net because of its immobility. No external lesions and no abnormalities of the viscera were seen. The gills, however, were pale and anaemic and parasites were found in great

abundance in heart blood smears. Nowicki (1940) performed several experiments to test the pathogenicity of _Tr. cyprini_ in splenectomized carp. He found that the parasite did not induce any disease symptoms in splenectomized fish but that the pathogenic action of strains of _Pseudomonas_ was greatly enhanced by the surgical splenectomy. Water temperature was also of little apparent influence on the pathogenicity. He concluded that a _Trypanoplasma_ infection of pond fish does not lead to a disease outbreak even if the fish are debilitated by other diseases or adverse environmental conditions. He also indicated that _Pleuronectes flesus_ was often infected with _Tr. flesi_ but did not give any indication of its pathogenicity. Katz (1951) described two new species of trypanoplasms: _Tr. salmositica_ from wild and hatchery reared silver salmon (_Oncorhynchus kisutch_) and _Tr. lynchi_ from wild _Cottus rhotheus_ and _Cottus aleuticus_. He did not remark on any pathogenic action of these parasites. However, Wales and Wolf (1955) demonstrated the pathogenicity of _Tr. borreli_ clearly in wild fishes as well as hatchery reared salmonids. Wild species such as _Oncorhynchus tshawytscha_, _Salmo gairdnerii_ and _Oncorhynchus kisutch_ were heavily infected and in addition _Salmo trutta_, _Catostomus snyderi_ and _Cottus_ sp. were found infected with trypanoplasms. Among the hatchery fish, both yearling and adult

rainbow trout (Salmo gairdnerii) were affected. Externally these exhibited marked exophthalmus, distended abdomems, raised scales and varying degrees of anaemia. Infected fish were lethargic and abnormally dark. High mortalities were also observed in yearlings of Oncorhynchus tshawytscha. Heavily infected fish had parasites in the skin, blood, ascitic fluid and also in muscle tissue, connective tissue and kidney parenchyma but not in the intestinal tract. Externally infected fish were anaemic and had the gills covered with a whitish, transmucent and gelatineous exudate. The viscera appeared normal except for pallor of the liver. Abnormal amounts of mucus were seen on the surface of such fishes. No secondary invaders were found.

Only very few trypanoplasms have been recorded from the marine environment. Mackeras and Mackeras (1925) described Tr. parmae from Parma microlepsis; Nowicki (1940) gave a short account of Tr. flesi from Pleuronectes flesus; and Bullock (1953) reported Tr. newingtoniensis from Pseudopleuronectes americanus but they did not give any indication of the pathogenicity of these parasites. Strout (1965) conducted a series of pathogenicity trials with Tr. bullocki using the intraperitoneal route of inoculation. Whenever trypanoplasms were injected

into apparently negative *Liopsetta putmani* and *Pseudopleuronectes americanus*, the parasites were readily detected in the blood within 48 hours. Transmission was equally successful to *Fundulus heteroclitus* and *Fundulus majalis* but failed with multiple doses of trypanoplasms to *Carassius auratus*, *Salvelinus fontinalis*, *Mugil cephalus* and *Microgadus tomcod* and to mice (*Mus musculus*) and frogs (*Rana pipiens*). Furthermore 0.5 ml doses of trypanoplasma-positive serum were force-fed to *Liopsetta putmani* and *Pseudopleuronectes americanus* to attempt infection of the intestine. However, no organisms were found in stomach and intestine 7 days post infection so that it appeared certain that the flagellates were not able to maintain themselves in the intestinal tract of the fish host. Kirmse (1975) found a *Trypanoplasma* sp. in a turbot (*Scophthalmus maximus*) but did not observe any pathogenic action associated with its presence. However, the trypanoplasm occurred concomittantly with an unidentified intraleucocytic haematoprotozoan parasite causing a proliferative disease condition in the blood of the same host.

2.3. Haemogregarines

Lebailly (1906) was not certain about attributing pathogenic action to the intracellular haematoprotozoan

parasites of the fish he examined. During the multiplication stage of the haemogregarines in the blood, he found that it could be so extensive that a large proportion of the red blood corpuscles were destroyed especially in *Solea solea*, *Blennius* sp. and *Callionymus lyra* infections. The erythrocytes were often enlarged in such a manner that the infection could alter the health of the fish host considerably and could even result in death. However, from the many cases of "chronic" disease which he observed he concluded that the diseased fish were well equipped to overcome such pathogenic activity by the parasite under normal circumstances.

Haemogregarines frequently produce considerable hypertrophy of the infected blood cell but marked changes in the morphology of the host cell apart from those associated with hypertrophy are less common. Wenyon (1908) reported that in *H. gracilis* from *Mabuya quinquetaeniata* the parasite had the property of distending the infected host cell at each pole and thus elongating it considerably. Ball, Chao and Telford (1969) also found a marked alteration in the shape of the infected erythrocytes which were frequently fusiform. This was the most noticeable feature of an infection of *Boa constrictor* with *Hepatozoon fusifex*.

Also in teleost fish, observations on the pathogenicity

of haemogregarines generally involved the infected blood cells. The results were varied according to the geographical area and the host for the haemogregarines. Reports are limited to marine fish haemogregarines since these parasites are so rarely encountered in freshwater fish. Neumann (1909) did not find any clinical symptoms in fish infected with haemogregarines and with high parasitaemias but observed a considerable leucocytosis in several cases. Henry (1913) remarked that in haemogregarine infections of _H. bigemina_, _H. quadrigemina_, _H. clavata_ and _H. simondi_ the host cells always underwent marked degenerative changes and destruction. Also schizogony in the circulating blood was always accompanied by enlargement of the whole erythrocyte, by rapid degeneration of its cytoplasm and nucleus and by its ultimate total destruction. The age of the fish appears to be of considerable importance. Laird (1952) observed that the youngest fish appeared to have the heaviest infection with 50 haemogregarines per 10,000 erythrocytes. At the large schizont stage of development the erythrocytes parasitized by haemogregarines became considerably hypertrophied and attained a size of 15 by 11 micron as compared with the 10.7 by 9.1 micron of normal cells. Saunders (1955) found that the pressure of the parasite as it grew appeared to indent or flatten the nucleus of the host cell mechanically. In heavily parasitized fish

disintegration of the host erythrocyte with abundance of free gametocytes, often still attached to the hypertrophied remains of the host cell nucleus, were observed. This nucleus was usually displaced in lateral direction and became longitudinally distorted as a result of being compressed between the gametocytes and the cell membrane. In H. bigemina the nuclei of the parasitized leucocytes were usually excentric but this was also common for unparasitized leucocytes so its significance was not known. Since post mortem examinations of infected fish have only rarely been performed during surveys for haematoprotozoan parasites no other pathogenic reports of haemogregarines are available. Saunders (1966) conducted the only investigations of the haematology of infected fish as compared with healthy ones, but they were very limited. Red cell counts were made of 4 uninfected and one H. bigemina infected Lutjanus apodus from Puerto Rico. The count for the apparently healthy fish was 4,130,000 erythrocytes per mm^3 of blood whereas for the haemogregarine infected fish 6,420,000 cells per mm^3 were counted. This would indicate that an infection by a haemogregarine might result in an increase in the number of erythrocytes in a fish. Most authors, however, agreed that in the majority of cases the host cells containing merozoites were not hypertrophied and their nuclei were seldom displaced

(Laird, 1952; Saunders, 1955, 1958; Laird and Morgan, 1973). So (1971) however, found a slight reduction in size of some host cell nuclei and regular marked nuclear displacement in H. platessae. Kirmse (1975) described an unidentified intraleucocytic haematoprotozoan parasite from farmed turbot. Intensive pathogenicity studies were undertaken from 1974 and are still in progress. After an incubation period of approximately 1-2 months, development of proliferative lesions and their metastatic distribution throughout the body was observed. Diseased fish did not differ in their behaviour from apparently healthy fish. Neither their feeding nor the growth rate appeared to be affected. However, infected fish seemed very susceptible to severe changes in temperature and this could result in sudden deaths. Under normal fish farming conditions the disease showed a rather chronic progress and fish with clinical symptoms only succumbed unfrequently. There was often a considerable increase in leucocytes of the circulating blood. No significant differences were seen in the packed cell volume (PCV) of infected fish and apparently healthy ones from the same tanks. Parasitized leucocytes, however, showed a striking difference in size from normal cells. Normal monocytes measured 11.3 by 9.3 micron whereas parasitized cells measured 15 by 13 micron in average. This was considered due to mechanical enlargement by the increasing

number of merozoites inside these cells which displaced the nucleus to one side and often caused the cells to burst. Intracellular schizonts would sometimes enlarge a leucocyte up to 25 by 22 micron. Parasites were never observed inside erythrocytes. The organisms were not transmissible by any route of inoculation to apparently healthy fish from the same aquaculture system.

3. MATERIAL AND METHODS

3.1. Collection of epizootiological data

A number of field trips were made to the two commercial aquaculture establishments at Hunterston (British Oxygen Company (B.O.C.) and Fitch Lovell(F.L.)) where a disease condition of farmed turbot (*Scophthalmus maximus*) similar to that seen in 1974 at the White Fish Authority (W.F.A.) fish farm at Hunterston, was observed in 1976/77. Similarly several visits were also made to the W.F.A. farm at Hunterston, where an anaemic condition of farmed sole (*Solea solea*) had been observed in June 1977. To gain additional information of the two disease conditions, questionnaires were submitted to the three fish farms involved (see Appendix).Diseased and apparently healthy fish of different age groups were collected from all three farms. Samples of 2-6 fishes according to size and age were packed into double polythene bags filled with seawater and aerated with compressed bottled oxygen and closed with steel wire or rubber loops for transport by car to the laboratory. Blood samples were collected from clinically diseased

and apparently healthy fish for a survey of the grade of parasitaemia. These were either examined on site in wet preparations under a binocular microscope or smears were made and air dried for transportation to the laboratory, where they were later fixed with methanol and stained with Giemsa. Occasionally the packed cell volume (PCV) was estimated on the fishfarm and also a leucocyte count was performed. This was especially necessary in the case of the very valuable brood stock of *Solea solea*. These fish were also surveyed for ectoparasites as leeches, copepods, isopods and trematodes. They were collected from the fish and taken to the laboratory in glass jars filled with seawater. Blood drawn from infected fish was filled into heparinized plastic vials for subsequent haematological studies, or into bijoux bottles filled with 2.5% glutaraldehyde for electron microscopical studies. In addition data were collected on the water quality, water temperature and nutrition in all three fish farms. At the W.F.A. the opportunity was also available to survey the general fauna of the holding tanks. A report on the findings at the W.F.A. farm at Hunterston was given to the farm management (see Appendix).

3.2. Examination of fish in the laboratory

3.2.1. Holding and feeding

Diseased and healthy fish were kept in separate tanks of either polythene or glass, filled with sea water pH 4.9-5.6 which was re-cycled through an Eheim Filter pumping mechanism. In addition one or two aerators were placed into each tank and a small amount of sand covering the bottom of the tanks was added. The fish were fed a diet of dry fish based pellet (WFA 6) or minced squid. The water temperature under normal conditions was kept at approximately 13° C.

3.2.2. Sampling of fluids and tissues of live fish

Live fish were anaesthetized with MS 222 (Sandoz) at a concentration of 1:50,000 (= 0.08 g/4 litres of water). Blood was then drawn either from the dorsal or ventral renal portal vein or by cardiac puncture. Wet smears and normal blood smears were prepared immediately after sampling; some of the whole blood was also fixed in 2.5% glutaraldehyde for electron microscopy.

3.2.3. Haematology

The packed cell volume values were estimated by collecting blood into heparinized haematocrit tubes, centrifuging at 12,000 rpm for 8 minutes and then reading the results from a Hawksley Microhaematocrit Guage expressed in percentage of cells. The enumeration of erythrocytes and leucocytes was performed with whole blood from diseased and healthy fish using a haemocytometer (Neubauer). The diluting fluid for the erythrocyte count consisted of Dacie's fluid (40% formaldehyde solution plus 3% trisodium citrate) as described by Blaxhall and Daisley (1973). For the leucocyte count the blood was diluted with Hain's solution consisting of 2% acetic acid plus 1% gentian violet. The differential blood cell count was done on stained slides using the battlement method by MacGregor, Richards and Loh,(1940).

3.2.4. Post mortem examination

Diseased fish were either already in a moribund state at the time of collection or they were sacrificed after laboratory observations had been made. Freshly dead fish were subjected to a thorough examination for external parasites by making scrapings from the upper and lower surfaces of the fish, the pectoral and

abdominal fins, the tails and the gills. Part of the gills was removed, suspended in saline in a petri dish and examined under the dissecting microscope. Scrapings were placed on glass slides in a drop of saline, covered with a cover slip and then examined at low power under the light microscope. After the collection of blood the fish was autopsied and impression smears were taken from all organs in the form of wet preparations and studied immediately under the light microscope or as permanent smears, air dried and fixed with methanol and subsequently stained with Giemsa. In addition pieces of each organ were dropped into 2.5% glutaraldehyde for electron microscopical studies and into 10% formalin for histology. Histological sections were routinely stained with haematoxylin & eosin, periodic acid-Schiff and Martius-scarlet-blue and Giemsa. Also pieces of lesion material were subjected to the same procedure.

3.3. Pathogenicity trials

In Scophthalmus maximus where a proliferative condition was present, clinically affected fish were observed for several months in the aquarium to study the growth of the lesions. During this time blood was withdrawn periodically from each of these fishes at irregular intervals to detect the presence or absence of parasites in the blood circulation and thus determine periodicity.

3.4. Transmission experiments

To test the transmissebility of the two haemato-protozoan parasites found in *Scophthalmus maximus* and *Solea solea*, respectively, a number of experiments were performed. Whole blood of highly parasitized fish as well as suspensions of lesion material of diseased *Scophthalmus maximus* were inoculated intravenously into apparently healthy *Pleuronectes platessa*, *Solea solea* and *Scophthalmus maximus*. Diseased *Solea solea* were kept together in the same tank with apparently uninfected *Pleuronectes platessa*, *Scophthalmus maximus* and hatchery reared *Solea solea* for possible contact transmission.

3.5. Therapy trials

Attempts were made to control the proliferative condition in *Scophthalmus maximus* therapeutically. For this purpose several drugs were employed *vide* the coccidiocide Amprolium-Hydrochloride plus Ethopabate (Amprol Plus)+; the antibiotic Oxytetracycline (Terramycin)++; and the antimalarial drug Cloroquine (Resorchin)+++. The dosage was estimated per kg body weight as recommended for mammals. It was generally in the range of 0.05-0.1 ml for a fish of 312-625 g weight which was the mean weight of *Scophthalmus maximus*

+)Merck Sharpe&Dohme ++)Pfizer +++) Bayer

of this age group. In a pilot experiment two fish were inoculated by various routes using higher dosage to determine toxicity levels. Amounts of 0.5-1.0 ml of Amprol Plus given orally; 0.3 ml (intravenously) and 1.0 ml (intraperitoneally) of Terramycin and 0.2 ml (intramuscularly) of Resorchin were found to be tolerated well by these fish.

A total of four diseased Scophthalmus maximus were subjected to the treatment with the coccidiocide. They were inoculated via a stomach tube with a single oral dose of 1.0 ml Amprol Plus,per day and fish,on four consecutive days.

The antibiotic Terramycin was used in the treatment of eight diseased fish. They each received a dose of 1.0 ml intraperitoneally,inoculated daily for four days. In addition two infected fish received 0.3 ml of the drug intravenously for the same length of time.

Two months later five of the originally treated fish were inoculated intramuscularly with 0.2 ml of a 10% solution of Resorchin and this antimalarial treatment was repeated daily over a period of five days.

In some of the infected fish efforts were also made to treat the lesions directly by injecting Terramycin into the stroma.

4. RESULTS

4.1. The pathogenicity of *Haemogregarina sachai* n.sp. in *Scophthalmus maximus*

4.1.1. Case History

New outbreaks of the proliferative condition of *Scophthalmus maximus*, first seen in 1973/74 at the W.F.A. farm in Hunterston, was observed in summer 1976 and October 1976, respectively, on two fish farming enterprises located adjacent to the previous outbreak. Staff at the Fitch Lovell site had noticed gross morphological changes in the form of "tumorous" lesions for the first time in summer 1976 and subsequently found that approximately 150 fish,i.e. 1% of their stock, in the age group 0-1 year were affected in 3 different tanks.

In October 1976 the same proliferative condition was also observed at the British Oxygen Company farm which had established a pilot scale production excercise in one large circular tank set up on the Fitch Lovell site.

Here also 1% of the fish population, i.e. 25 turbot of the year group 1+ was involved. This tank was stocked at a density of 20-60 kg/m^3 and was self cleaning, whereas the three tanks of Fitch Lovell were cleaned daily but their stocking rate could not readily be ascertained.

All fish originated from North Wales where they had been seine netted from the wild at approximately 5-6 months of age. In addition hatchery derived Scophthalmus maximus, Solea solea and Salmo gairdneri were held in tanks at the same site. The overall conditions of water source, temperature and pH as well as the ionic levels in the water appeared to be the same for both aquaculture systems and are summarized in the field questionnaires (Appendix I and II).

The only difference related to the two different tank systems. In the case of B.O.C. the self-cleaning system of this circular tank allowed repeated feeding 6-7 times a day with a necessary flow rate of 15,000 litres of water per hour, whereas Fitch Lovell fed only twice a day to obviate excessive accumulation of waste products and a water flow rate of 200 g/p/m (approximately 54,000 litres per hour) was required. Apparently no faecal casts or food waste were found in either system

but it was interesting to note from the questionnaires that other marine life including small fish, mussels, crabs and barnacles as well as sea weeds had entered both systems. A further survey of the marine life found in tanks of the W.F.A. gave an even more dramatic impression of the inefficiency of the filtering system for these tanks at Hunterston (Table12).

The Unit of Aquatic Pathobiology was first notified of this disease condition in January 1977 and most of the diseased fish had until then been disposed off either frozen and despatched with the refuse (B.O.C.) or they had just been flushed (Fitch Lovell). However, none of the infected fish with obvious visible clinical symptoms had been marketed. From January 1977, diseased fish were collected in separate tanks and submitted to the Unit for further studies on the pathogenicity of the disease.

In addition, blood smears were collected of apparently healthy fish from the same groups. Of the 200 fish examined at B.O.C. non showed parasitaemia. However, 7 out of 138 fish examined at Fitch Lovell had moderate to high parasitaemias without any clinical signs of the disease. After the end of March 1977 no further clinically diseased fish were observed and also no reoccurence of

this proliferative condition was seen during summer and autumn 1977. Furthermore no haematoprotozoan parasites were detected on repeated blood sampling despite the fact that no therapeutic control had been attempted.

4.1.2. Haematology

The blood of the moderate and highly parasitized fish, when observed under phase contrast in wet preparations and using a number of different vital stains often showed a high degree of infection with extra- and intracellular haemogregarine parasites. No emigration of intracellular parasites was observed but the extra-cellular haematoprotozoans were moving slowly through the field of observation in a gliding manner. The PCV of infected *Scophthalmus maximus* with high parasitaemias averaged 28 whereas apparently uninfected fish from the W.F.A. at Ardtoe showed a PCV of 35.

In blood smears from such parasitized fish the levels of cellular infection varied. In those infected fish without visible clinical signs a parasitaemia involving 3.2% of all blood cell types was seen. In clinically affected fish differences in levels of parasitaemias were observed regardless of the clinical condition of

the fish. Light infections had a range of 0.5-1.4% of all cells parasitized. Medium infections ranged from 10.0-12.2%. Moderate infections ranged from 21.2-24.0% and finally very heavy infections, mainly from moribund cases, showed parasitaemias involving up to 36% of all cells. To facilitate the monitoring of individual infections over longer periods of time, infections were designated as follows: light (+); medium (++); moderate (+++) and heavy (++++).

In healthy Scophthalmus maximus the total erythrocyte count was 1.355,080. The total leucocyte count including the thrombocytes was 65,615. Lymphocytes (including small and large lymphocytes) numbered 22,822 and thrombocytes numbered 28,528. Thus the ratio of lymphocytes to thrombocytes in normal turbot blood is about 1:1.25. Neutrophils make up 14.6% of the total leucocyte count and monocytes 6.2%.

The differential analysis of healthy turbot blood cells indicated 95% erythrocytes, 0.2% erythroblasts, 2% thrombocytes, 0.1% large lymphocytes; 1.5% small lymphocytes; 0.7% neutrophils and 0.3% monocytes.

In moderate infections erythrocyte levels had dropped to 86%, thrombocytes to 0.5% and erythroblasts had inreased to 2.2%. There was also apparant a monocytosis

(3%) and a considerable increase in neutrophils (5.4%). Small lymphocytes (1.4%) and large lymphocytes (1.7%) did not appear to change significantly. In very heavy parasitaemias the anaemic condition of the fish was not only characterized by the thin,watery and pale-red blood but also by the distinct erythrocytopenia (73.5%), a further increase in monocytes (6.8%), neutrophils (15%) and erythroblasts (4%) and also a decrease in thrombocytes (0%), small lymphocytes (0%) and large lymphocytes (0.7%).

The total erythrocyte count in very heavy infections was down to 1,105,460 and the total leucocyte count went up to 320,940.

The majority of the cells found to be infected with haemogregarines were neutrophils and monocytes. An average of 7% (range 1.0-16.0) of all cells were infected neutrophils and an average of 4.5% (range 1.0-8.0) of all blood cells were infected monocytes. There appeared to be a difference in the numbers of cells involved according to the severity of infection. In light infections 88.6% of all neutrophils and 90% of all monocytes were infected, whereas in heavy infections only 61.6% of all neutrophils and 32.9% of all monocytes were parasitized. This may be explained by the overall increase in the total numbers of these cell types during heavy infections.

Although infections of these cells with a single parasite were commonly observed and found in 28.9% of all neutrophils, the most common infection was that with two parasites and seen in 53.4% of all infected monocytes.

Possible fluctuations in parasitaemia were monitored in a number of Scophthalmus maximus over a period of of several weeks. An obvious rhythm in the periodicity of the occurrence of the haemogregarines within the blood circulation was observed in fish with clinical manifestations of the disease. These results are summarized in Table 13 (page 222) and compared with results from a similar experiment by Laveran (1912). The cycle observed in the present study appeared to have a duration of 25-45 days.

It was also interesting to observe that peripheral blood smears of infected fish, as was sometimes also the case in healthy fish, showed an erythrocytic poikilocytosis and anisocytosis with often remarkable changes occurring in the nuclei and frequent segmentation of the latter into small lobes.

4.1.3. Pathology and Histopathology

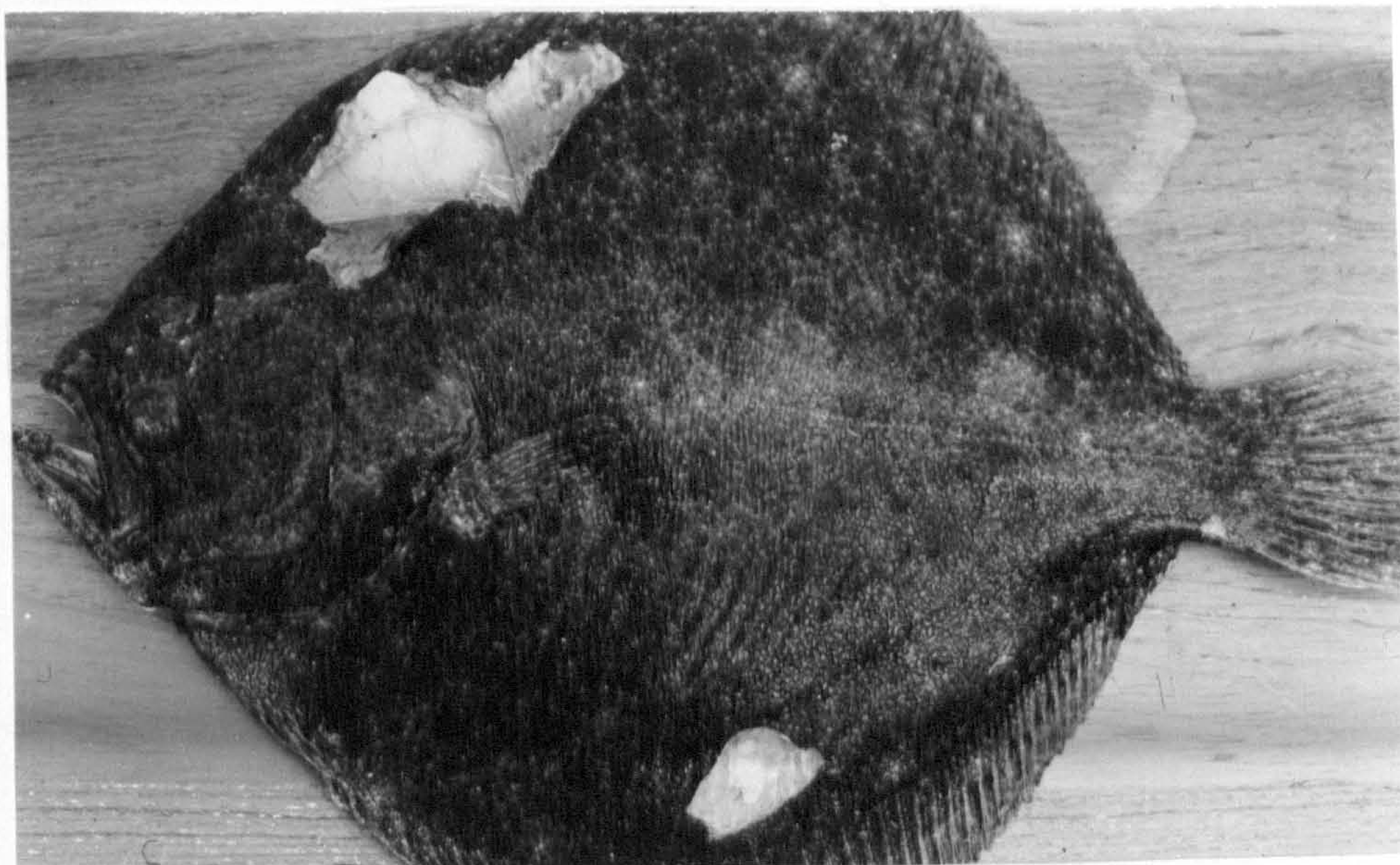
In contrast to earlier observations on this proliferative condition in Scophthalmus maximus (Kirmse,1975) the clinical signs in these outbreaks appeared much more pronounced than before. All of the fish on which necropsies were performed had conspicuous lesions grossly visible both on the upper and lower surfaces (Fig.89). In many cases the whole musculature of specific areas was replaced by "tumorous" masses. Often the lateral lymphatic ducts were grossly enlarged and showed clearly through the skin. Gross lesions were not observed in the heart, brain, liver or intestine, although the heart and brain were often encompassed by large masses of proliferative lympho-myeloid tissue (Fig.90). Although the eyes were not involved per se ,they were often pushed forward by retrobulbar "tumorous" masses producing exophthalmus. As a general pathological finding the spleen was always enlarged (to 2.5 by 1.0 cm in size), dark red-brown and very soft and congested. It was common to find "tumorous" masses or multiple, circumscribed small "tumours" in the oesophagus and stomach, often making deglutition impossible. Kidneys and gonads were regularly involved and their parenchymatous tissue was replaced at least in part by "tumorous" masses.Lesions more frequently involved the gonad of

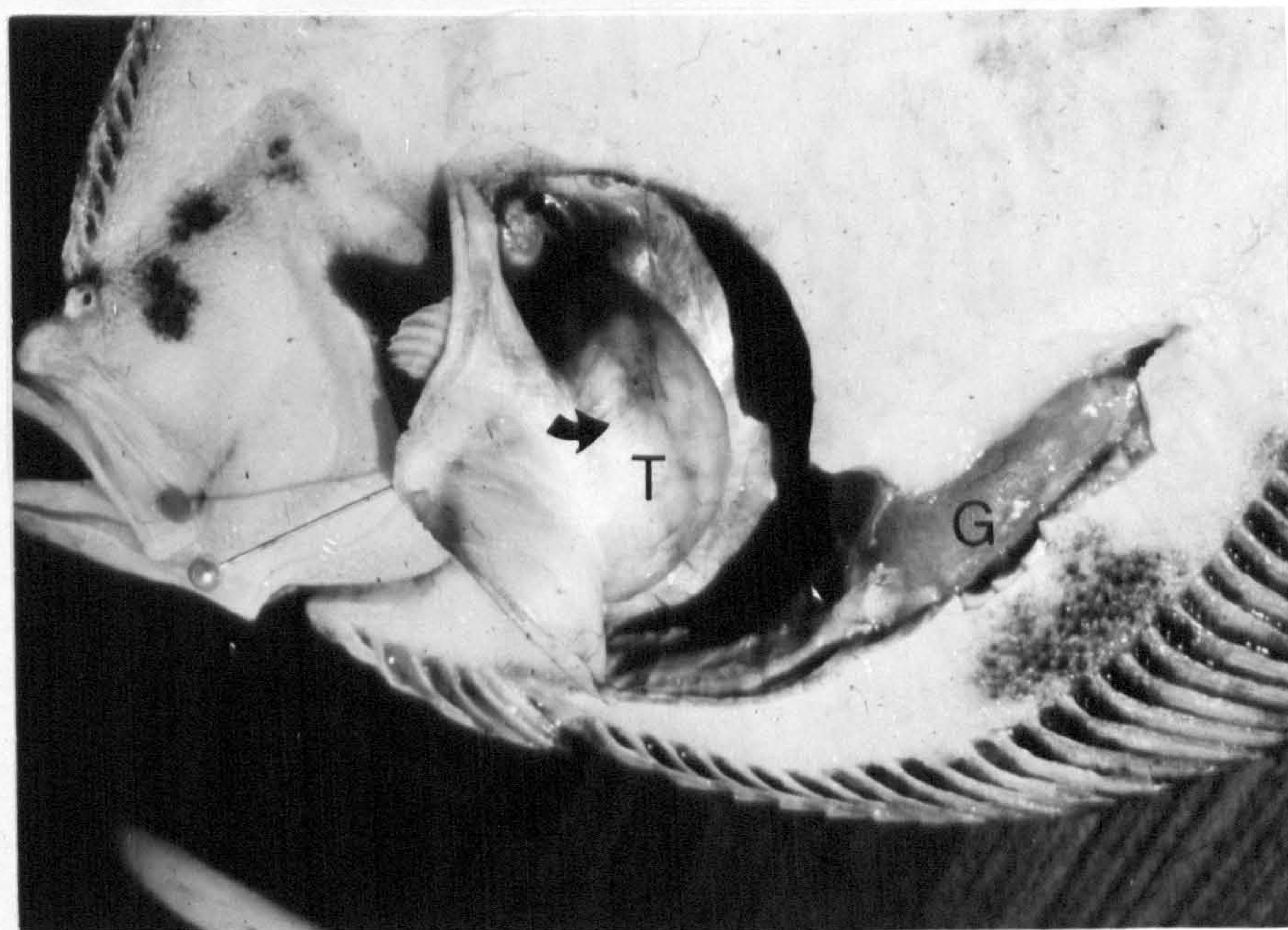
FIGURE 89.

Scophthalmus maximus with "tumorous" lesions involving the lateral lymphatic ducts. Upper surface, skin partly reflected.

FIGURE 90.

Lower surface of *Scophthalmus maximus* with opened abdominal cavity. The internal organs are removed with the exception of the female gonads (G). A "tumorous" (T) mass encompassing the heart is seen (arrow), bulging into the abdominal cavity.

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the female and often the whole ovary was affected giving rise to lesions which extended through the entire thickness of the fish including skin, muscle and bone. An enlargement of the dark blue-green gall-bladder to 3.0 by 1.5 cm was also regularly seen in the majority of cases. Many of the small lesions could only be detected by palpation through the skin or by cross sections. The smallest lesions were in the range of 0.2 by 0.3 cm.

Large"tumours" were sometimes surrounded by a fibrous capsule, the interior of which was of a creamy pinkish colour. This consisted of liquefied necrotic tissue with a solid caseous centre. Impression smears of "tumours" (Fig.91 a,b,c) and various organs revealed an abundance of free or intracellular haemogregarines. They were predominantly seen in organs with "tumour" involvement but were also seen even in organs which showed no gross lesions e.g. brain and liver, although to a lesser degree. These haemogregarines were observed in capillary spaces only, where they appeared either free or inside of mainly macrophage type cells and also large and small lymphocytes and neutrophils. No evidence was found that these parasites invaded tissue cells.

Histologically the lesions found in various organs were characterized by an accumulation of parasitized

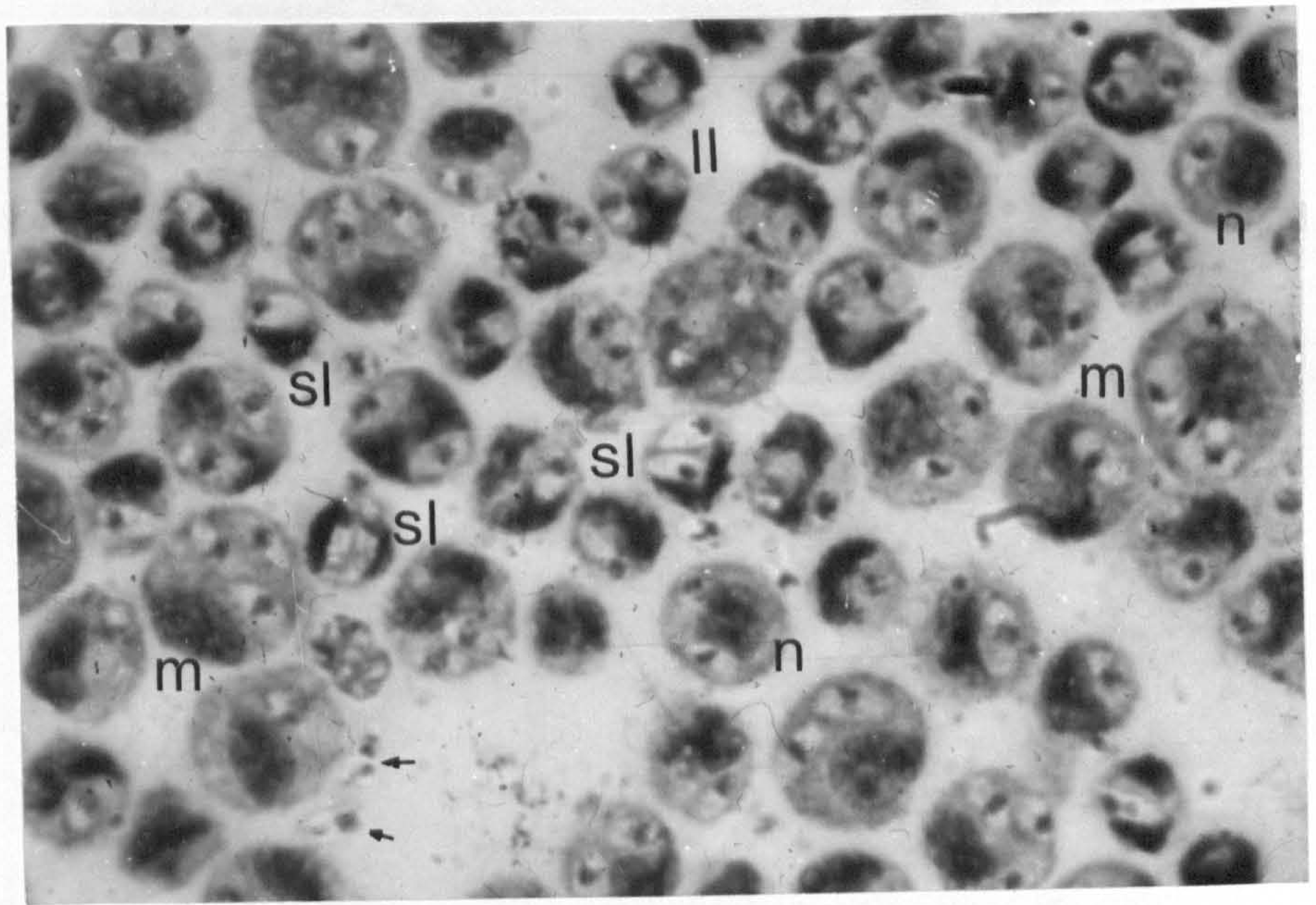
FIGURE 91.

Impression smear of "tumorous" lesion. Most of the cells appear parasitized with Haemogregarina sachai n.sp.

(a) Small lymphocytes with one or two intracellular parasites. Large lymphocytes, neutrophils and monocyte-macrophage type cells.Few free organisms (arrow).

(b) A large immature neutrophil(?) appears uninfected,also a few small lymphocytes. Division stages of parasites indicate active multiplication inside host cells.

(c) A schizont with 18-20 nuclei and a flattened host cell nucleus is seen (arrow).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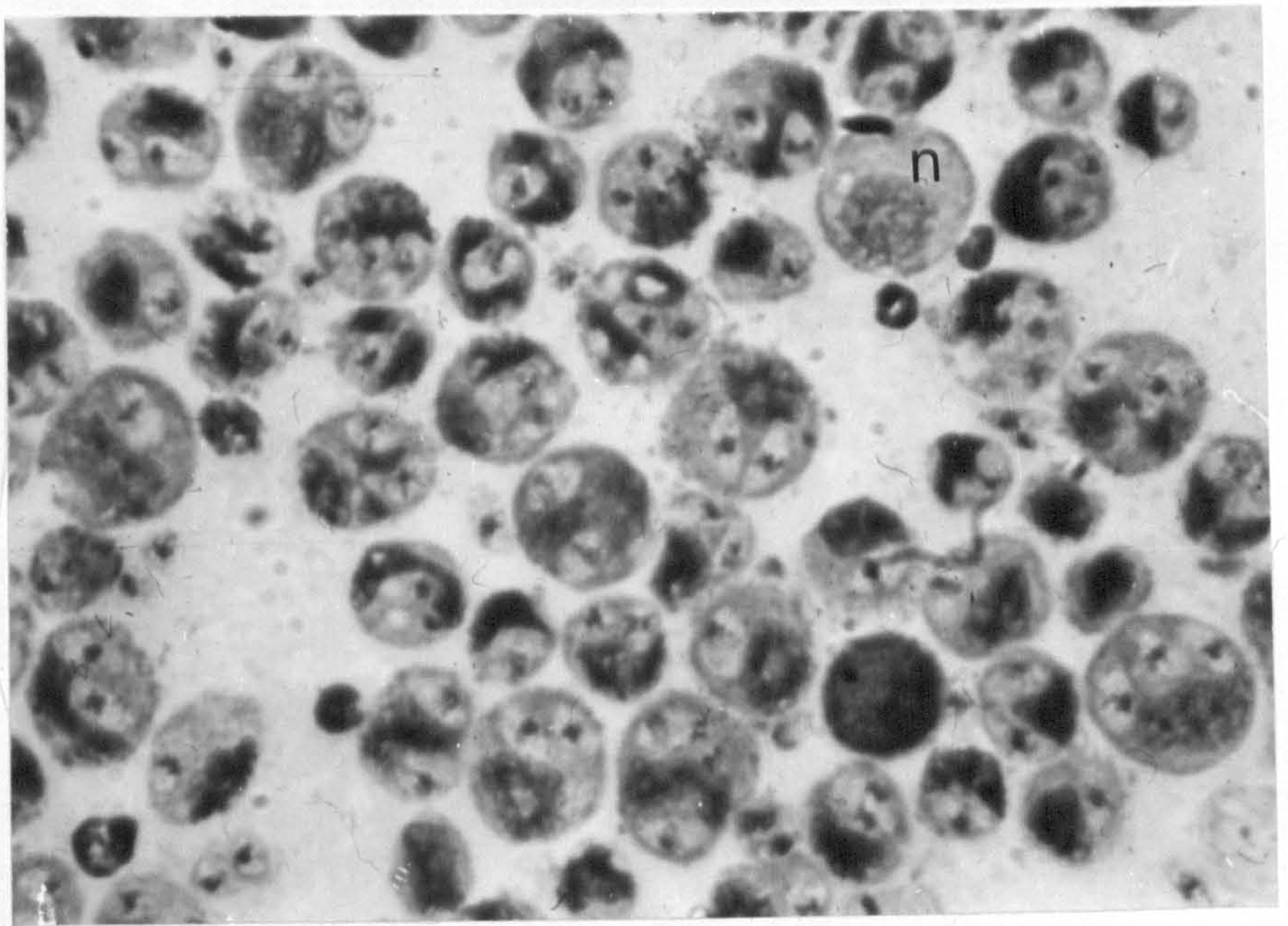
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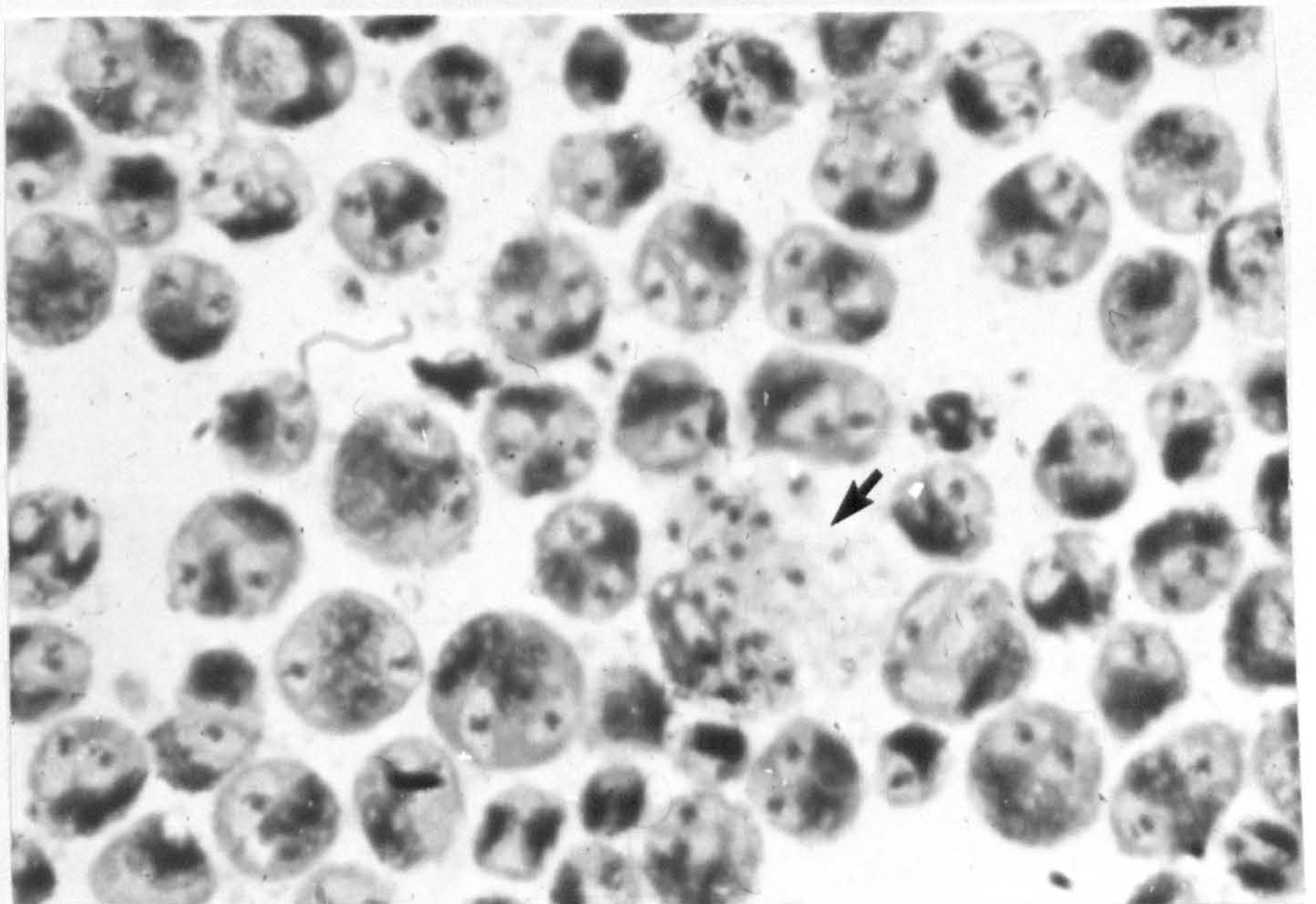


macrophages, cell debris and pyknotic nuclei. There was sometimes demarcation of these clusters of macrophages by fibrous strands. However, only few plasma cells and no lymphocytes were seen and giant cells were also absent. Parasitized macrophages were most abundant in the melano-macrophage centres of kidney and spleen and parasites were observed also occasionally in cells of the haematopoietic tissue. The description of the histopathology has been reported in detail elsewhere (Kirmse,1975).

4.1.4. Transmission experiments

All the attempts to transmit *Haemogregarina sachai* n.sp. from infected *Scophthalmus maximus* to apparently healthy *Pleuronectes platessa*, *Solea solea* and *Scophthalmus maximus* by inoculation or by contact were unsuccessful.

4.1.5. Therapy trials

The pilot scale attempts to control the proliferative condition in *Scophthalmus maximus* therapeutically failed. Neither a progression nor a regression of the "tumorous" lesions were observed in connection with the administration of various drugs and the general health of the experimental fish seemed unaffected by the different treatments. Also no changes were observed in the parasitized peripheral blood of these fish.

4.2. The pathogenicity of Haemogregarina simondi in Solea solea

4.2.1. Case history

In April 1977 a total of 200 adult Solea solea of unknown age were caught for the White Fish Authority by trammel nets at Selsey about 10 miles from Portsmouth in the English Channel from a depth of 4 fathoms and 1/2 - 5 miles off shore. These fish, almost all of them with mature gonads and captured when gathering at the spawning grounds, were transported to Hunterston to be introduced as brood stock. Only 70 fish survived the transport. They were separated on arrival into two lots of 34 and 36 fish, respectively, and accomodated in two square tanks of 11 m^3 size within a wooden shed at a stocking density of 1.3 kg/m^3 or 15 kg per tank and fed mussels (Mytilus sp.) and lugworms (Arenicula sp.). They were fed 3-5 times per week and the tanks were cleaned after each feed to get rid of excessive waste. The flow rate of the seawater was 33 m^3/ day / tank and the temperature of the water, measured during July, varied between 15.1 and 16.7 C. No warm effluent cooling water from the adjacent power station was used

during the warm weather period. There had been no mortalities registered since the arrival of the fish in Hunterston and no other marine fauna had been observed in the tanks.

The first sign of a general malaise was noticed at the end of May when the fish began feeding poorly. A general weakness was apparent in June when one fish was suddenly found dead and another fish in a moribund state. At close inspection both fish were found to be infested heavily with ectoparasites. The dead fish had 26 copepods identified as the blood feeding adult female stage of the anchor-worm *Lernaeocera* sp. (24 on the gills and 2 on the upper surface of the body). The moribund fish was 37 cm long and had a weight of 533 g. It was infected with 20 adult *Lernaeocera* sp. on the gills and died shortly after treatment with methylene-blue given as a bath. After the possible onset of the infestation with *Lernaeocera* sp., the feeding of the fish had ceased alltogether for 2 weeks.

An investigation of the problem was first undertaken in July 1977 when blood samples of infested fish were taken and another moribund fish was collected and taken to the laboratory at the Unit of Aquatic

Pathobiology. After the examination had revealed an additional infestation with the ectoparasites *Hemibdella soleae*, a blood feeding marine leech; *Entobdella soleae*, a mucus feeding marine trematode and an infection with the intracorpuscular haemato-protozoan parasite *Haemogregarina simondi*, a preliminary report was prepared for the W.F.A. (see Appendix IV). Both ectoparasites had been observed on these fishes when they were captured in April. Further surveys showed that approximately 25 *Solea solea* from both tanks at Hunterston were infested with from 1-22 specimens of *Lernaeocera* sp. and that at the same time all fish were infested with up to 45 individual *Hemibdella soleae* and those examined for haematoprotozoan parasites were all infected with *Haemogregarina simondi*. All fish infested with *Lernaeocera* sp. showed an increased ventilation rate, readily visible in the water. At close examination a distinct pallor of the gills suggesting an anaemic condition was detected. The fish appeared rather weak and sluggish in their movements and almost reluctant to swim and they were easy to capture by hand net. No attempt was made to remove the ectoparasites mechanically by crushing them with a pair of forceps as suggested by Slinn (1967) however,the fish were all routinely treated at the

end of the spawning period (June/July) by immersing them individually in tap water for 20-30 minutes which apparently killed the leeches and flukes but did not have any effect on the *Lernaeocera* sp. An additional formalin treatment was also tried without success. In a further trial 5 infested fish were treated with Dipterex+ in a concentration of 8 ppm in August which apparently cured them of the infestation with *Lernaeocera* sp. No further casualities were recorded and the fish fed again normally. An obvious decline of the *Lernaeocera* sp. was observed in autumn 1977 after the number of parasites had already decreased during June/July (see questionnaire Appendix III). No egg deposits or other juvenile stages of the parasite were seen in the tanks. It is interesting also to note that brood stock caught earlier that year (February 1977) and stock from the year before had never been observed to be infested with *Lernaeocera* sp. In late autumn 1977 many of the infested fish, however, and others from various capture sites and times, were still infested with live *Hemibdella soleae* and those examined for haematoprotozoan parasites in their blood were all infected with Haemogregarina simondi. During October 1977 a freshly captured lot of adult *Solea solea* from the North Sea

+ Dipterex 80 (trichlorphon) Bayer

near Lowestoft appeared equally infested with *Hemibdella soleae* and harboured the haematoprotozoan *H.simondi* in their blood.

4.2.2. Haematology

Examination of wet blood smears at the W.F.A. fish farm at Hunterston and in the laboratory in Stirling revealed a large number of banana-shaped organisms occurring both intra- and extracellularly, but with little or no evidence of motility. Using the hanging drop method for motility two intraerythrocytic schizonts were observed for 4 hours without recognizing any movement of individual merozoites and an additional pair of schizonts was observed for 12 hours without any attempt by the haemogregarines to leave their host cells being observed. Examination of Giemsa stained blood smears of infected fish from both tanks showed a heavy infection with the marine haemogregarine *H.simondi* in various stages of development (see Section A). There was a pronounced leucocytosis with a general increase in lymphocytes, neutrophils and monocytes.

In very heavy infection the differential cell count analysing the percentage distribution of the various cell types showed 76.6% erythrocytes, 1.8% erythroblasts,

1.4% thrombocytes, 6% small lymphocytes, 3.2% large lymphocytes, 3.0% neutrophils and 7.6% monocytes. The average PCV for diseased fish was 9, for healthy fish it was 13. From 1-8 parasites were counted parasitizing leucocytes as large lymphocytes, neutrophils and monocytes and mature erythrocytes. No parasites were detected in small lymphocytes and erythroblasts. Both, infected leucocytes and erythrocytes were greatly hypertrophied in association with the growth of the parasite and were often ruptured as a consequence to the repeated schizogonies taking place. Intraerythrocytic schizonts increased the host cell up to 22.1 by 11.7 micron as compared with the size of an average uninfected erythrocyte of 12.6 by 6.9 micron. Intraleucocytic schizonts enlarged the host cells also quite considerably and up to 19.5 by 10.4 micron in contrast to normal monocytes measuring on average 14.9 by 11.1 micron and normal neutrophils measuring on average 10.4 by 9.9 micron.

In healthy Solea solea the total erythrocyte count was 1.007,248. The total leucocyte count including thrombocytes was 31,150. Lymphocytes numbered 11,422 and thrombocytes 13,499 thus giving a ratio of 1:1.18 of lymphocytes to thrombocytes in the blood. In contrast the total erythrocyte count in very heavy infections decreased to 493,891 and the total leucocyte count went up to 113,401. Especially remarkable was the difference in neutrophils which had

increased from 13.3% to 16.6% and monocytes which went up from 6.7% to 42.2% of the total number of leucocytes.

4.2.3. Pathology and histopathology

The moribund fish collected in July and brought to the laboratory, died on arrival. It was an adult female fish of 27.5 cm in length and 9.2 cm in width. Skin scrapings from the upper and lower surfaces of the fish revealed 5 ectoparasitic trematodes on the lower surface identified as Entobdella soleae. In addition there was found one specimen of the marine leech Hemibdella soleae on the upper surface near the head attached by its posterior sucker. In addition 8 adult female Lernaeocera sp. brown to dark red in colour and with ripe egg sacks were found anchored deeply in the gills and induced considerable amounts of haemorrhage when mechanically removed. Ingested blood from the fish host was seen pulsating through the Lernaeocera sp. when viewed under the dissecting microscope. Scrapings of the fins and gills did not show any other parasites to be present.

At post mortem examination the spleen was enlarged and dark red brown. The liver appeared pale yellow, friable and fatty. The posterior kidney was also enlarged and dark brown in colour. The digestive tract was flaccid and 49 cm long but stomach and intestine were empty. No specific changes were seen grossly

in any of the other organs examined. Impression smears of the internal organs showed numerous intravascular schizonts resembling those of *H. simondi* as observed in the circulating blood. They were especially common in spleen, kidney and heart but were also found in the capillary spaces of other organs including the brain. Also the smears made of all *Lernaeocera* sp. and the *Hemibdella soleae* obtained from this fish revealed numerous merozoites and other developing stages of *Haemogregarina simondi*. These were described elsewhere.

The most interesting feature in the histopathology of the internal organs was the excessive haemopoiesis taking place in the kidney. However, no auxiliary haemopoietic tissue was detected in the heart and liver as is normally the case in teleosts with a longstanding anaemia. The spleen was characterized by large fibrinous deposits within the melano-macrophage centres and this was the case to a lesser extent in the liver. Large bean-or kidney shaped schizonts of *H. simondi* were seen in the heart, spleen, liver and kidney but they occurred exclusively intravascularly.

4.2.4. Transmission experiments

The attempted transmission of *H. simondi* to *Scophthalmus maximus*, *Pleuronectes platessa* and hatchery reared *Solea solea* both by parenteral and oral routes was unsuccessful. Also contact transmission between diseased *Solea solea* and apparently uninfected fish of the same or other species was not achieved.

5. DISCUSSION

5.1. Parasitaemia

5.1.1. Periodicity of parasitaemia

Periodicity with reference to haematoprotozoan parasites is determined by taking blood film at more or less regular intervals and recording the periods of absence of the parasite in the blood circulation of the host. Absence can be due to either an intervening period between inoculation of sporozoites and the appearance of the parasites in the blood as seen in the schizogonies of haemosporidian parasites (Garnham,1966) and in Theileria parva infection of cattle (Neitz,1957); a temporary disappearance of mature extracellular blood parasites from the peripheral circulation and their retreat to the capillary level of the internal organs as seen in Trypanosoma congolense (Losos,1972) and Ikede and a primarily restriction of blood parasites to intracellular sites as seen in T.brucei, T.rhodesiense and T.gambiense (Losos and Ikede,1972) or T.cruzi (Hoare,1972).

In the present study a periodicity of parasites was observed in the blood of *Scophthalmus maximus*. The fluctuation in blood levels of parasitaemias was very marked even in fish with similar clinical lesions. In only three specimens of *Scophthalmus maximus* was a total cycle between two negative periods observed. These were fishes No.0769; 0770; and HU 287 (Table 13). The cycle for fish No.0769 was apparently 50 days; the cycle for fish No.0770 was 42 days; and the cycle for fish No.HU 287 was 25 days. It thus appears that the average time interval between two negative periods or the approximate time in which a cycle takes place is 39 days(25-50).

These were rather limited observations and only show that there is indeed present a periodicity of the parasites as observed also in other haematoprotozoan parasites of fish and mammals. They were,however,no less limited than the observations by Laveran and Mesnil (1912) who experimentally infected members of the family *Esocidae* with blood containing trypanosomes and derived from the same family of fish. They observed a periodicity of the trypanosomes in the blood of their two infected fish. In fish A a cycle of approximately 44 days and in fish B a cycle of approximately 28 days were observed. It appeared that the average time in which a cycle took place was 36 days (28-44).

TABLE 13

PERIODICITY OF *HAEMOGREGARINA SACHAI* N.SP. IN *SCOPHTHALMUS MAXIMUS*

No.of fish:	0769	0770	0771	0772	0773	0774	0775
Date:							
Feb.9	-	-	++++	-	+++	+	+
Mar.3	+	++	-	++++	++++	+++	++
Mar.23	+	-	+	+	+	-	++++
Mar.31	-	+++	+++	++	-	-	died

No.of fish:	0776	F771	F773	F774	F779	F783
Date:						
Feb.9	+	++	+	+	++++	++
Mar.3	++	++++	+++	++	-	+
Mar.23	++++	++++	-	+	-	-
Mar.31	+	died	+	-	-	-

No.of fish:	HU224	HU 102		HU 287	HU 288
Date:			Date:		
Mar.8	+	-	May 5	-	-
Mar.14	-	-	May 9	+	+
Mar.23	-	+	May 12	++	+
			May 30	-	++

Periodicity has also been observed in other *Haemogregarinidae*. Hull and Camin (1960) showed a cyclical occurrence of *Haemogregarina* sp. in reptiles which was in the range of 6-8 days between peaks of parasitaemias.

5.1.2. Levels of parasitaemia

In the present study the levels of parasitaemia of the fish hosts varied in agreement with the periodic nature of the parasitaemia. In the case of *Haemogregarina simondi* from farmed *Solea solea* these levels ranged from 0.7-2.4% and in the case of *Haemogregarina sachai* n.sp. of farmed *Scophthalmus maximus* they ranged from 0.5-36.0%. This was in keeping with results obtained by other authors.

Marceau (1901) found usually only few gametocytes in blood smears of *Lacerta muralis* infected with *Karyolysus lacertarum* but observed parasitaemias with up to 17-20% in individual cases. Reichenow(1913) confirmed this by describing one individual *Lacerta muralis* where almost every single erythrocyte was parasitized. Also Hull and Camin(1960) found in one individual specimen of *Thamnophis marcianus* out of 154 examined an extremely high level of parasitaemia of 82.4% of all erythrocytes infected with *Haemogregarina* sp

Bergle (1971),however, found an average level of parasitaemia of only 0.1-1.5% in Lacerta sicula infected with Karyolysus sp. and rarely up to 4%. He suggested that this fluctuation of parasitaemia was seasonal and was very much dependent on the time of the year when blood was sampled. However, he did not find any indication of a true cyclical periodicity as observed in the present study. That indeed the taking of blood samples and the finding of different levels of parasitaemias is very much related to the time when these samples are taken has been shown with other haematoprotozoan parasites such as trypanosomes,where even a diurnal periodicity has been described (Hornby and Bailey,1931).

Such cyclical variations in evidence of infection make blood sampling of dubious value as a method for establishing the presence or absence of infection in any particular fish population.

5.2. Transmission experiments

Considering the large number of haematoprotozoan parasites that have been found to date in wild marine and freshwater fishes, very few authors have made an effort to study the pathogenicity of these parasites for their hosts and for other species of fish by means of transmission experiments. Where transmission studies have been attempted the usual technique employed has been the inoculation of parasite infected blood.

In the present study the repeated attempts to transmit *Haemogregarina sachai* n.sp. by inoculation of blood and lesion material or by contact to apparently uninfected *Scophthalmus maximus*, *Solea solea* and *Pleuronectes platessa* were unsuccessful.

Only two earlier studies were concerned with the pathogenicity of haemogregarines from marine fish. Kohl-Yakimoff and Yakimoff (1915) described a new haemogregarine (*Haemogregarina yakimowi-kohl*) from *Gobius capito*. To test if this haemogregarine was hostspecific for this fish they inoculated the following marine fish species intraperitoneally with the blood of the infected host: *Gobius paganellus*(3);

Blennius ocellaris(3); *Blennius trigloides*(1); *Sargus annularis*(3); *Motella tricirrata*(3); *Conger conger*(1); *Serranus cabrilla*(1); *Scyllium* stellare(1); and *Gobius capito*(2). Both of the *Gobius capito* (the original host for the parasite) were successfully infected, however the attempted transmission to the other fish species failed altogether even after repeated examination of the blood over extended periods of time. This is the only recorded successful blood transmission of a haemogregarine although it cannot be excluded that the apparently uninfected *Gobius capito* used in this experiment might have been latently infected and the haemogregarine might have shown a periodicity similar to the one observed in *H.sachai* n.sp. in the present study.Entirely negative results were also obtained by Kirmse (1975) who used various routes of inoculation in an attempt to transmit the haemogregarine of *Scophthalmus maximus* to apparently healthy fish of the same species, but then again a low level of infection in the apparently uninfected fish and undetected by routine blood smear examination could not be excluded.

Varying results were also obtained by Lebailly (1906) who studied the pathogenicity of trypanosomes of marine fish. He inoculated one *Anguilla anguilla*

being supposedly free of trypanosomes with a mixture of saline and blood of a *Limanda platessoides* heavily infected with *Trypanosoma limandae*. A frequent examination of the blood of this *Anguilla anguilla* to recover the trypanosomes was always negative. However, after 5-6 such examinations there suddenly appeared a very motile example of *Trypanosoma granulosum* var. *magna* (the trypanosome described from *Anguilla anguilla*). This appears very instructive and shows that one has to be extremely careful in declaring a fish in an experiment as free of trypanosomes or any haematoprotozoan parasites. Lebailly also inoculated citrated blood of *Anguilla anguilla* highly parasitized by trypanosomes both intraperitoneally and subcutaneously into *Conger conger* but with negative results. He also attempted to transmit by inoculation various trypanosomes such as *T. platessae*, *T. callionymi* and *T. granulosum* to *Conger conger*, *Pholis gunellus* and *Onos* sp. but was again unsuccessful.

Attempts to transmit trypanoplasms of marine fish to apparently healthy fish by inoculation of blood appear to have been more successful in the past. Strout (1965) detected these flagellates already

after 48 hours in the blood of apparently uninfected *Pseudopleuronectes americanus*, *Liopsetta putnami*, *Fundulus heteroclitus* and *Fundulus majalis* using the intraperitoneal route of inoculation which is considerably shorter than the 16-20 days it takes *Trypanoplasma borreli* to appear in the peripheral blood of freshwater fishes (Keysellitz,1906). However,when Strout attempted to transmit these marine trypanoplasms to various other fish species that were not the original hosts for these parasites such as *Carassius auratus*, *Salvelinus fontinalis*, *Mugil cephalus* and *Microgadus tomcod* he was unsuccessful.This again makes it doubtful if the apparently uninfected pleuronectids and cyprinodontids used by Strout were in fact free of parasites. The stress of handling and inoculating these fish might have induced a relapseof an already established low level infection.

5.3. Infection rates of intermediate and final hosts

5.3.1. Intermediate host

All of the Hemibdella soleae leeches examined from Solea solea contained developing stages of Haemogregarina simondi but in the Calliobdella nodulifera leeches haemogregarines were rarely observed and a seasonal fluctuation was apparent in trypanosomes which appeared to be completely absent from the alimentary tract during winter but accounted for an infection rate of as high as 45.8% in mid summer.

Observations in other Haemogregarinidae differed widely. The infection rate of Glossina palpalis infected with Hepatozoon pettiti was 0.9% (Chatton and Roubaud,1913) and only 2% of the Culex fatigans infected with Hepatozoon breinli and Hepatozoon mesnili,respectively, were found by Mackerras (1962).Also Bergle(1971) found only 5% of the vector Ophionyssus lacertinus infected with Karyolysus sp. However, Brumpt(1938) found 9.5% females and 47.9% males of Hyalomma syriacum infected with Hepatozoon mauritanicum and Robin(1936) reported even an infection rate of 96% in Culex fatigans infected with Hepatozoon mesnili.

5.3.2. Final host

The infection rate of Solea solea could not be well established because of the value of the fish and the reluctance of the farmer to have them examined but it appeared to be rather high. Certainly all of the fish which were available for examination were infected with H.simondi.In the case of wild Solea solea examined in France the infection rate varied from 27.8-50.0%.

In the Scophthalmus maximus the infection rate with H.sachai n.sp. appeared to be very low never reaching a level greater than 1%. No infection was detected in wild fish of the same species.

Varying degrees of infection rates with haemogregarines of marine fish were also observed by others (Table 5). Low infection rates were found by Laird(1952) with 0.3% of Hoplichthys haswelli infected with H.hoplichthys and 0.15% of Leptoscopus macropygus infected with H.leptoscopi. Noble(1957) observed 1.1% of Microstomus kitt infected with H.platessa but an infection rate as high as 70% in Callionymus lyra infected with H.quadrigemina Extremely high infection rates were also obtained by Saunders(1960) who observed 50-75% infection with H.bigemina in the families Acanthuridae and Scaridae from the Red Sea.

In addition Noble(1957) and Laird(1952) made the observation that young fish had the highest rates of infection. This was fully in agreement with the findings in the present study where the highest rate of infection of <u>Scophthalmus maximus</u> was also in the group 0-1.

The infection rate of the final host may be dependent on the nature of the biotope. Lebailly (1904) noticed high infection rates in fish that live on sand or on the bottom of the sea. Neumann (1909) also found a high percentage of bottom living fish species infected and believed this to be an indication of the importance of the environment.

Higher water temperatures may also facilitate the multiplication of parasites and increase the infection rate of the final host. The warm effluent water employed at Hunterston to boost the growth rate of <u>Scophthalmus maximus</u> and <u>Solea solea</u> might have influenced the propagation of <u>H.sachai</u> n.sp. and <u>H.simondi</u>. It is already well established that high temperatures may speed up the rate of sporogony in haemosporidian parasites (Garnham,1966). Such an effect would also help to explain the high infection rates in the Red Sea observed by Saunders (1960).

There appears also a seasonal variation in infection rates.Noble (1957) found very heavy infection of *Callionymus lyra* with *H.quadrigemina* in summer (24%) and only light infection in winter (7%). Similarly Laird and Morgan (1973) observed an infection rate of 23% in *Trinectes maculatus* infected with *H.platessae* in summer but no infection when the same fish species was examined in winter at the same location. They suggested either a seasonality of infection or a higher susceptibility of the fish in summer because of spawning stress but a simple effect of high temperature on sporogony is just as acceptable in the absence of detailed information.

Such a seasonal variation in parasitism might well depend on the presence or absence of a vector for these parasites as had been suggested already by Neumann(1909) and Noble(1957) and in addition there might also be individual differences in susceptibility between species and even individual fish.

5.4. Pathogenicity of haemogregarines as observed in aquaculture

5.4.1. Effects of the parasites at the cellular level

In *Haemogregarina sachai* n.sp. a mechanical hypertrophy was observed in cells of the leucocyte series of infected *Scophthalmus maximus*. All infected cells were enlarged according to the number of individual parasites they harboured. They were always enlarged by at least one third over the size of uninfected cells (Table 14).Infected erythroblasts,which were occasionally found also infected measured up to 10.4 by 9.7 micron as compared with the measurements of normal cells (average 7.3 by 7.2 micron).Erythrocytes, however, seemed to maintain their normal dimensions whether they were parasitized or not. The hypertrophy appeared most pronounced in monocytes,which normally averaged 9.9 by 9.3 micron,but holding several individual parasites,they enlarged to 13.0 by 12.3 micron. When multiplication of the parasites in these cells ensued, intracellular schizonts with up to 36 or more nuclei would hypertrophy the host cell to such a degree that the cells would rupture and release the organisms. Even in those greatly enlarged cells the nucleus was apparently not fragmented although often distorted and greatly enlarged.

In *Haemogregarina simondi* blood cell enlargement was mainly seen in the large lymphocytes,neutrophils and monocytes of the leucocyte series,being most pronounced in the case of infected large lymphocytes and neutrophils (Table 15). Here only the erythrocytes and not the erythroblasts were found infected. These reached an average size of 22.1 by 11.7 micron when containing large schizonts with up to 8 individual parasites as compared with the size of normal erythrocytes of *Solea solea* (12.6 by 6.9 micron). Also here the nucleus of the host cell appeared to be either greatly enlarged or took on an irregular morphology so that the origin of the host cell became difficult to determine. Fragmentation of the nucleus was rarely observed in the present study (Fig.36).

Marked degenerative changes of infected erythrocytes and hypertrophy with degeneration of host cell cytoplasm and host cell nucleus were observed also in other marine fish parasitized by haemogregarines (Henry,1913; Laird,1952;Saunders,1955;and So,1971). However, some authors did not find any changes in haemogregarine infected erythrocytes and their nuclei (Saunders,1958; Laird and Morgan,1973). Not much is known about the pathogenic action of haemogregarines on leucocytes. Laird(1953) describing the life cycle of *H.bigemina*

TABLE 14

SIZE DIFFERENCES BETWEEN NORMAL AND HAEMOGREGARINE-INFECTED BLOOD CELLS OF *SCOPHTHALMUS MAXIMUS* (L.)

	normal cell size	infected cell size
	Small Lymphocytes	
L	3.7 (3.6-3.9)	5.8 (5.7-5.9)
W	3.6 (3.3-3.9)	5.5 (5.2-5.8)
	Large Lymphocytes	
L	6.1 (5.9-6.5)	7.9 (6.5-8.8)
W	5.5 (5.2-6.2)	6.6 (6.5-7.1)
	Neutrophils	
L	9.1 (8.0-9.5)	11.5 (10.4-11.7)
W	8.2 (7.8-8.5)	10.1 (9.1-11.0)
	Monocytes	
L	9.9 (9.1-10.4)	12.4 (11.2-13.0)
W	9.3 (9.1-9.75)	11.5 (9.8-12.3)
	Erythroblasts	
L	7.3 (6.5-7.8)	9.0 (8.4-10.4)
W	7.2 (6.4-7.8)	8.7 (7.1-9.7)
	Erythrocytes	
L	11.7 (10.5-15.0)	11.7 (10.5-14.5)
W	6.5 (6.2-8.0)	6.6 (6.4-7.8)

L = length (range)
W = width (range)

TABLE 15

SIZE DIFFERENCES BETWEEN NORMAL AND HAEMOGREGARINE-INFECTED BLOOD CELLS OF *SOLEA SOLEA* (L.)

	normal cell size	infected cell size
	Small Lymphocytes	
L	4.1 (3.9-4.5)	not found infected
W	3.9 (3.9-4.1)	
	Large Lymphocytes	
L	7.8 (6.5-9.1)	12.3 (11.8-12.5)
W	7.3 (6.5-8.5)	9.4 (8.75-10.0)
	Neutrophils	
L	10.4 (9.1-11.7)	15.9 (13.0-19.5)
W	9.9 (7.8-11.7)	7.5 (5.2-10.4)
	Monocytes	
L	14.9 (13.0-19.5)	16.5 (14.9-19.8)
W	11.1 (9.1-14.3)	9.1 (6.5-9.8)
	Erythroblasts	
L	12.8 (12.5-13.5)	not found infected
W	12.8 (12.0-13.3)	
	Erythrocytes	
L	12.6 (11.7-13.0)	19.1 (16.9-22.1)
W	6.9 (6.5-7.8)	9.3 (5.8-11.7)

L = average length (range)
W = average width (range)

found that the nucleus of the host cell was usually indented by the parasite and especially in larger leucocytes he found that the haemogregarine penetrated the host cell nucleus and became completely surrounded. Saunders (1955) describing intraleucocytic stages of *H.bigemina* in *Menticirrhus littoralis* only rarely observed indentation of the host cell nucleus. This is in contrast to the observations made in the present study on farmed marine fishes.

A more or less significant hypertrophy of the host erythrocyte as seen in the present study in *H.simondi* parasitizing *Solea solea*, but not observed in case of the intraerythrocytic stages of *H.sachai* n.sp.,has been reported also by Labbé(1894),Breindl (1914) and Ball (1958) for other Haemogregarinidae. In addition, Ball, Chao and Telford (1967) found the erythrocytes of *Drymarchon corais* parasitized by *Hepatozoon rarefaciens* quite distinctly enlarged to 34 by 15 micron as compared with their normal size of 17 by 10 micron. However, Marceau(1901) found the erythrocytes of *Lacerta muralis* only marginally enlarged when they were parasitized by *Karyolysus lacertarum* and Reichenow(1912) considered the parasite to be responsible for pathogenic action only in termsof mechanical activity,which resulted in a morphological change of the host cell due to the movements of the intracellular organism. This was later confirmed by Bergle(1971) in the case of *Karyolysus* sp.

from *Lacerta sicula*. Also the changes seen in the present study in leucocytes and erythrocytes of *Solea solea* and *Scophthalmus maximus* parasitized by haemogregarines are considered the result of a purely mechanical action of these parasites.

5.4.2. Changes in blood parameters associated with haemogregarine infection

Although studies on the haematology of fish in relation to disease have been given greater prominence of late (Mulcahy,1975) there are still only a few publications on blood parameters of healthy and diseased fish. In addition variations between individual fish and between species have made interpretation of results somewhat hazardous.

In the present study a marked anaemia was observed in specimens of *Scophthalmus maximus* and *Solea solea* parasitized by haemogregarines. However, in *Solea solea* this condition was no doubt highly influenced also by the presence of large numbers of the ectoparasitic bloodsucking copepod *Lernaeocera* sp.

In both infected fish species there was a considerable decrease in circulating erythrocytes as compared to controls. Such an erythrocytopenia was also observed

in other diseases of marine fish such as in vibriosis of various pleuronectids and *Gadus morhua* (Anderson and Conroy, 1970) and in lymphoma of *Esox lucius* (Mulcahy,1975).

In addition,in *Scophthalmus maximus* deformed erythrocyte nuclei were frequently encountered with anisocytosis and poikilocytosis as described in earlier investigations of the same problem (Kirmse,1975). This has been also observed in *Oncorhynchus kisutch* fed a folic acid deficient diet (Smith,1968).An avitaminosis of vitamin B has also been observed in leishmaniasis and it has been suggested that B vitamins may be essential to multiplication of the parasite and thus proliferation of the parasite may induce depletion of vitamins that would otherwise be available for the host (Sen Gupta *et al*.1952).

In *Solea solea* infected with *H.simondi* the blood showed also a marked increase of progranulocytes and erythroblasts indicating an active haemopoiesis to cope with the anaemic condition. This was confirmed histologically by the changes observed in kidney,spleen and liver.

Changes in leucocytes were more pronounced than those seen in erythrocytes. In infected Scophthalmus maximus there was an especially marked increase in neutrophils(from 0.7-15.0%) but there was also an increase in monocytes (from 0.3-6.8%) and erythroblasts (0.2-4.0%). The mean total numbers of all leucocytes including thrombocytes increased from 65,615 to 320,940 per mm^3.In infected Solea solea the increase of leucocytes was also quite pronounced and they increased from 31,150 to 113,401. Similar changes although less significant were also seen in Esox lucius with lymphoma where an increase from 111,500 to 132,000 leucocytes per mm^3 was observed (Mulcahy,1975). A considerable drop in the number of lymphocytes was also noticed as seen in the present study in infected Scophthalmus maximus (1.6 to 0.7%).

The only other investigations that have been conducted on the parameters of healthy and haemogregarine infected marine fish were the rather limited tests by Saunders (1966). She found an increase in the number of erythrocytes from 4,130,000 per mm^3 in apparently uninfected Lutjanus apodus to 6,420,000 per mm^3 in a single specimen of the same species which was infected with H.bigemina.

More studies on the parameters of marine fish comparing healthy and diseased fish of the same species are indicated to assist in the epizootiology of diseases.

5.4.3. Pathogenic action of haemogregarines on clinical level

Ferguson and Roberts (1975) first described the clinical pathology and histopathology of the proliferative condition in *Scophthalmus maximus* now known to be associated with *Haemogregarina sachai* n.sp. More extensive investigations into the nature of this disease were carried out by Kirmse(1975) who gave a detailed description of the pathology and histopathology and discussed the clinical symptoms in comparison with observations made on similar disease manifestations such as toxoplasmosis,leishmaniasis and theileriosis of higher animals.It was concluded that the disease might well result from immunological unresponsiveness of the fish host.

Such a delay or depression of the primary immunological response to the parasite (immunological paralysis) and the unusual rapid multiplication of the parasite might have been caused by high levels of parasitic antigens which the individual fish could not cope with or stresses associated with the husbandry system. Normally in wild fish only low levels of infection with haemogregarines have been noted and no clinical disease manifestations were observed, thus there

appears to have been a slow and balanced adaptation of parasite to host. However, high levels of parasites and clinical disease have often been observed when parasites are transmitted to an aberrant host. In *Theileria parva* infection of cattle, wild bovines are considered to be the natural hosts for the parasite showing only low level infections without clinical manifestations. When the same parasite is transmitted to introduced domesticated bovines the parasite multiplies so rapidly that the host defence breaks down and the animal dies. Similar observations have been made with *Leucocytozoon simondi* endemic in local birds in subarctic Canada. When domestic geese were introduced into the area they were killed by this parasite(Laird and Bennett,1970).Another aberrant host for *Leucocytozoon* sp. appear to be psittacine birds (Walker and Garnham,1972).

The question as to whether *Scophthalmus maximus* is infected in nature or not remains open. Since it was only in individual cases that the disease occurred one can assume that the transmission also took place only accidentally and that the parasite has not been adapted to the host.This was in contrast to the situation in *Solea solea* wh ere a frequent re-infection was assured by the presence of the intermediate host *Hemibdella soleae*.

5.5. Economic importance of haemogregarines in marine flatfish in aquaculture

In the case of the proliferative condition of *Scophthalmus maximus* farmed at Fitch Lovell and B.O.C. and caused by *Haemogregarina sachai* n.sp. both companies agreed that the disease was of no economic importance to them as long as the losses remained in the range of 1%. However, the disease would become significant in case of higher morbidity and mortality.

In the case of farmed *Solea solea* parasitized by *Haemogregarina simondi* at the White Fish Authority, no apparent economic impact was observed because this fish farm considered the infestation with *Lernaeocera* sp. and the resulting anaemic condition as only a temporary problem. The losses occurred only seasonally and were limited to the brood stock. Ectoparasites appeared to be controlled momentarily when the fish were treated with insecticides. Thus when compared with the much greater loss of 130 *Solea solea* during transportation to Hunterston with the value of one brood stock fish given as approximately 25 pound sterling on the basis of scarcity value, the economic importance of the multiple infestation of *Solea solea* with various ectoparasites and *H.simondi* resulting in

the death of two fish only seemed for them negligible.

However, such assessments based solely on mortality and failing to consider the debilitating effects such haematoprotozoan parasites can have appears not to be a very effective way of approaching this situation. Apart from the effects it can have for the host such as poor growth rates and higher susceptibility to infections with other agents such as bacteria and viruses, such infections with haematoprotozoan parasites under aquaculture conditions may also have an impact on spawning of brood stock and can result in production of poor eggs and offspring. The resistance of fish in an aquaculture system is anyway lowered because of the many stress factors involved and in less favourable conditions of husbandry and management the percentage of diseased and unmarketable fish might soon rise to a level where it can become of quite important economical consequence.McVicar and MacKenzie (1977) have pointed out,that the crowding and high temperature effects in a marine fish monoculture system as seen in Hunterston can facilitate reproduction and transmission rate of parasites. This will be even further increased if it is possible for the intermediate host to obtain optimal conditions to live and reproduce somewhere within the same aquaculture system.

Although it appears easier to control parasites with multiple-host life cycles than parasites with single-host life cycles, the fish farms involved in this study seemed unaware that such control measurements -could well be undertaken and thus would prevent further economic losses through haematoprotozoan parasites,such as haemogregarines,under aquaculture conditions.

5.6. Possible control of haemogregarine infection in aquaculture

Since it was shown that in the case of _Solea solea_ the vector for _Haemogregarina simondi_,i.e.the marine leech _Hemibdella soleae_, was present in large numbers throughout the year in the same tanks as the fish hosts it is postulated that control of vectors would be advantageous. The same may also apply to the situation in _Scophthalmus maximus_, although no vector has as yet been established for _Haemogregarina sachai_ n.sp., but from the survey of the holding tanks on the affected farms for other marine animals which included a number of potential vectors for haemogregarines it is also evident that vector control is necessary. This would also be a feasible approach since many ectoparasites can be exterminated by regularly cleaning of the tanks and their disinfection but it might also be an advantage to secure the screening of the inflow of seawater by installing proper devices such as irradiation systems to keep out all marine life including plants so that new vectors cannot establish themselves. Another more direct approach would be a mechanical removal of ectoparasites which have not responded to therapeutic treatment when fish are graded. It is also suggested that various drugs which are already well established as controls of ectoparasites and haematoprotozoans of animals and birds be tried under aquaculture conditions.

The therapeutic treatments used in a pilot trial in the present study should be used on a larger scale and in a controlled environment, possibly at a laboratory, before they are examined under aquaculture conditions, taking in account the numerous variables such as salinity,temperature, ion levels, diet and different stress factors. In addition a reduction of the parasite load can well be achieved by using good husbandry standards including a balanced diet and the avoidance of overcrowding and handling stress.

S E C T I O N D

THE COMPARATIVE ULTRASTRUCTURE OF SOME OF THE HAEMATOPROTOZOAN PARASITES OF MARINE FISHES

1. INTRODUCTION

During the last decades ultrastructural research has brought an immense volume of new and valuable information to the field of protozoology. Especially a large amount of work has been performed on the detailed structure of the Sporozoa and related organisms. A number of excellent publications have elucidated the ultrastructure of the Eimeria (Scholtyseck and Piekarski, 1965); Toxoplasma (Scholtyseck, Mehlhorn and Müller, 1973); Leucocytozoon (Desser, 1973); Plasmodium (Garnham, Bird and Baker, 1963); Haemoproteus (Sterling and De Giusti, 1972); Babesia (Friedhoff, Scholtyseck and Weber, 1972); and Theileria (Büttner, 1967). The great variety of described organelles and their fine structures not only brought more depth to the understanding of these protozoan organisms but also set new dimensions for the modern systematics and taxonomy of the Sporozoa. Levine (1970) on the basis of ultrastructure grouped together the "classical" taxon Sporozoa with the only recently characterized Toxoplasmea and the Piroplasmea under the name Apicomplexa.

The name apical complex, first employed by Senaud (1967), describes a number of fine structured elements, i.e. polar-ring, micronemes, rhoptries, subpellicular microtubules and generally a conoid. All of these organelles need not be present at the same time but they have to appear at least once during the various stages of development. Levine (1971) has also resolved the differences and the various synonyms for the variously described organelles and has defined and standardized the nomenclature of the Apicomplexa. For examples the names "paired organelle"; "toxoneme";"lankesterelloneme"; "eimerianeme" and "dense body" were synonyms for the now generally used term "rhoptrie" coined by Sénaud (1967). In the present study Levine's terminology is generally adopted with only limited number of exceptions, i.e. "refractile body" instead of "clear globule".

The ultrastructure of marine Sporozoa was not investigated until quite recently. Heller (1969) described the ultrastructure of merozoites, macrogametes and spores of <u>Aggregata eberthi</u>, a sporozoan parasite from the intestine of <u>Sepia officinalis</u>. Ferguson and Roberts (1975) while investigating a "myeloid leucosis" infection in cultured turbot found that the lesions of these fish when observed under the electronmicroscope contained sporozoan parasites. Their ultrastructure showed a few organelles which could be identified as micronemes

and subpellicular microtubules and the authors suggested that this sporozoan might belong to the Coccidia and possible could be placed within the Toxoplasmatinae. Kirmse (1975) in a subsequent study on the ultrastructure of the merozoite stage of this parasite was able to group it definitely among the Apicomplexa because of the presence of an apical complex and other typical organelles. Although there appeared a discrepancy in the number of microtubules when compared with the fine structure of merozoites of toxoplasma-like organisms such as *Toxoplasma* sp., *Sarcocystis* sp., *Frenkelia* sp. and *Besnoitia* sp., all members of the Apicomplexa. there was indeed a remarkable similarity with the Toxoplasmatinae. A relationship to the *Haemogregarina* which was observed during the lightmicroscopical investigations could not be further elucidated because of the lack of comparative material and an obvious gap in the ultrastructural research of the haemogregarines. Buchanan (1977) described recently two sporozoan parasites and their ultrastructure from the marine bivalve *Tellina tenuis*. These parasites are also members of the Apicomplexa and belong to the Microsporoidea. The ultrastructure of marine haemoflagellates is limited to culture forms of *Trypanosoma rajae* from the elasmobranch *Raja clavata* (Brooker and Preston, 1967).

The discovery of quantities of haematoprotozoan parasites during the present investigations not only in marine teleost fish but also in marine leeches made it possible to study the ultrastructure of these parasites for the first time in greater detail and compare their fine structure with other members of the Apicomplexa. Since the parasite from the turbot (*Scophthalmus maximus*), now *Haemogregarina sachai* n.sp. was the first haemogregarine from the marine environment to have its ultrastructure described in detail (Kirmse, 1975), the results of this first description are included here for reasons of continuity.

2. REVIEW OF THE LITERATURE

2.1. Ultrastructure of Haemogregarines

Most species of haemogregarines have been studied through the medium of blood smears alone and comparative little is known about their life cycles. Ultrastructural investigations of haemogregarines have only been started quite recently. The first description of the fine structure of a Haemogregarina sp. was by Stehbens and Johnston (1967). They studied sections of the blood of the gecko Gehyra variegata containing intraerythrocytic stages of the parasite and described various organelles such as the conoid, micropores, microtubules and micronemes which are considered characteristic for the Sporozoa. Around the same time Baker and Lainson (1967) described the ultrastructure of the gametocytes of an unidentified haemogregarine from the blood of the frog Rana montezuma. Numerous ribosomes were found in the parasite's cytoplasm but no endoplasmic reticulum was apparent. A conical anterior end with two concentric rings was suggestive of a penetrative mechanism. Though schizonts were encountered frequently in smears of heart and kidney,

these were not included in the ultrastructural investigations. Stehbens and Johnston (1968) continued their studies of the fine structure of *Haemogregarina* sp. from the gecko and found "cystic bodies" in the lung which had been described earlier in the lightmicroscope by Mackerras (1962) as stages of *H.heteronotae* in the lung of the lizard *Heteronota binoei*. The ultrastructure of the enclosed parasite resembled that of other Sporozoa with regard to differentiated organelles. The schizonts observed by light microscopy were not found and were assumed to have been lost from the parasitophorous vacuole during processing for electronmicroscopy. The "cystic bodies" were considered to be dormant stages or perhaps the earliest form of a schizont. Desser and Weller (1973) described the ultrastructure of intra-and extracellular gametocytes of *Haemogregarina* sp. from the frog *Rana berlandieri*. The pronounced pellicular folds they found in extracellular gametocytes were thought to represent the peristaltic waves of constriction passing from anterior to posterior pole of the parasite along the body. These had been observed by phase contrast in light microscopy of motile parasites. All of the common organelles were seen except the rhoptries. Bergle (1974) described the ultrastructure of another haemogregarine, a *Karyolysus* sp. from the lizard

Lacerta sicula bagnolensis. The intraerythrocytic gametocytes from the blood showed structural variation according to age. In addition so-called "activated" gametocytes were described from the intestine of Rhodnius prolixus which had fed experimentally on infected lizards. They were characterized by a wealth of ultrastructural details and the presence of a "refractile body". Schizonts were found within phagocytic cells of the capillary spaces of the liver but not in hepatocytes. Mature schizonts had an average of 15-25 merozoites in cross sections. Macroschizonts with distinctly fewer merozoites were seen only rarely. The merozoites showed the same typical structural elements as the blood gametocytes. In addition so-called "trophozoites" which had not been observed by light microscopy were found in the liver. These also were found solely within the phagocytic reticulo-endothelial cells in the capillaries. The author assumed that these developing stages of the parasite might be sporozoites which had only recently invaded the final host. Kirmse (1975) described the ultrastructure of the merozoites of an unidentified haematoprotozoan parasite from "neoplastic" lesions of Scophthalmus maximus, a marine flatfish. Most of the parasites were found intracellularly in cells of the macrophage series and exhibited the characteristic organelles of the Sporozoa (Apicomplexa). They were found inside a

parasitophorous vacuole in the host cell cytoplasm either singly, or from two to five, and as such were considered immature schizonts. The merozoites of the turbot haematoprotozoan parasite in question when compared with similar stages of other sporozoans, resembled closely the merozoite of Toxoplasma gondii, with the exception of thirty-one microtubules instead of twenty-two; and two micropores instead of one. However, a suspected relationship to the Haemogregarina could not be elucidated further because of the lack of other haemogregarine material for comparison.

2.2. Ultrastructure of Trypanosomes

The ultrastructure of trypanosomes has been thoroughly described because of their importance as disease agents in higher animals. However, most reports on the fine structure are concerned with pathogenic salivarian trypanosomes. The presence of microtubules constituting a deformable corset maintaining the elongate shape of the body was first demonstrated by Schulz and MacClure (1961). One of the most striking structural features,the undulating membrane formed by the flagellum running along the pellicle, was shown as being composed of two parallel rod-like structures only one of which

had its origin in the basal body (Ray et al.1955). The kinetoplast was described by Clark and Wallace (1960) as an apparently modified mitochondrion and was unique as a cytoplasmic structure in containing high quantities of DNA. A comparison of the fine structure of blood and vector forms of Trypanosoma brucei by Vickerman (1962) showed that the flagellum was bound by a coated membrane of similar appearance to the pellicular membrane. The ultrastructure of the surface coat and the flagellar adhesion of trypanosomes was demonstrated by Vickerman (1969).

There have been few ultrastructural studies only of trypanosomes from lower vertebrates. The fine structure of the amphibian haemoflagellate Trypanosoma mega was demonstrated by Steinert and Novikoff (1960). They also showed the presence of a cytostome in the cultured form of these trypanosomes and its relation to endocytosis. A cytostome was also observed in Trypanosoma conorrhini by Brooker and Preston (1967).

The only report on the ultrastructure of a trypanosome from the marine environment is from the elasmobranch Raja clavata infected with Trypanosoma rajae (Brooker and Preston, 1967; Preston,1969). They found a distinct cytostome just as in the other trypanosomes of lower

vertebrates. By contrast, this organelle has not been observed in trypanosomes of higher animals. The presence and function of a prominent cytopharyngeal complex as a constant feature of the epimastigote and trypomastigote forms of this cultured trypanosome were also described.

3. MATERIAL AND METHODS

Tissues from lesions of diseased turbot Scophthalmus maximus, organ tissues from parasitized turbot and sole Solea solea, whole blood of turbot and sole with high parasitaemias as well as the leeches Calliobdella nodulifera and Hemibdella soleae and the ectoparasitic copepod Lernaeocera sp. were prepared for electron microscopy.

3.1. Embedding of Tissues

The tissues from the organs of all fish and the proliferative lesions from the diseased turbot were fixed as blocks of 1 mm^3 in a 2.5% solution of glutaraldehyde in 0.05 M Sym-Collidine buffer of pH 7.2 for a minimum of two hours at room temperature. The tissues were then rinsed in 4-6 changes of Sym-Collidine buffer solution, drip dried on blotting paper and fixed for one additional hour at 4° C in 1% osmium tetroxide. After osmication the tissues were dehydrated in graded series of alcohols. First in 50% with two changes of 15 minutes, next in 70% with two changes

of 15 minutes, then in 80% with two changes of 15 minutes, then in 90% with two changes of 15 minutes and finally in 100% alcohol for four changes of 15 minutes each. The next step consisted of immersing the tissues in a mixture of propylene oxide and Araldite/Epon resin 75%-25% for one hour; followed by a mixture of 50%-50% for one hour and finally in 100% resin overnight. The tissues were then embedded in blocks and covered with 100% resin and cured in an oven at 60° C for twenty-four hours.

3.2. Embedding of Blood

Immediately after withdrawal from the renal portal vein, renal artery or heart, the blood of the turbot or sole was immersed directly in 2.5% glutaraldehyde in 0.05 M Sym-Collidine buffer of pH 7.2 in the field and stored in small Eppendorf vials, i.e. centrifuge vials destined for holding serum samples of maximal 2 ml. After returning from the field these vials were refrigerated for a short time at 4° C. The blood pellet was then resuspended and centrifuged twice for 15 minutes each at 2.200 rpm. The supernatant glutaraldehyde was poured off and the sample was centrifuged twice with 0.05 M Sym-Collidine buffer. After the supernatant buffer was discarded the blood pellet was centrifuged again with 1% osmium tetroxide

and the sample was then kept for one hour at 4° C. Dehydration was carried out in a graded series of alcohols as in 3.1 above. Each sample was centrifuged with the respective alcohol dilution and the supernatant discarded. After the final rinse in 100% alcohol the plastic vials were opened with scissors and the pellet was dropped into a bijoux bottle for further propylene oxide / resin mixture processing. Since certain difficulties were experienced with these blood samples concerning their consistency a different approach was addopted to hold the blood pellet together. After the blood pellet had been treated with glutaraldehyde centrifuged and rinsed in buffer, the supernatant excess fluid was discarded and liquefied 2% ion-agar was poured on top of the blood pellet. After the agar had gelled the vials were opened and the blood pellet could be removed as a whole and immersed in 1% osmium tetroxide solution. The blood was then further treated like an organ tissue as outlined in 3.1.

3.3. Embedding of Ectoparasites

The two species of marine leeches were sectioned into 3 parts (anterior, central and posterior) or left _in toto_. Processing was conducted as described for organ tissues. An attempt was also made to dissect

the leeches and remove pieces of the crop or the whole intestinal tract for immersion into glutaraldehyde and then to proceed as described before for the blood samples. The ectoparasitic copepod was immersed as a whole into glutaraldehyde. After osmication it was sliced into appropriate sized blocks for further treatment as described for the tissues.

3.4. Section Cutting

Sections were cut first at 2 micron thick from resin embedded blocks of tissues either on a LKB 11800 Pyramitome or on a Huxley hand microtome using glass knives prepared on a LKB Knife Maker. These thick sections were stained with AMB-stain (1% Toluidine blue) and viewed under a binocular microscope. This allowed rapid location of the desired area for further ultra-thin sections. The blocks were trimmed accordingly with a razor blade and then ultra-thin sections were cut with glass knives on either a ReichertOMU-3 (Austria) ultramicrotome or a LKB Ultrotome III 8800 (Sweden) until the silvery color of the sections indicated an approximate range of 60-90 millimicron. The sections were then removed with copper grids which were stained with uranyl acetate and lead citrate or in a combined 1% potassium permanganate / 1% uranyl acetate stain

in double-distilled water, a process which provides enhanced contrast. The sections were viewed either in an AEI Corinth electron microscope or a Carl Zeiss EM 9 S-2 electron microscope.

4. RESULTS

4.1. The Ultrastructure of *Haemogregarina sachai* n.sp. from *Scophthalmus maximus*

4.1.1. The Merozoites

Mostly intracellular and only occasionally free parasites were observed in ultra-thin sections of lesion material, spleen and blood. Intraerythrocytic merozoites were seen under the light microscope in a few cases of infected turbot but it was difficult to detect merozoites in erythrocytes under the electron microscope. What appeared as an invading parasite penetrating into an erythrocyte from a blood vessel in the spleen showed such a poor structural differentiation that no detailed description of its fine structure could be given here (Fig.92). Observations are therefore limited in this study to the ultrastructure of intraleucocytic merozoites. The earliest stages encountered in the circulating blood were single parasites inside lymphocytes (Fig.93,94) or neutrophils (Fig.95). The lymphocytes are usually characterized by a large nucleus which is often

indented and surrounded by a thin rim of cytoplasm containing numerous large mitochondria. The plicated cell membrane shows occasionally small pseudopodia. The nucleus of the neutrophils is irregular and often multilobed so that sometimes two or three nuclei appear to be present in a single cell in cross section. The nuclear chromatin is dense and numerous ovoid granules of fibrillar appearance are found in the cytoplasm. The merozoites observed were found lying inside the host cell in a single parasitophorous vacuole (PV) which was not always very distinct (Fig.94,97,98,101). Characteristic for these early stages of development were the large numbers of ovoid or irregularily rounded vacuoles (V) in close proximity to eachother (Fig.98,101). They were located with preference in the middle of the parasite and appeared sometimes surrounded by thin membranes. The merozoites (ME) found in leucocytes as neutrophils (NE) and lymphocytes (LY) were seen in various longitudinal, diagonal and transverse sections and had the following characteristics. Their pellicle seemed to be composed of two unit membranes, each of them composed of a dark and highly osmiophilic layer and a less electron dense and lighter layer. Thus the outer membrane (OM) measured 0.028 micron in width and the inner membrane (IM) measured 0.017 micron. A distinct apical complex was observed at the anterior

end of the parasite consisting of a polar ring (P) and a conoid (C) apparently pushing at times through the polar ring (Fig.129). The inner diameter of the polar ring measured approximately 0.25-0.35 micron and its strength was 0.06 micron. In transverse sections through the apical complex of a merozoite about 6-8 club-shaped structures were seen lying in the cytoplasm and apparently originating from inside the polar ring and conoid (Fig.96,129. They measured an average of 0.07 micron in width at their necks or ductules (DRH) and 0.16 micron in width through the tubular posterior and dense osmiophilic part. Their length was in the range of 0.9-1.1 micron and they were considered to be so-called "paired organelles" or rhoptries (RH). The parasite seemed to be covered with a net of subpellicular microtubules (MT) which also originated at the polar ring and extended over the whole surface of the merozoite towards the posterior pole. In tangential sections near the apical complex they appeared as distinct parallel rods with a distance of approximately 0.125-0.15 micron between eachother (Fig.97). A total number of 31 micro-tubules was counted and this result was confirmed in a cross-section of a merozoite where these organelles appeared in form of osmiophilic concentric rings with a less electron dense centre of 0.009 micron in diameter (Fig. 98. Micronemes (MN) were also found in abundance

and close to the surface of the parasite so that in cross section often a dense ring of micronemes appeared at the periphery (Fig.98). The number of micronemes was in excess of 60 and they measured approximately 0.08 micron in diameter. Several rounded or oval-shaped structures with double outer membrane and vesicular interior were seen in the cytoplasm. They were considered to be mitochondria (MI) having well developed tubular cristi. In addition a Golgi apparatus (GO) was observed together with numerous profiles of rough endoplasmic reticulum (ER). Ribosomes (RI) were scattered throughout the cytoplasm. A well defined nucleus (N) was seen lying in the centre of the parasite with a double nuclear membrane (NM) interrupted by several nuclear pores (NP). A spherical electron dense nucleolus (NU) was present in most cases (Fig101,102).The outer membrane of the merozoite was occasionally seen intensively folded (Fig.98). Up to two micropores (MP) were seen in individual merozoites (Fig.99). At the micropore the inner membrane of the pellicle appeared to be interrupted whereas the outer membrane was invaginated (Fig.100). The depth of the micropore was measured as 0.1 micron, the inner diameter was 0.05 micron and the outer diameter in the range of 0.12-0.13 micron. Division by binary fission was observed in an early merozoite lying in a single parasitophorous vacuole (Fig101).

This stage of development was characterized by numerous large and thin-membrane surrounded vacuoles, micronemes and several tubular mitochondria. The result of such a division,i.e. the presence of two merozoites inside a single parasitophorous vacuole and by some authors considered an early schizont was observed also (Fig.102). The intraleucocytic merozoites described here measured approximately 3.2 micron in width as judged from several cross sections. Their length from longitudinal sections appeared in the range of 4.41-5.67 micron..

4.1.2. The Schizonts

Multinucleate schizonts (SCH), as seen under the light microscope, were found in all types of leucocytic cells. The host cell did not appear to be altered morphologically. The parasites were seen in pairs (Fig.103), threes and fours and up to six within a single parasitophorous vacuole. These schizonts measured from 4.0 by 5.0 micron to 7.35 by 13.6 micron. No residual body was encountered. In the spleen sometimes single schizonts filled with apparently degenerated merozoites and cell debris were observed. In melano-macrophage centres of the spleen few remainders of disintegrated merozoites were seen among the cell debris (Fig. 111). Near the edge of one melano-macrophage centre two intraleucocytic intact merozoites were found among numerous erythrocytes (Fig109).

4.1.3. The Sporozoites

What appeared to be a single sporozoite (SP) was encountered free in the intracellular spaces of the spleen. The parasite was kidney shaped and surrounded by a pellicle with an outer and inner unit membrane. The usual organelles such as microtubules, micronemes and mitochondria were observed in the cytoplasm which was densely covered with ribosomes. The centrally situated nucleus was large and was surrounded by a distinct perinuclear space (PNS). The characteristic feature of this parasitic stage were two large, more or less electron lucent spherical refractile bodies (RB) of approximately 0.4 micron in diameter which were located at either side of the nucleus and led to the presumption that this parasite might be considered a sporozoite (Fig.112). No other similar stage of development was observed. The length of this parasite was 3.2 micron, the width was 1.76 micron and the nucleus measured 0.8 by 1.0 micron.

No gametocytes have been observed so far under the electron microscope in organ tissues or in pellets of circulating blood.

4.2. The Ultrastructure of Haemogregarina simondi from Solea solea

4.2.1. The Merozoites

The intra- and extracellular merozoites showed typical structural elements of the Sporozoa. The first stage of development encountered was a single parasite inside a neutrophil (Fig114). Characteristically this parasite was found lying in a distinct parasitophorous vacuole (PV) which was a common feature for all merozoites whether they were found inside a leucocyte or an erythrocyte. The parasite itself was surrounded by a triple-layered pellicle consisting of an outer membrane (OM) of high electron density, a central membrane of low electron density (MM) and an inner membrane (IM) of less osmiophilic character than the outer membrane and clearly distinguished only at higher magnifications (Fig125). All three membranes had unit membrane characteristics and measured together 0.07-0.09 micron. At the apical pole of the merozoites a typical polar ring (P) was seen with a width of approximately 0.4-0.5 micron at its upper opening (Fig122). The maximal thickness of the wall of this polar ring was 0.2-0.21 nm = 0.02-0.021 micron. A large number of subpellicular microtubules (MT) was observed extending

from the polar ring and covering the surface of the parasite (Fig.123). Their number was difficult to estimate but from the distance between individual microtubules (0.04-0.08 micron) and the diameter of the parasite (average 1.33 micron) they were estimated as being in the range of 45-61 microtubules. It appeared also as if the inner membrane of the pellicle did not end at the polar ring but overlapped the ring, turning inwards. A conoid (C) was clearly observed at the apical pole extending at times through the aperture of the polar ring (Fig. 122). This conoid measured 0.3 micron in diameter at its base and showed an approximate height of 0.22 micron. Approximately 4-6 rhoptries (RH) were found in the form of typical electron dense, elongate and club-shaped structures which appeared to arise from inside the conoid (Fig.122,124). They measured 0.7-1.1 micron in length and 0.15 micron in width near the polar ring (DRH) whereas their club-shaped, posteriorly directed ends measured up to 0.37 micron in width. A large number of micronemes (MN) were observed throughout these parasites. They were more densely packed towards the anterior end, had a diameter of 0.08-0.09 micron and their numbers were in the range of 169 and more. Rarely were cytoplasmic inclusions of low electron density detected and only in apparently recent invasions of young merozoites (Fig.114).

They measured an average of 0.35-0.6 micron in diameter and were considered to be amylopectin granules (AM). However, they might perhaps also belong to the two rather illdefined spherical structures lying anteriorly and posteriorly in close proximity of the nucleus and resembling "paranuclear" or "refractile bodies" (RB) as seen in sporozoites of some Sporozoa. The elongate and protruding apical complex of this early merozoite together with the two refractile bodies, the amylopectin granules and the enlarged pouch of the parasitophorous vacuole behind theposterior pole of the parasite seemed to indicate that the parasite had just invaded the cell and was probably a sporozoite transforming into a merozoite. The nucleus (N) of these merozoites appeared to be located in the centre of the organisms taking up more than 3/4 of the width of the parasite. It was surrounded by a distinct nuclear membrane (NM) with several nuclear pores (NP). Areas of condensed, osmiophilic chromatin granules were distributed within the nucleus. In the cytoplasm of the merozoites were found numerous ribosomes (RI) and rounded or oval-shaped structures with vesicular interior which were considered mitochondria (MI)(Fig123). The number of intracellular merozoites in both erythrocytes and leucocytes appeared never to exceed 8. Usually from 1-8 merozoites were seen. Their length was estimated from measurements taken of different tangential and longitudinal sections as being in the

range of 6.5-7.35 micron. However, some intracellular merozoites were much longer and measured from 8.6-12.7 micron (average 10.95) in length and from 0.9-2.0 micron (average 1.33) in width and were considered micro-merozoites. The average width of merozoites as measured from spherical cross sections was 1.5 micron.

4.2.2. The Schizonts

Schizonts were encountered in either leucocytes (Fig. 117) or erythrocytes (Fig.119) from the capillaries of the spleen and in sections of blood pellets. Intraleucocytic schizonts with 8 merozoites measured from 8.0-9.1 micron in length (average 8.6) and 5.6-6.0 micron in width (average 5.8). Intraerythrocytic schizonts from the spleen with 8 merozoites measured 7.9 by 5.5 micron in average. Schizonts in the blood were surprisingly less numerous despite the fact that they had been observed in large numbers by light microscopy. It was assumed that they might have been lost during the process of fixation and dehydration. Those found measured an average of 8.5 by 4.5 micron (Fig.118). Schizonts in various stages of development were observed. Intracellular schizonts appeared to be covered by a distinct envelope of 0.6 micron thickness thought to be of host cell origin (HCM). The host cell

nucleus (HCN) took various shapes during the process of schizont development. It appeared kidney- or halfmoon shaped (Fig117), seemed to become fragmented (Fig126) or flattened and pushed to the periphery (Fig114). Finally the schizont ruptured and the merozoites were released (Fig121). A direct lysis of the host cell nucleus was not observed. Some of the schizonts appeared to be inside phagocytosing cells of the monocyte-macrophage type but normally no damage was seen to schizonts or individual merozoites nor was any lytic effect observed on them from the host cell. However, in one instance (Fig128) a capsulated area measuring 10.5 by 8.6 micron and containing the remnants of disintegrated parasites and cellular debris was seen in the spleen. This was considered to be the edge of a melano-macrophage centre mopping up an intraerythrocytic schizont. The flattened host cell nucleus appeared to be compressed at the periphery. A residual body was never found in these schizonts. No distinction between micro-and macroschizonts was observed. In mature schizonts often more than 8 parasites were found in longitudinal and transverse sections. This could be explained by the large dimensions of the intracellular merozoites, especially the micromerozoites, which have to curl up inside the host cell to be accomodated. These merozoites are thus sectioned twice (Fig. 120).

4.2.3. The Gametocytes

What appeared to be a differentiation between macro- and micro-gametocytes, as seen by light microscopy, was only observed as far as it concerned the micro-gametocytes, but macro-gametocytes were not detected. The micro-gametocytes were found free in the plasma of the capillaries of the spleen (Fig127) or intra-erythrocytic where they were still described as micro-merozoites. They measured 11.8 by 1.5 micron in average and were elongate,curved structures with a broader apical pole and a tapering posterior end as seen under the light microscope. The large nucleus (N) was situated in the broader anterior 1/3 of the parasite. It was surrounded by a nuclear membrane (NM) interrupted occasionally by nuclear pores (NP). The karyoplasm showed a granular structure with more or less irregularily spaced osmiophilic areas. At the periphery, lying towards the posterior end, was a distinct rounded electron dense body of granular consistency which was considered to be the nucleolus (NU) measuring 0.3-0.5 micron in diameter (Fig127). The pellicle of this parasitic stage appeared as a triple pellicular complex intracellularly (Fig122) but in free micro-gametocytes only two distinct osmiophilic lines were seen separated by an intermediate electron lucent layer (OM+IM) measuring altogether 0.05 micron in width. Immediately

underlying the inner membrane cross-sections of ring structures, considered to be subpellicular microtubules (MT), were seen. These tubular structures were also clearly observed in parallel arrays at the apical pole and extending from there towards the posterior end. Throughout the body of the parasite numerous micronemes (MN) were seen and a total of 194 were counted. It is assumed, however, that there were probably more than 200. In addition, several tubular mitochondria (MI) were found and the cytoplasm contained large numbers of ribosomes (RI) of approximately 0.012-0.018 micron in diameter which formed the ground substance of the cytoplasm. A large and completely homogenous structure of 0.9-1.1 micron in diameter and not surrounded by any distinct membrane was found in close proximity to the nucleus. It appeared to correspond to a similar structure resembling a large vacuole and was seen as a constant feature under the light microscope. This was considered to be the refractile body (RB). In free micro-gametocytes it appeared singly and was located in the immediate vicinity of the nucleus (Fig127). But occasionally two or even three spherical refractile bodies were observed in free or intracellular parasites lying in juxtaposition to the nucleus or farther towards the posterior pole (Fig.115,125,126,127). Their function remains unknown. In addition accumulations

of more or less rounded or irregular vacuoles with homogenous contents of very low electron density and often surrounded by distinct membranes were seen in the vicinity of both poles. Their diameter measured between 0.16 and 0.4 micron and they resembled amylopectin granules (AM).

4.3. Ultrastructure of developmental stages of haematoprotozoan parasites in possible invertebrate vectors

4.3.1. *Haemogregarina simondi* in *Hemibdella soleae*

Despite prolonged searching in ultrathin sections of these leeches no clearly distinguishable developmental stages of this haemogregarine corresponding to the light microscopical observations were detected. The only stage found to date was an early *zygote* (Fig.135) showing 3 nuclei in different phases of division.

4.3.2. *Haemogregarina simondi* in *Lernaeocera* sp.

The electron microscopy of this ectoparasitic copepod showed only free stages comparable to the mature merozoites found in schizonts of *H.simondi* from the blood and

intravascular spaces of the spleen (Fig.125,126). They were characterized by a pellicle consisting of an outer membrane (OM), a central membrane (MM) and an inner membrane (IM). Also seen were numerous micronemes (MN) and subpellicular microtubules (MT) arranged in concentric rings. The presence of a distinct refractile body (RB) was suggestive for their readiness to leave the host cell Fig.136).

4.3.3. *Trypanosoma* sp. in *Calliobdella nodulifera*

Unidentified developmental stages of a trypanosome were observed in ultra-thin sections of this leech. Clearly visible was the lobed nucleus (N), an elongate mitochondrion (MI) with cross-sections of cristae (CR) and were numerous electron dense granules (G). Also present was what appeared to be part of the shaft of a flagellum (F) (Fig.137). In transverse sections the nucleus appeared elongate with a distinct nuclear membrane (NM) and nuclear pores (NP). Numerous vesicles of varying electron opacity were scattered intracytoplasmically. Rough endoplasmic reticulum(RER) and a few large irregular shaped vacuoles (V) were also present. Pellicular microtubules (MT) were also quite obvious (Fig.138).No kinetoplast was seen and no other organelles to assist an exact identification of the developmental stage of this parasite.

5. DISCUSSION

5.1. The Ultrastructure of Organelles of the Haemogregarina as compared with other Sporozoa

The fine structure of the developing stages of the Sporozoa is characterized by common organelles as the pellicle consisting of two or three layers of unit membranes, the polar rings, the subpellicular microtubules, the conoid, the rhoptries, the micronemes and the micropore. All of these structures were found in the present study in both haemogregarines from the turbot and sole. It was thus shown that these parasites belong to the Apicomplexa. Each of these organelles will be discussed in relation to other members of this subphylum.

(i) The Pellicle, the cell boundary of extra- and intracellular merozoites consisted of an outer and an inner membrane in the case of *H.sachai* n.sp. (OM+IM) and appeared to be triple layered in *H.simondi* where the distinction between an outer, central and inner membrane was made (OM+MM+IM). Each of these individual membranes was composed of two unit membranes of different

electron density. The outer membrane was continuous and enclosed the whole parasite and was only invaginated where a micropore interrupted the other membrane(s). The innermost unit membrane terminated near the anterior end of the polar ring and also composed the inner walls of the micropore. The pellicle varies in width in the different species. It measured 450 Å in H.sachai n.sp. and 700-900 Å in H.simondi . Scholtyseck (1973) gives the average width of this organelle as being in the range of 400 Å. A considerable difficulty appears to be the interpretation of the layers of the pellicle. Some authors describe as "layer" a single unit membrane, two relatively close connected or completely fused unit membranes and sometimes even the electron lucent space between two components. Others consider the pellicle composed of two osmiophilic membranes interspaced by a lighter zone of lesser electron density as "triple layered". Bergle (1974) described the pellicle of so-called trophozoites of Karyolysus sp. as consisting of an outer layer of high electron density and a width of 250-320 Å but composed of two unit membranes. It was followed by an electron lucent space of 200-250 Å in width and an inner unit membrane of moderate electron density. This pellicular complex would correspond to a triple layered pellicle composed of three unit membranes and postulated as being characteristic for all motile and infecting stages of the Sporozoa,i.e. sporozoites and merozoites,by Vivier and Petitprez (1969). The

authors suggested that earlier descriptions of only two unit membranes might have been due to imperfect fixation or insufficiant enlargement. However, in the present study magnifications of more than 100,000 times did not reveal three unit membranes in intracellular merozoites of *H. sachai* n.sp. Merozoites of *Plasmodium* sp. were covered with a three-layered pellicle including the outer unit membrane, a labyrinth structure revealed by negative staining and a layer of microtubules(Aikawa, 1967). No comparable structure, however, was observed in other Sporozoa. Although the number of unit membranes of the pellicle of merozoites is still disputed, it appears that there is more consistency concerning the pellicular complex of gametocytes. Two unit membranes seem to be characteristic for the *Haemogregarina* and were observed for *Haemogregarina* sp. by Baker and Lainson (1967), Stehbens and Johnston (1967) and Scholtyseck, Mehlhorn and Hammond (1972) and for *Karyolysus* sp. by Bergle (1974). This was confirmed also in free micro-gametocytes of *H. simondi* in this study. Three unit membranes were seen in microgametocytes of *Leucocytozoon simondi* and *Haemoproteus columbae* (Scholtyseck, Mehlhorn and Hammond,1972) and of *Klossia helicina* (Fleischmann,1977). But in *Toxoplasma gondii* and most of the Eimeridae only one limiting unit membrane has been observed (Scholtyseck,1973).

(ii) The Polar Ring, is usually one structure only, as observed in the two contrasted parasites here and in *Karyolysus* sp. (Bergle,1974). However, two polar rings have been observed in sporozoites of *Lankesterella hylae* (Stehbens,1966) in sporozoites of *Eimeria nieschulzi* (Colley,1967) and in sporozoites of *Isospora canis* (Roberts, Mahrt and Hammond,1972). Three polar rings were described in merozoites of *Plasmodium fallax* by Aikawa (1966). These polar rings serve for the attachment of the subpellicular microtubules and appear to serve also in connection with the latter for the mobility of the infective stages of the parasite.

(iii) The Microtubules originate at the polar ring and run subpellicularly posteriorly at least as far as the nucleus and often far beyond as seen in *H.sachai* n.sp. In *H.simondi* they extended from the apical pole to the posterior end of the parasite covering the whole surface with a fine net- of fibrillar structures. In cross section they seemed to be composed of an osmiophilic central core and a densely staining osmiophilic cortex. The number of microtubules differs according to the different stages of development and from species to species so that they can be taken to some degree as a distinguishing characteristic between related organisms. Sporozoites and merozoites of many *Eimeria* sp. have 24 MT;

Lankesterella hylae sporozoites 27; Isospora sp. merozoites 18-22; Toxoplasma and related organisms 22; Plasmodium sp. merozoites 24-26; Babesia bigemina merozoites 32; Theileria annulata merozoites 38-40; and Klossia helicina merozoites 70 MT. Among the Haemogregarina the higher number of microtubules is apparent. As compared with the number of microtubules in other members of the Apicomplexa, the hypothesis of a higher phylogenetic age of the haemogregarines was postulated by Bergle (1974). He found 70-80 MT in Karyolysus sp. Stehbens and Johnston (1967) found 70-73 MT in gametocytes of Haemogregarina sp. and in Hepatozoon erhardovae approximately 70 MT were observed, (Mehlhorn and Scholtyseck,1974). In other Haemogregarina sp. 80-100 MT were described (Baker and Lainson,1967; Desser and Weller,1973). In the present study H.simondi had between 45-61 microtubules whereas H.sachai n.sp. had 31 microtubules which would indicate that this parasite might be phylogenetically much younger than H.simondi.

(iv) The Conoid is an organelle running through the polar ring in all sporozoites and merozoites of the Coccidia. It is apparently not present in the Haemosporidia and the Piroplasmea (Scholtyseck,1973) and stands in no connection with the polar ring as seen in the present study. In contrast to the conoids of the Eimeriidae

the conoid of the Haemogregarina sp. (Baker and Lainson, 1967; Desser and Weller,1973) is considerably wider and often twice as wide as high. In H. simondi the conoid was also wider (0.3 micron) than its height (0.22 micron) and this was also observed in H. sachai n.sp. here. This can be seen also in other Adeleids as Aggregata eberthi and Eucoccidium dinophili (Scholtyseck,Mehlhorn and Friedhoff, 1970) and in Klossia helicina (Fleischmann,1977).

(v) The Rhoptries are located in the anterior region of these parasites and belong to the apical complex. They were first described in sporozoites of Plasmodium gallinaceum (Garnham, 1966). They vary in shape from club-shaped, drop-like, elongate or tortuous. The anterior neck portions are narrow and extend into the conoid area as seen here in H. simondi and H.sachai n.sp. They also differ in numbers from species to species. Two rhoptries are found in Plasmodium sp.,Isospora sp., Babesia bigemina and many Eimeria sp. More than two club-shaped structures are seen in some Eimeria sp. (2-5); Toxoplasma gondii (5-9); Sarcocystis sp. (more than 11); Frenkelia sp. (5-8); Besnoitia sp. (3-5) and Karyolysus sp. (4-6). In the present study H. sachai n.sp. had 7-10 rhoptries and H. simondi from 4-6. In merozoites of some Sporozoa as in Toxoplasma gondii (Scholtyseck and Piekarski,1965); Babesia ovis (Friedhoff and

Scholtyseck, 1968) and _Theileria annulata_ (Mehlhorn, Weber, Schein and Büscher,1975) rhoptries cannot be distinguished from micronemes. Structural connections between rhoptries and micronemes were not observed here but were observed by others (Aikawa,1966; Sénaud, 1967). These organelles are considered to secrete proteolytic enzymes to assist the mechanical function of the conoid in the penetration of the host cell.

(vi) _The Micronemes_ also vary in their numbers of individual spherical or oval, drop-like structures from species to species. _H. sachai_ n.sp. had more than 60 micronemes which is a rather small number and as such compares more favourably with _Toxoplasma gondii_ (30-50) and _Frenkelia_ sp. (50-70). In _H. simondi_ more than 169 MN were counted in intracellular merozoites whereas their number was even more in micro-merozoites and free micro-gametocytes (over 200). This would compare with the _Eimeriidae_ which have always large numbers of micronemes (Scholtyseck,1973) but also with _Sarcocystis_ sp. (300-400) and members of the _Haemogregarina_ as _Karyolysus_ sp.(140). They appear to be covered by a membrane in _Isospora_ sp.,_Babesia gibsoni_ and _Haemogregarina_ sp. (Scholtyseck,1973) but this was not the case with _H. sachai_ n.sp. and _H. simondi_.

(vii) _The Micropores_ or ultracytostomes are invaginations of the pellicle of the parasite and serve as organelles

for the metabolism. They are present in almost every stage of the life cycle of the Sporozoa. In most cases only one micropore is observed. However two micropores are found in Lankesterella sp., Besnoitia sp. and Plasmodium sp. (Scholtyseck,1973) but also in the adeleid Klossia helicina (Fleischmann,1977) and in Haemogregarina sp. (Stehbens and Johnston,1967). In the present study two micropores were a characteristic feature of H. sachai n.sp. but so far no micropores have been detected in H. simondi.

5.2. The Ultrastructure of different stages of Development of the Haemogregarina as compared with other Sporozoa

5.2.1. The Sporozoites

There are only few reports in the literature of electron microscopy that are dealing with sporozoites. The most characteristic feature of the sporozoites of Eimeria sp. are the "refractile bodies" (RB)(Scholtyseck, 1973). Such a structure has been first described under the name of "paranuclear vacuole" or"paranuclear body" by Stehbens (1966) from sporozoites of Lankesterella hylae. Since then "refractile bodies" have been found

frequently and are considered the typical organelle of the sporozoites but also of trophozoites and 1st generation schizonts of Eimeria sp. and related Sporozoa (Hammond, Speer and Roberts, 1970). Levine (1971) included them as "clear globules" in his ultrastructural terminology of the Apicomplexa. They were also found in merozoites of Eimeria sp. (Colley, 1968). Usually these refractile bodies are present as two refractile globules in the anterior and posterior part of the parasite. Occasionally there is only one refractile body present. In sporozoites of Eimeria tenella this seems to be the case and only one refractile body is found in the posterior part (Strout and Scholtyseck, 1970). Such structures were found only once during this study in H. sachai n.sp. from the spleen of an infected turbot. The refractile bodies were in bipolar position with no indication of a limiting membrane and therefore this stage of the parasite was considered to be a free sporozoite. In H. simondi a somewhat similar appearance of two paranuclear structures tentatively identified as refractile bodies were seen in a single parasite inside a neutrophil. This stage exhibited a protruding conoid at what appeared to be the apical pole and seemed to have just recently invaded the host cell. It was therefore also considered as a sporozoite transforming into an early merozoite. Shortly

after entrance of a sporozoite into a host cell it appears that the refractile bodies disintegrate. This was observed in *Eimeria bovis* sporozoites by Fayer and Hammond (1967).

5.2.2. The Merozoites

This is probably the stage of development most thoroughly described in the *Sporozoa*. Merozoites are generally only distinguished from sporozoites by the absence of inclusions as the "refractile bodies". They have the same morphology as the sporozoites of *Aggregata*, *Eucoccidium*, *Coelotropha*, *Myriosporides* and *Angeiocystis* where refractile bodies do not occur (Scholtyseck, 1973). But in some species as *Eimeria nieschulzi* (Colley, 1968), *E.callospermophili* and *E.bilamellata* (Hammond, Speer and Roberts, 1970) the merozoites have also refractile bodies. This was not observed in *H.sachai* n.sp. nor in *H.simondi* but single intracellular merozoites were characterized by high vacuolization. This seems to indicate that these are young merozoites and a similar vacuolization was also reported by Bergle (1974) in young merozoites and trophozoites of *Karyolysus* sp. The pellicle of the merozoites remains a structure for discussion. Vivier and Petitprez (1969) believe that the pellicular complex is composed of three unit membranes. However,

Mehlhorn and Scholtyseck (1974) are of the opinion that generally all motile stages of the Apicomplexa including the merozoites have a pellicle consisting of two layers, each layer being a unit membrane. In the present study a two-layered pellicle was observed in H.sachai n.sp. and in H. simondi merozoites three unit membranes were present. From this it is concluded that perhaps at some time in the development of the parasite a triple membrane can be seen but that normally only two membranes are detected.

5.2.3. The Gametocytes

In the present study only micro-gametocytes were discovered under the electron microscope in contrast to the clear differentiation between micro- and macro-gametocytes as observed under the light microscope. Micro-gametocytes were only seen in H. simondi and free in the plasma. Bergle (1974) found both gametocytes of Karyolysus sp. exclusively intracellular inside a parasitophorous vacuole and this was also observed by Baker and Lainson, (1967) in Haemogregarina sp. Perhaps the, micro-merozoite of H. simondi described here (intracellular) might correspond to their micro-gametocyte. However, a capsular membraneous sheath giving the impression of a cystic membrane was not seen here but was described as a capsule surrounding the mature gametocytes by Stehbens and

Johnston (1967) and Baker and Lainson (1967) for *Haemogregarina* sp. and by Bergle (1974) for *Karyolysus* sp. Also Desser and Weller (1973) who had tried to elucidate the ultrastructure of the gametocytes of *Haemogregarina stepanowi* failed because this capsule apparently prevented the proper fixation of the parasites and caused a distinct decrease in their size. The pellicle of the gametocytes has been in general described as double layered, each layer, i.e.the unit membrane, consisting of two neighbouring membranes of different degrees of electron density. This was seen by Stehbens and Johnston (1967) the width of the pellicle given as 0.02-0.033 micron (200-330 Å); Baker and Lainson (1967) given as 0.02-0.025 micron;and Bergle (1974) given as 0.028-0.036 micron (280-360 Å) for members of the *Haemogregarina sensu latu*. In *H. simondi* the average width of the pellicle was 0.025 micron for free micro-gametocytes and up to 0.05 micron for the intracellular micro-merozoites. The first description of "refractile bodies" from gametocytes was by Bergle (1974) who found a sphaerical refractile body of maximal 1.0 micron in diameter in the cytoplasm of *Karyolysus* sp. It is the second time here that it was observed in the micro-gametocyte of *H.simondi* measuring 0.9-1.1 micron in diameter. The life span of such refractile bodies appears to be not more than 24 hours and its function is seen in connection with the activation,

penetration and establishment of the parasites inside the host cell (Vetterling,Pacheco and Madden,1973). This might explain that this organelle is only found immediately before or after invasion of a cell by the sporozoite and in mature gametocytes before leaving the host cell and when liberated in the plasma. Roberts and Hammond (1970) suggested the storage of enzymes inside the refractile bodies. These might also have common features with the "crystalloid body" frequently encountered in the Haemosporidia (Trefiak and Desser,1973). A viral nature was suggested by Terzakis (1969) who found the "crystalloid body" composed of aggregates of dense particles in a less dense matrix. This could not be confirmed in the present study for the refractile bodies of sporozoites and gametocytes.

5.2.4. The Schizonts

No difference between macro- and micro-schizonts was observed in *H.sachai* n.sp. and *H.simondi* in the present study although this has been reported from *Karyolysus* sp. (Bergle,1974) and from *Plasmodium praecox* (Mudrow and Reichenow, 1944). It was also not seen in *Klossia helicina* (Fleischmann,1977).

The number of merozoites in a schizont appears also to differ from species to species. In H.sachai n.sp. schizonts with up to 6 merozoites were detected electron microscopically and the schizonts of H.simondi appeared to contain up to 8 merozoites. Fleischmann (1977) observed also only up to 10 merozoites in cross-sections of the schizonts of the adeleid Klossia helicina but Bergle (1974) found schizonts with up to 50 merozoites in ultra-thin sections of Karyolysus sp. the average being 15-20. In Haemogregarina sp. technical problems prevented Baker and Lainson (1967) and Stehbens and Johnston (1968) to demonstrate schizonts by electron microscopy because the latter would fall out of the parasitophorous vacuoles when ultra-thin sections were prepared. In the present study no residual body was encountered in schizonts of the two haemogregarines and it was also not observed in Klossia helicina (Fleischmann,1977). A residual body of 6 micron in diameter was quite obvious,however, in Karyolysus sp. where it was mainly seen in older schizonts and was surrounded by a distinct membrane (Bergle,1974).

Also very characteristic in the present study was the observation that schizonts of both haemogregarines were lying in a distinct parasitophorous vacuole

and were not surrounded by what some authors claimed to be a cystic membrane (Stehbens and Johnston,1968) or the outer unit membrane of the pellicle from the initial invading sporozoite or merozoite as suggested by Bergle (1974).

The schizonts in the present study were encountered exclusively inside the vascular spaces of the spleen and other organs. Similar observations were made by Bergle (1974) who found the schizonts of Karyolysus sp. in blood capillaries in the liver and exclusively outside the liver parenchyma. It is now understandable why a misinterpretation of the site of development resulted from light microscopical observations because the large schizonts fill easily the lumen of the finest capillaries and thus give the impression that a development takes place in the parenchyma of the various organs. So far no electron microscopy has been performed on developmental stages of the 3rd genus of the Haemogregarinidae, i.e.Hepatozoon sp. It might prove rewarding to examineultrastructurally if the development of Hepatozoon perniciosum is indeed taking place in hepatocytes as suggested from light microscopical observations (Miller,1909) or if all Haemogregarina sensu latu develop only intravascularly.

TABLE 16 a

ABBREVIATIONS FOR ULTRASTRUCTURE MORPHOLOGY

AM	Amylopectin
C	Conoid
CR	Crista of mitochondrion
DRH	Ductules of the rhoptries
E	Erythrocyte
ER	Endoplasmic reticulum
F	Flagellum
G	Granules
GO	Golgi apparatus
HCC	Host cell cytoplasm
HCM	Host cell membrane
HCN	Host cell nucleus
IM	Inner membrane of the pellicle
LY	Lymphocyte
ME	Merozoite
MI	Mitochondrion
MIG	Micro-gametocyte
MM	Centre membrane of the pellicle
MN	Micronemes
MP	Micropore
MPV	Membrane of the parasitophorous vacuole
MT	Microtubules
N	Nucleus
NE	Neutrophil

TABLE 16 b

ABBREVIATIONS FOR ULTRASTRUCTURE MORPHOLOGY

NM	Nuclear membrane
NP	Nuclear pore
NU	Nucleolus
OM	Outer membrane of the pellicle
P	Polar ring
PV	Parasitophorous vacuole
RB	Refractile body
RH	Rhoptries
RER	Rough endoplasmic reticulum
RI	Ribosomes
SCH	Schizont
SP	Sporozoite
T	Tail of merozoite
TH	Thrombocyte
V	Vacuole

FIGURES 92 - 112.

ULTRASTRUCTURE OF HAEMOGREGARINA SACHAI N.SP.

FROM SCOPHTHALMUS MAXIMUS

FIGURE 92.

General view of intravascular space in spleen with two merozoites (ME),one of them apparently entering an erythrocyte x 3,400

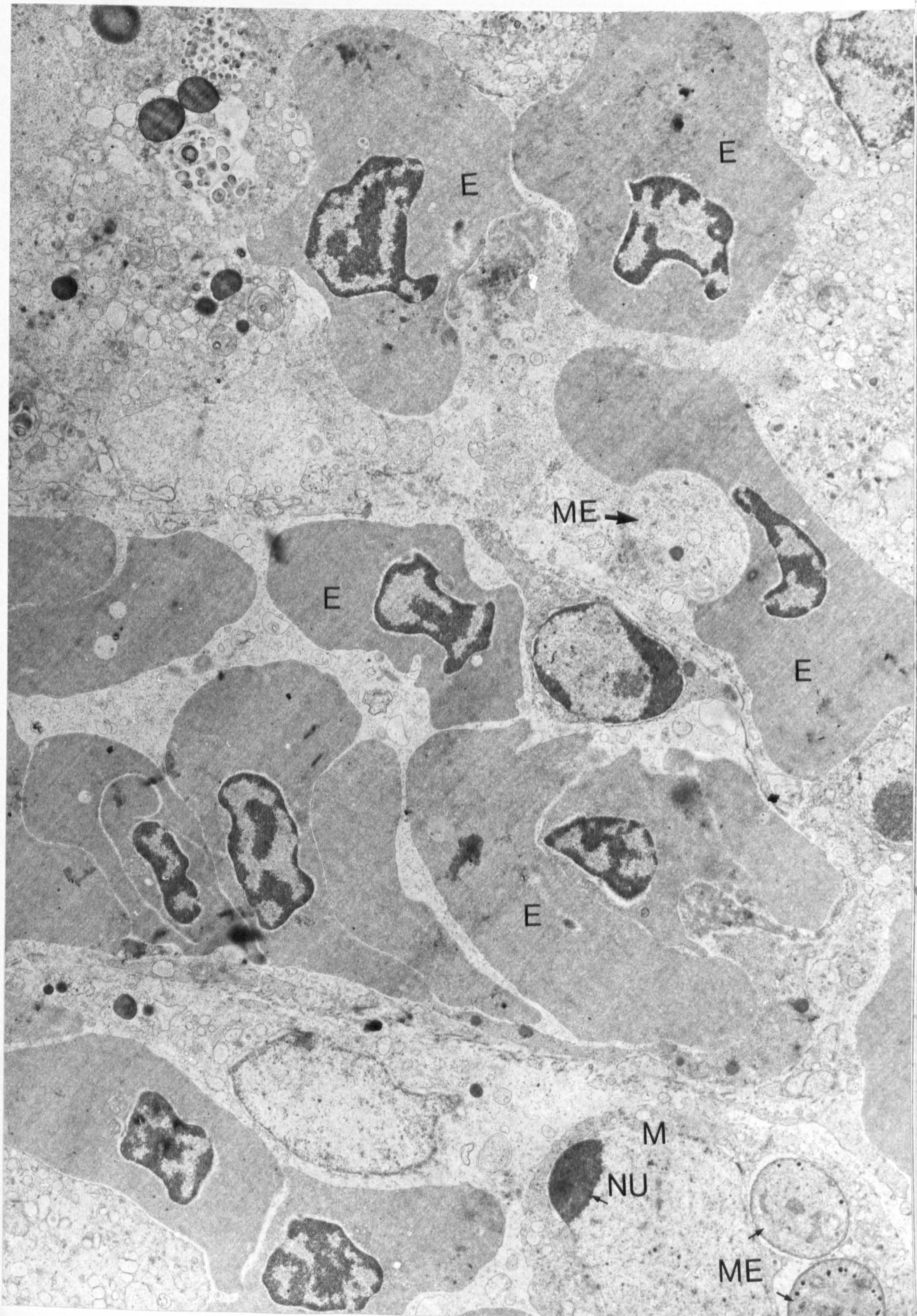

E
E
ME
E
E
E
M
NU
ME

FIGURE 93.

Lymphocyte with intracellular, juvenile merozoite recently invaded characterized by large numbers of amylopectin vacuoles, a double outer pellicle and what appears to be the cross section through the club-shaped posterior portions of 2 rhoptries x 23,500

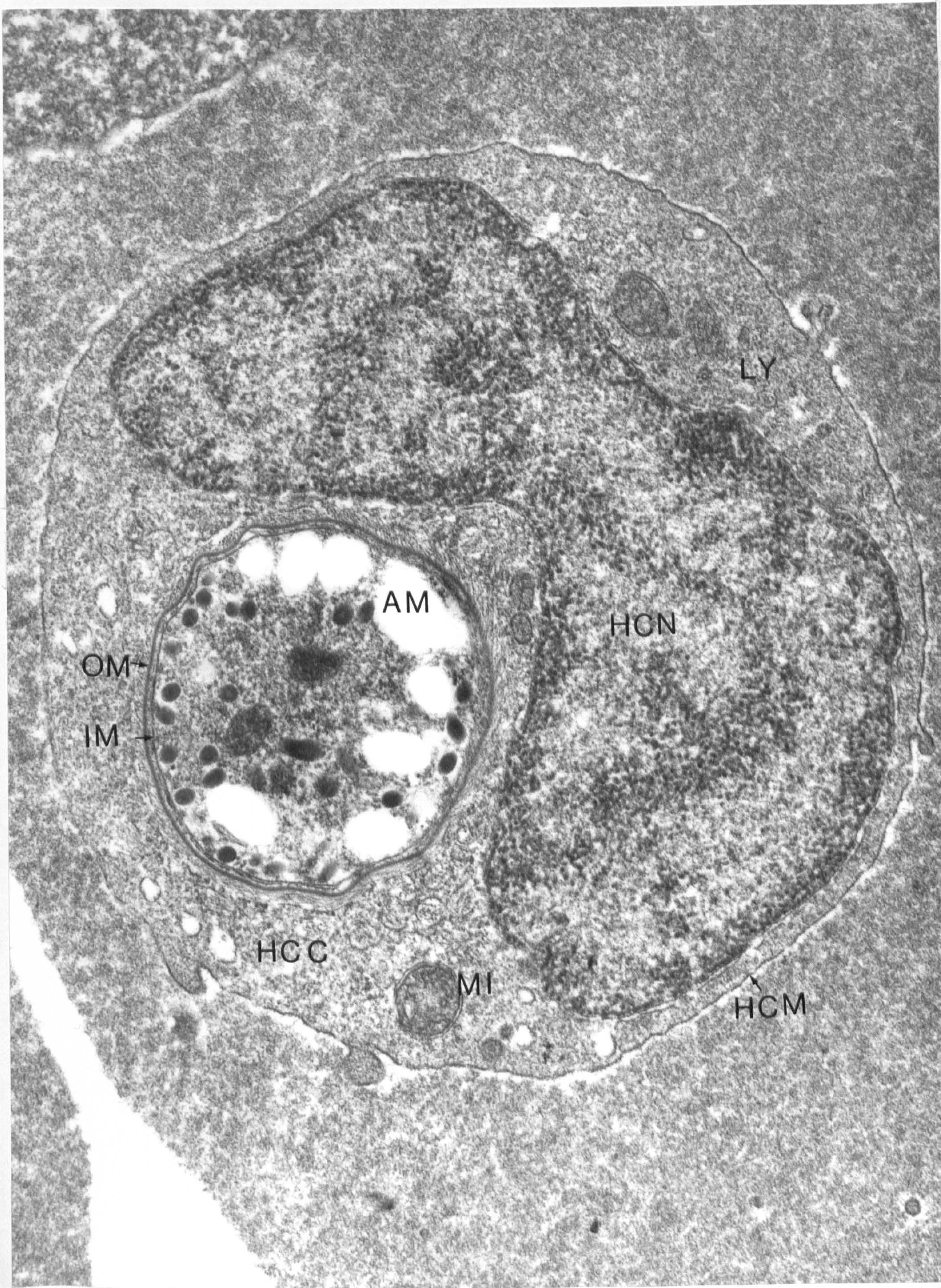

LY
AM
HCN
OM
IM
HCC
MI
HCM

FIGURE 94.

Intralymphocytic merozoites with distinct nuclei containing electron-dense inclusions, micronemes and several irregularly shaped vacuoles. There is also an indication of a parasitophorous vacuole (PV) and in one parasite transverse sections of 4 rhoptries (RH) are visible
x 14,200

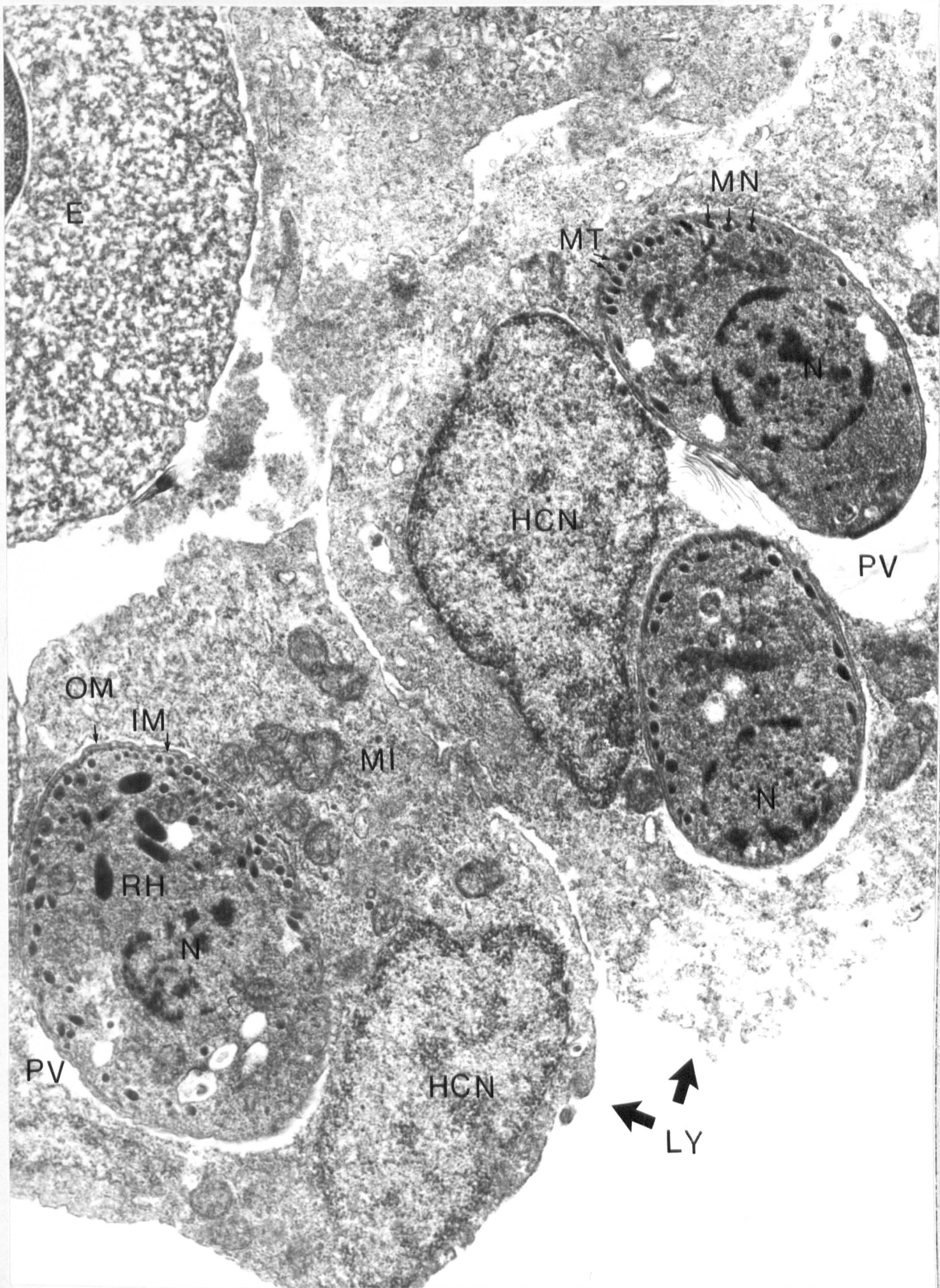

MN
MT
E
N
HCN
PV
N
OM
IM
MI
RH
N
PV
HCN
LY

FIGURE 95.

Two neutrophils with intracellular merozoites.Note the characteristic irregular and multilobed host cell nuclei and the numerous ovoid granules of fibrillar appearance in the host cell cytoplasm x 7,200

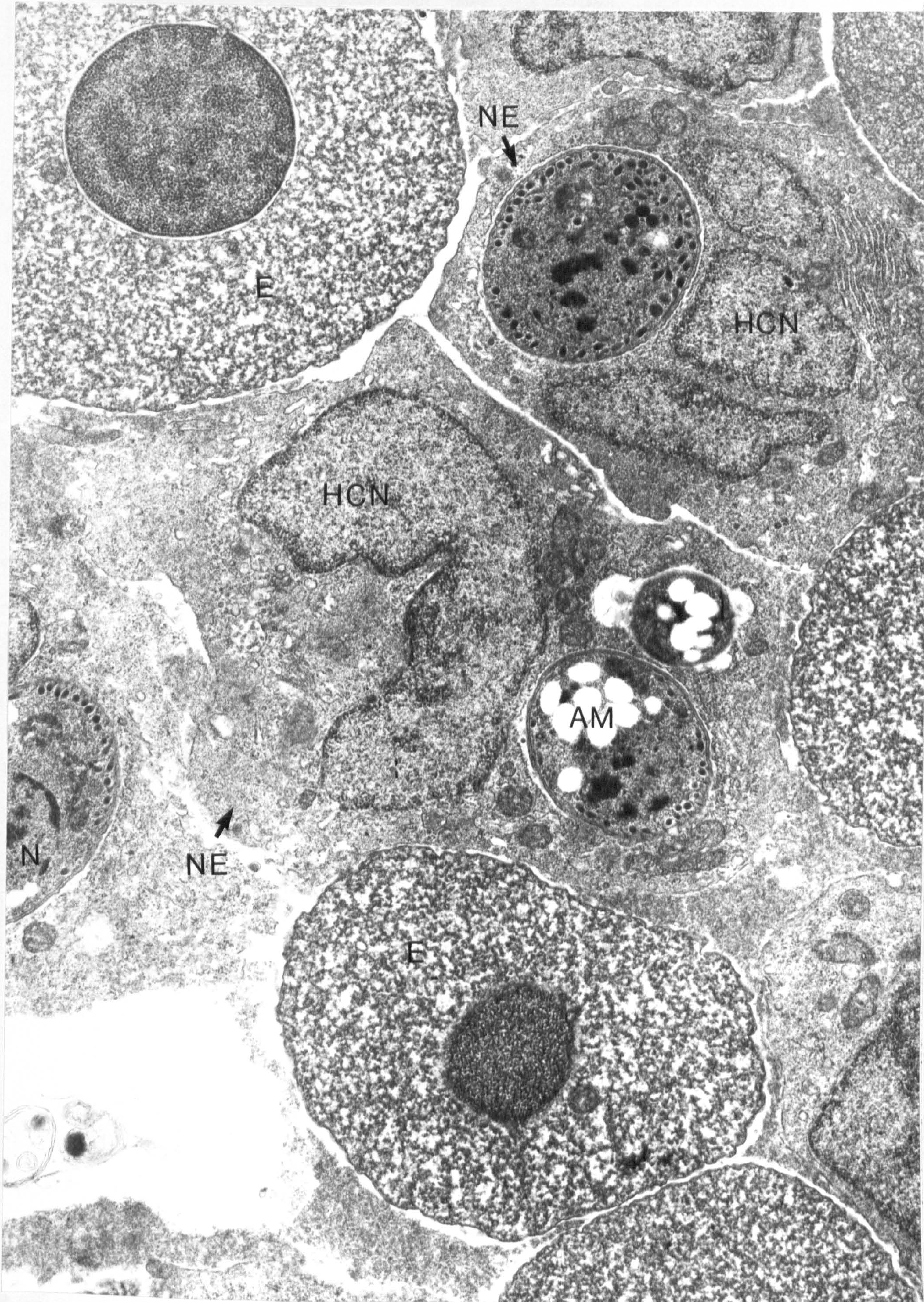

NE
E
HCN
HCN
AM
N
NE
E

FIGURE 95 a.

Merozoite inside a lymphocyte. A conoid at the apical pole is clearly visible inside a polar ring (P). Large numbers of micronemes and a few mitochondria are also present (MI) x 11,750

FIGURE 95 b.

Merozoite with distinct double pellicle in a cross section which apparently cut through the anterior and posterior halves of the parasite lying in a curved position inside a large parasitophorous vacuole (PV) x 26,000

LY
C
P
HCN
95 a

PV
N
GO
95 b

FIGURE 96.

Transverse section through the apical pole of a merozoite with peripherally arranged micronemes(MN), mitochondria(MI),distinct large vacuoles(V) and showing the length sections of 5 club-shaped rhoptries(RH) and the ductules of rhoptries(DRH) x 56,400

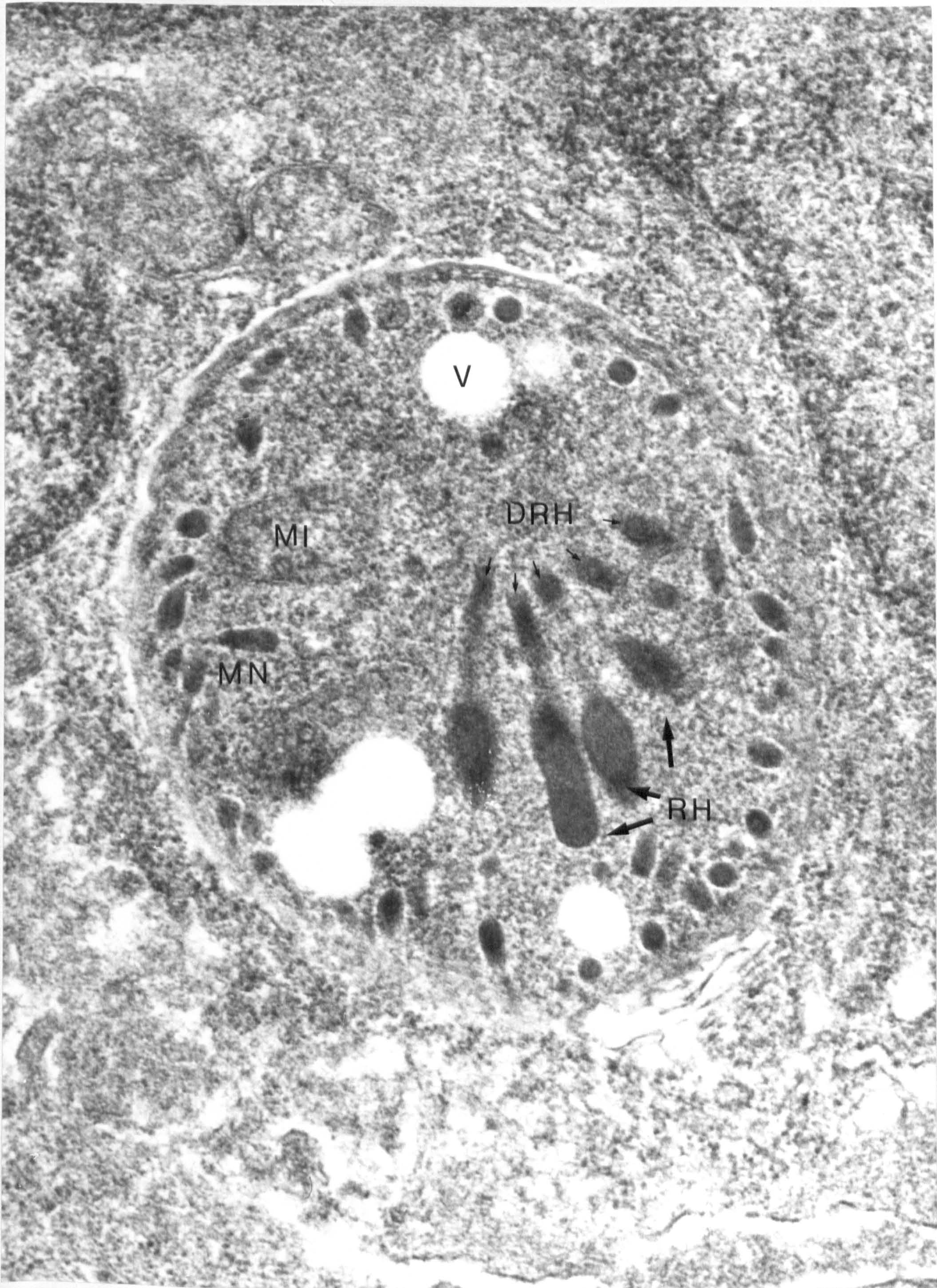

V
MI
DRH
MN
RH

FIGURE 97.

Transverse section through the apical pole of a merozoite showing distinctly the 31 microtubules(MT) and the club-shaped posterior poles of approximately 8 rhoptries(RH) x 61,750

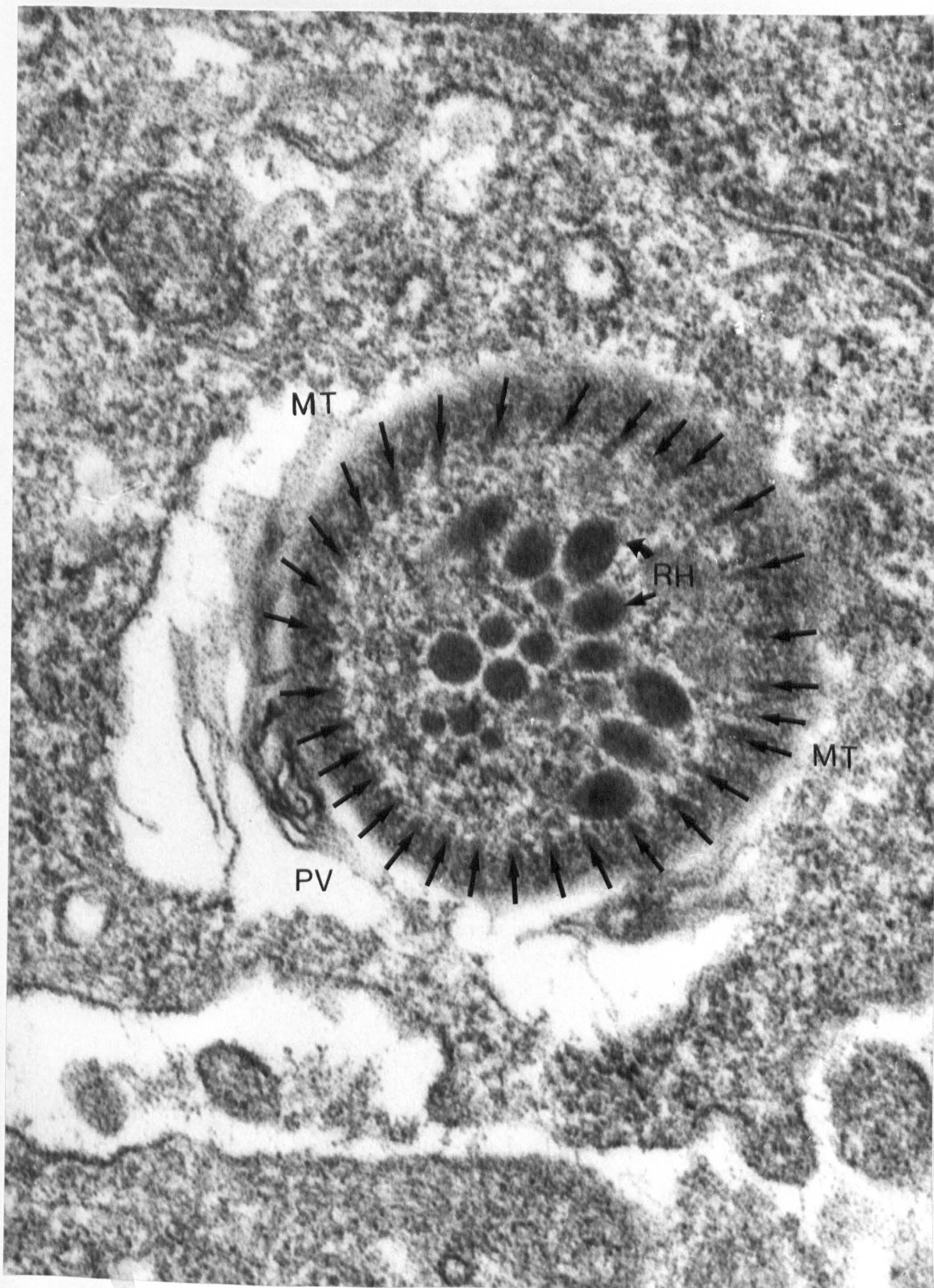
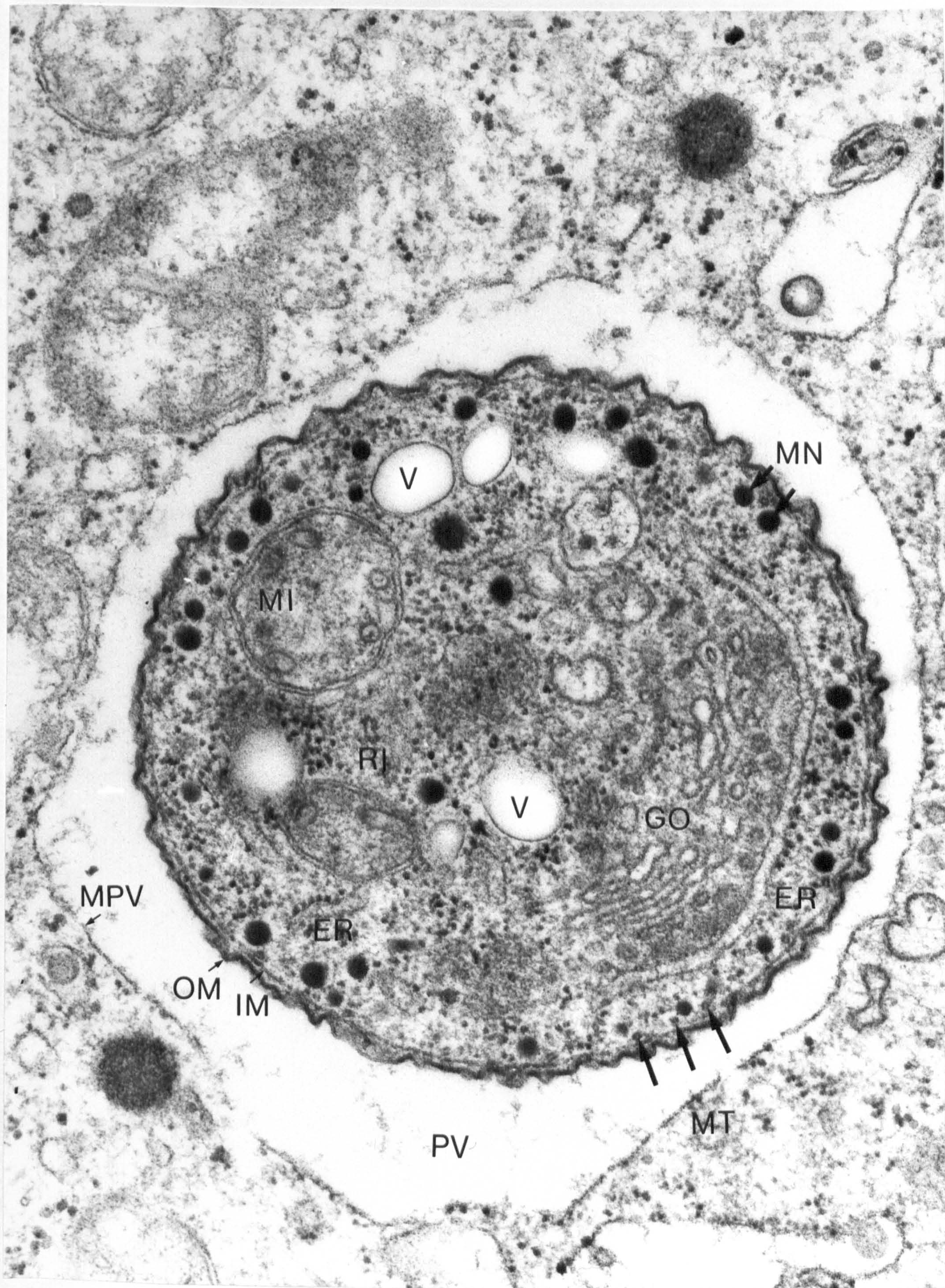

MT
RH
MT
PV

FIGURE 98.

Cross-section through merozoite lying in a distinct parasitophorous vacuole(PV).A double unit membrane characterizes the pellicle consisting of outer and inner membrane(OM+IM).The concentric rings of the subpellicular microtubules are clearly seen.In addition seen are a golgi-apparatus(GO),micronemes (MN),mitochondria (MI),vacuoles with distinct membranes(V),endoplasmic reticulum (ER)and numerous ribosomes(RI) x 115,000



V
MN
MI
RI
V
GO
ER
MPV
ER
OM
IM
MT
PV

FIGURE 99.

Section through merozoite with large nucleus,vacuoles,micronemes and pellicle to show a characteristic micropore (MP) x 160,000

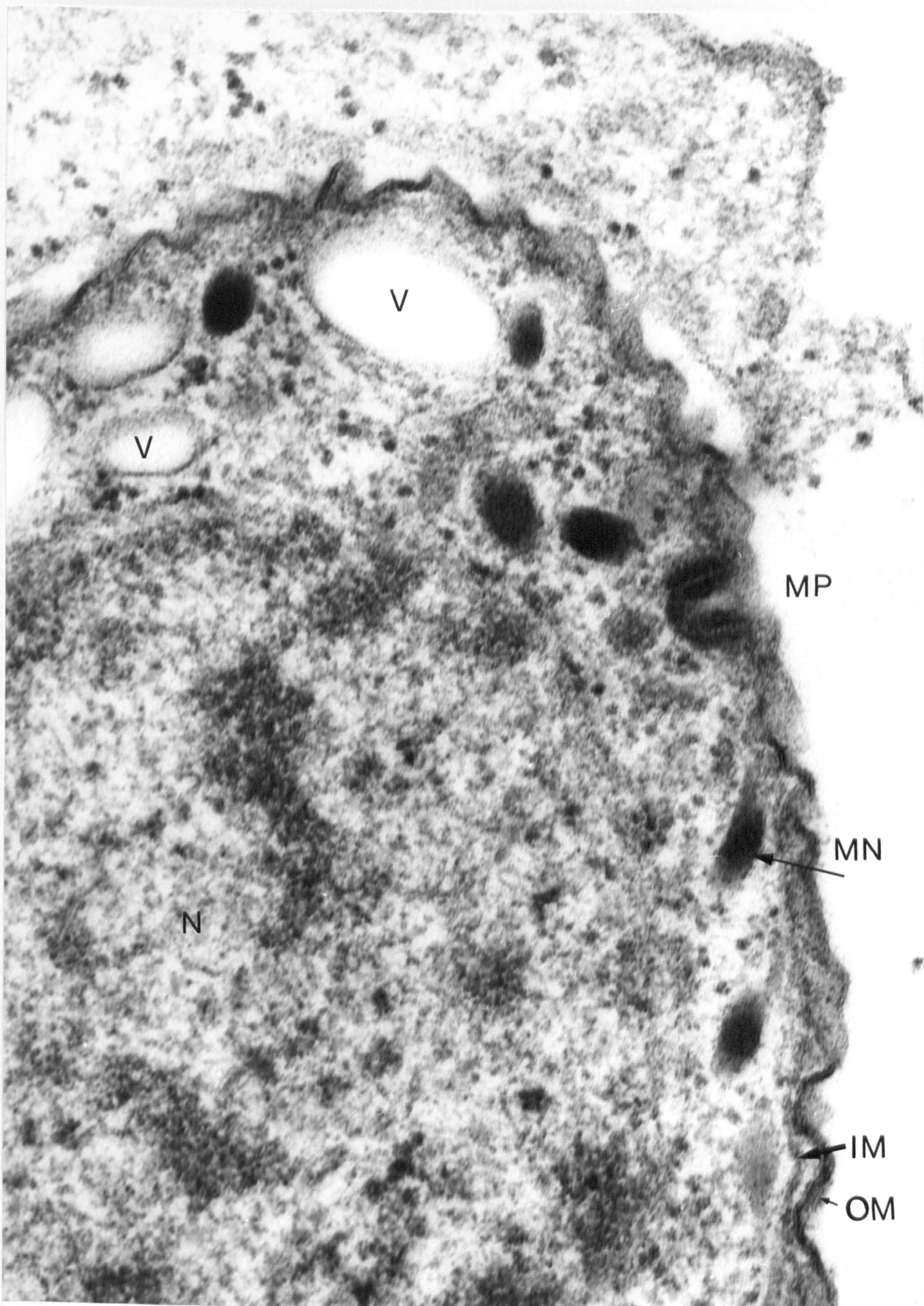

V
V
MP
MN
N
IM
OM

FIGURE 100.

Micropore enlarged (MP)
x 400,000

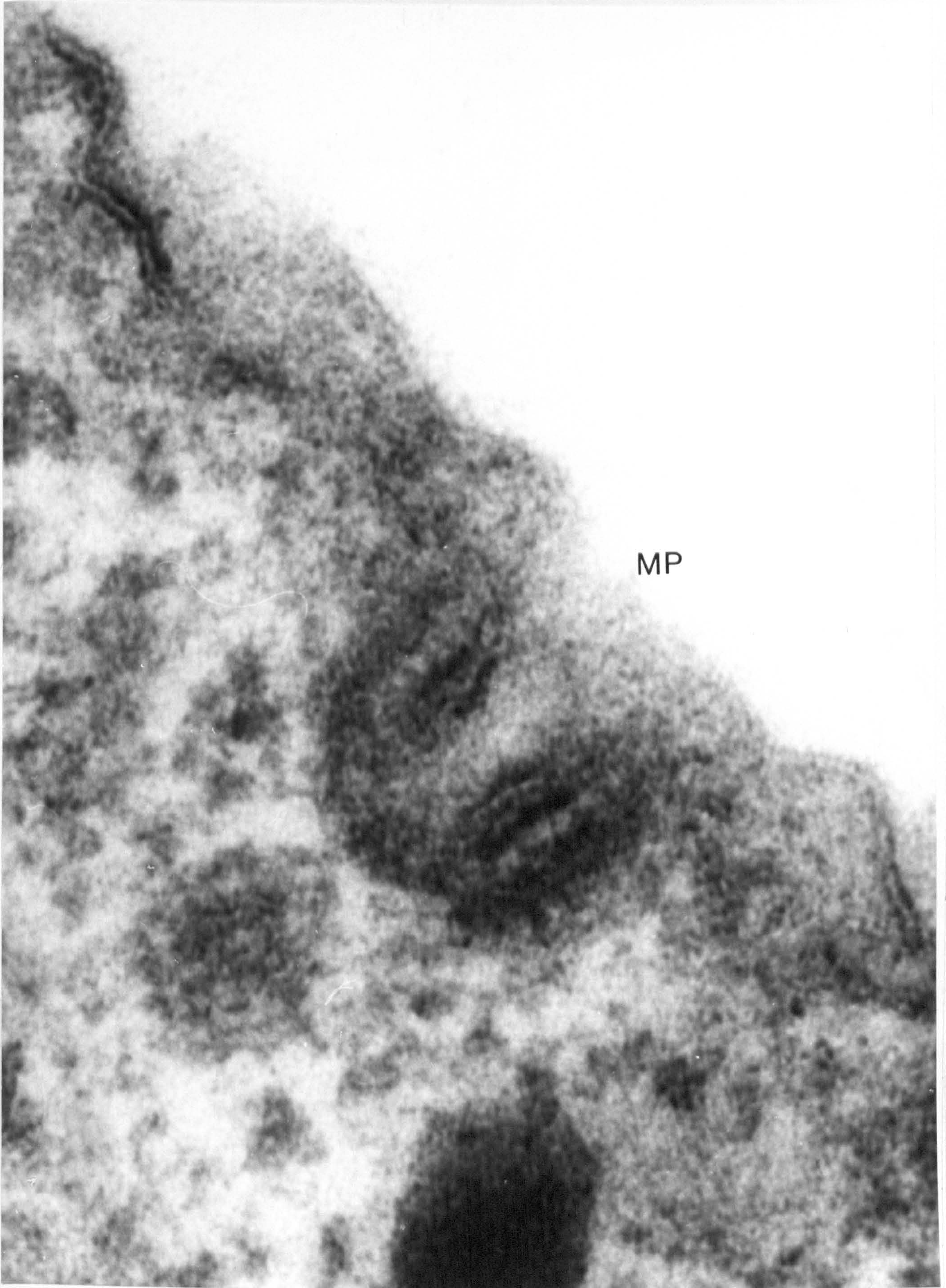

MP

FIGURE 101.

What appears as possible division by binary fission is more likely a curved merozoite as seen in the transverse section Fig.95 b since no division of the nucleus is apparent here x 52,000

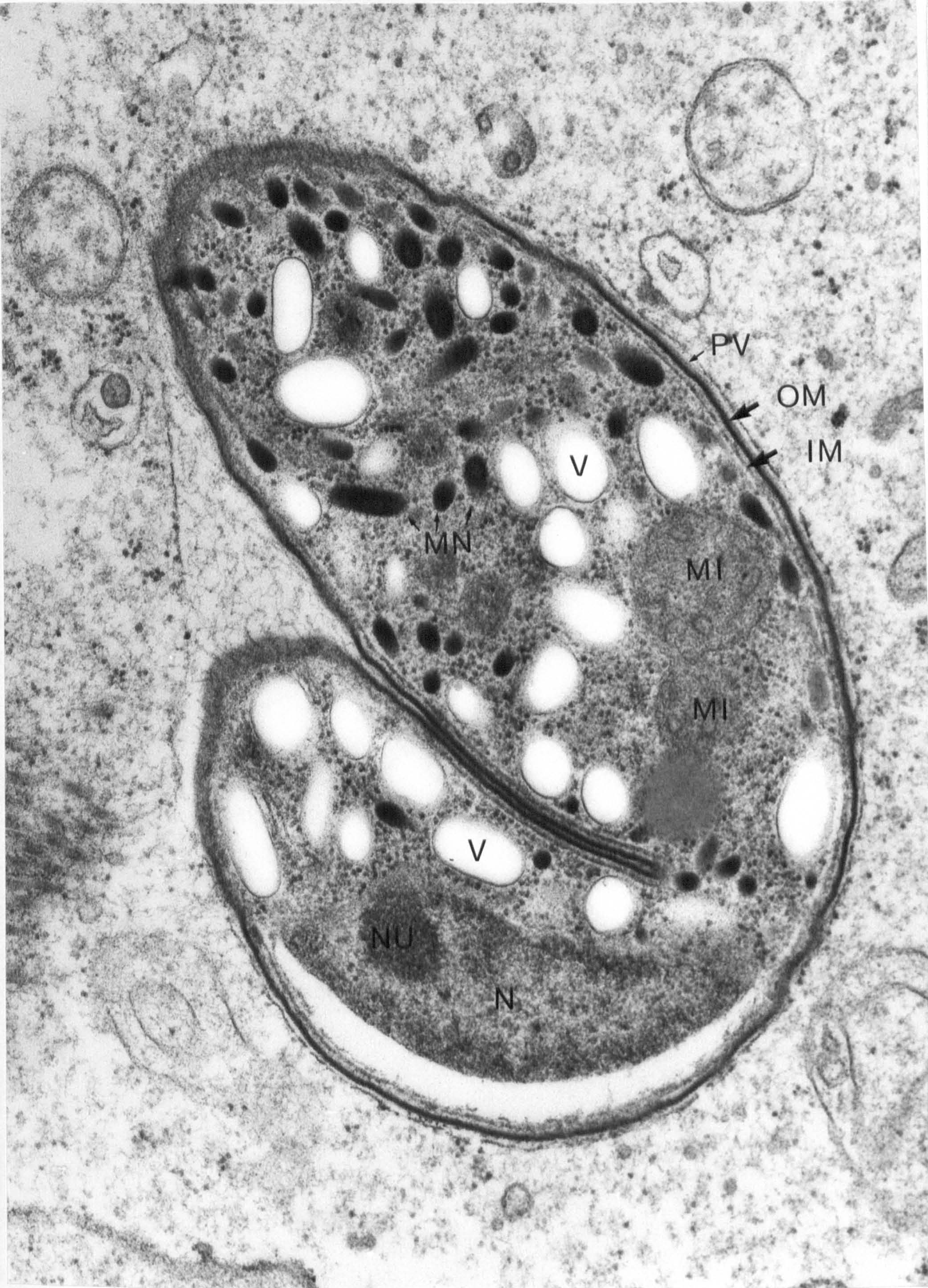

PV
OM
IM
V
MN
MI
MI
V
NU
N

FIGURE 102.

Young schizont with 2 merozoites inside a distinct parasitophorous vacuole common to both parasites (PV) probably shortly after division x 23,500

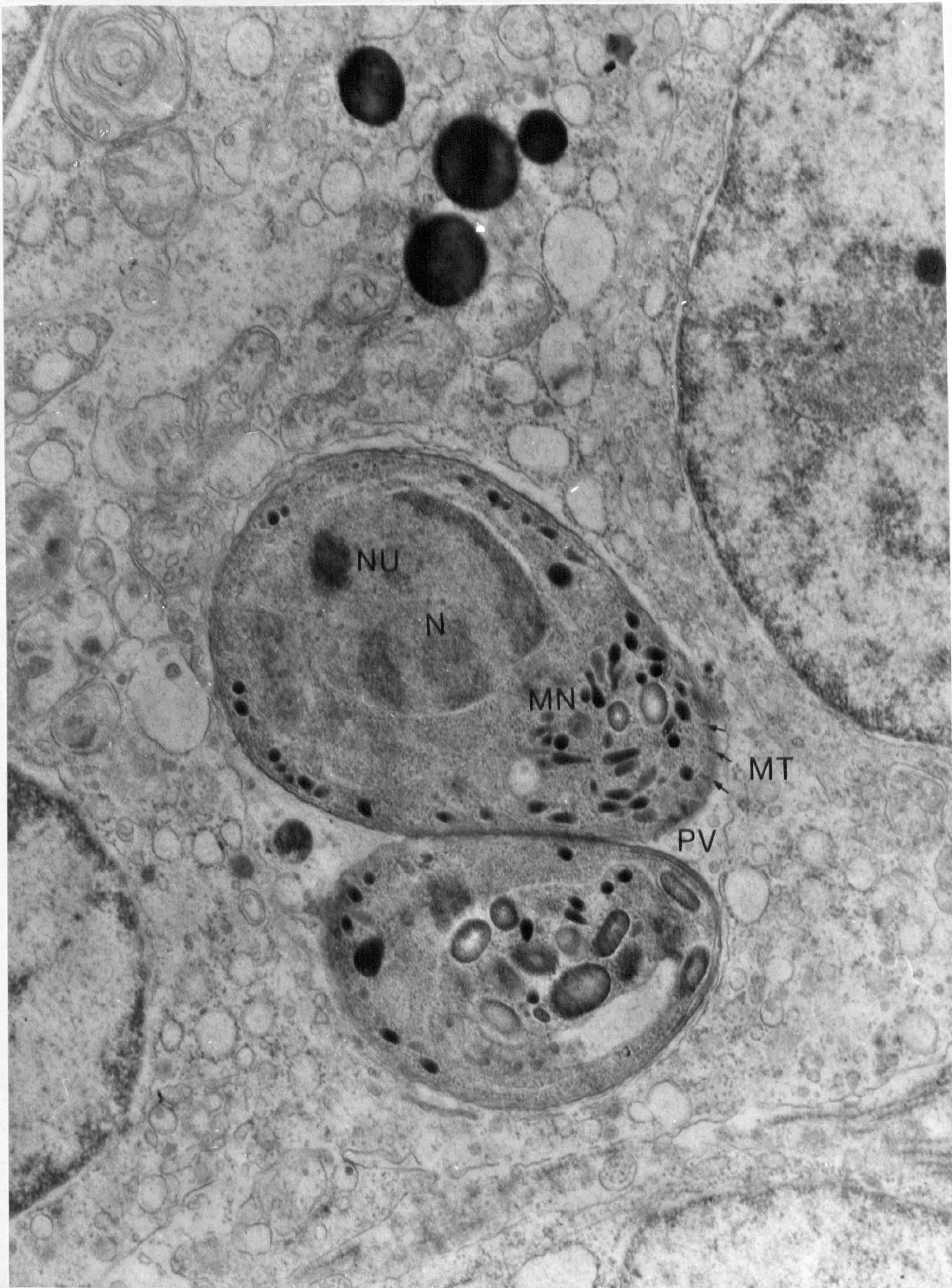

NU
N
MN
MT
PV

FIGURE 103.

Intraleucocytic schizont with
2 merozoites showing distinct
nuclei with concentric,osmiophilic
nucleoli(NU) and large amylopectin
vacuoles (AM) x 18,800

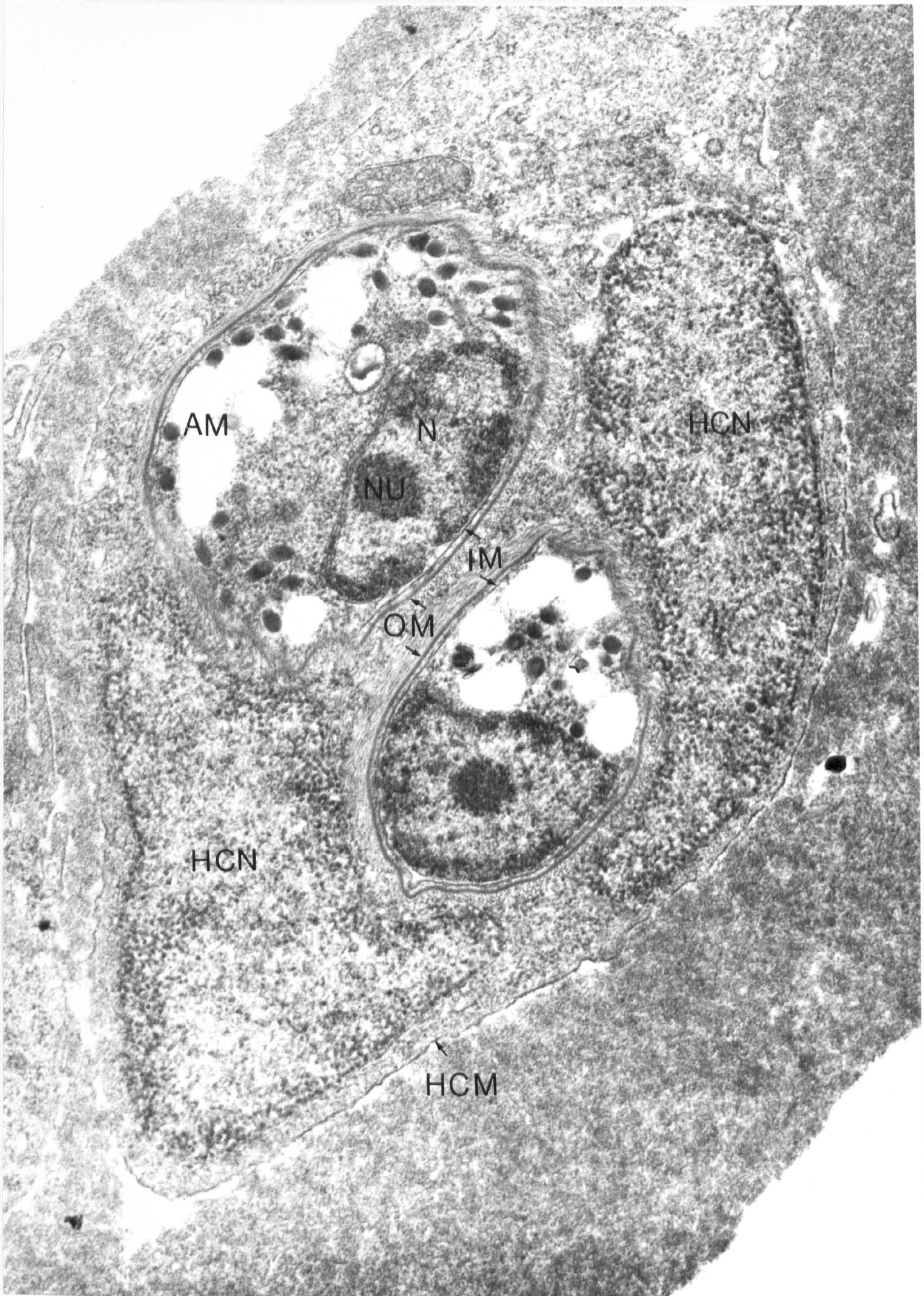

AM
N
NU
HCN
IM
OM
HCN
HCM

FIGURE 104.

Schizont with 4 merozoites
inside a joint parasitophorous
vacuole (PV) x 60,000

SCH
PV

FIGURE 105.

Schizont with 4-5 merozoites
in large parasitophorous
vacuole(PV) x 40,000

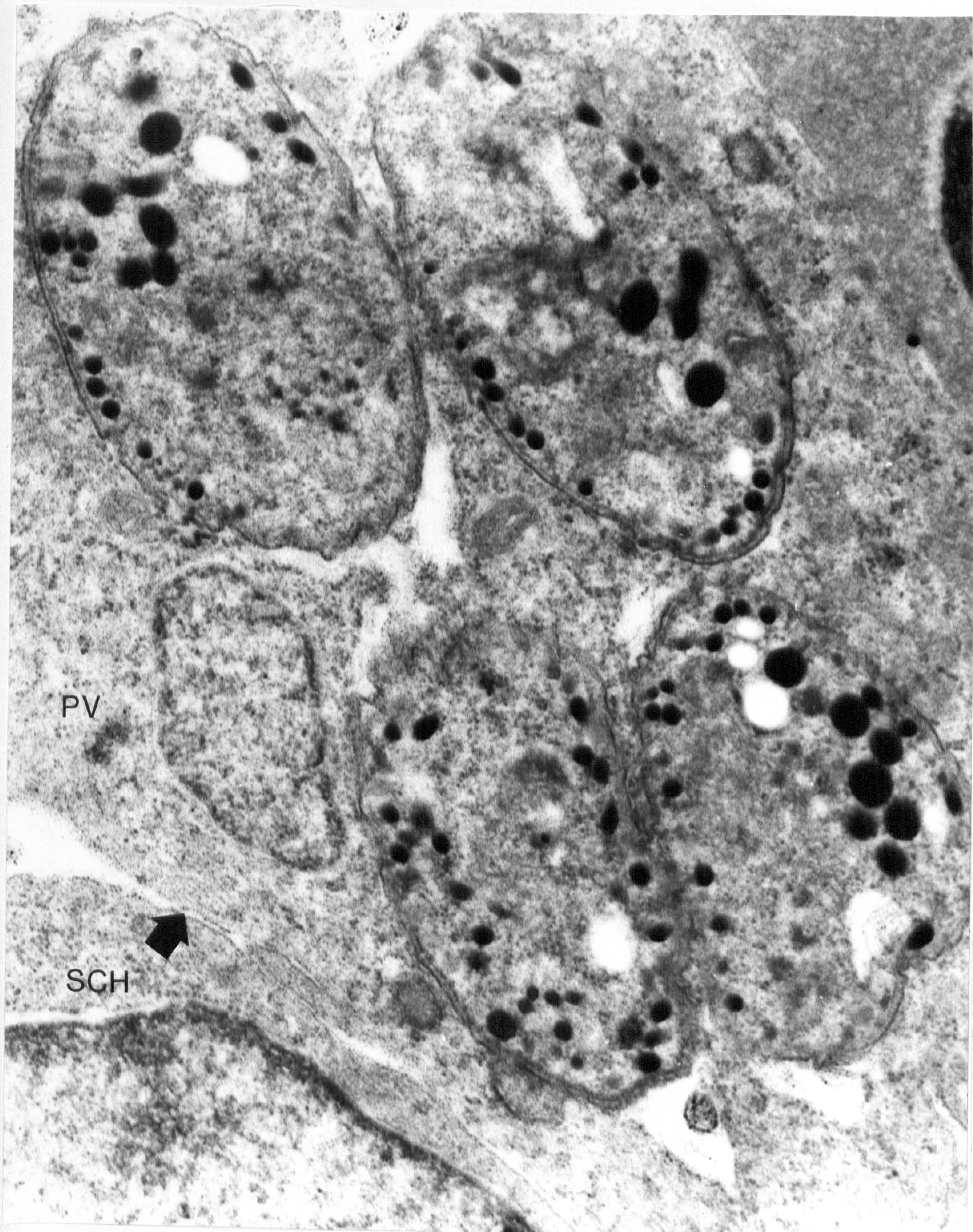
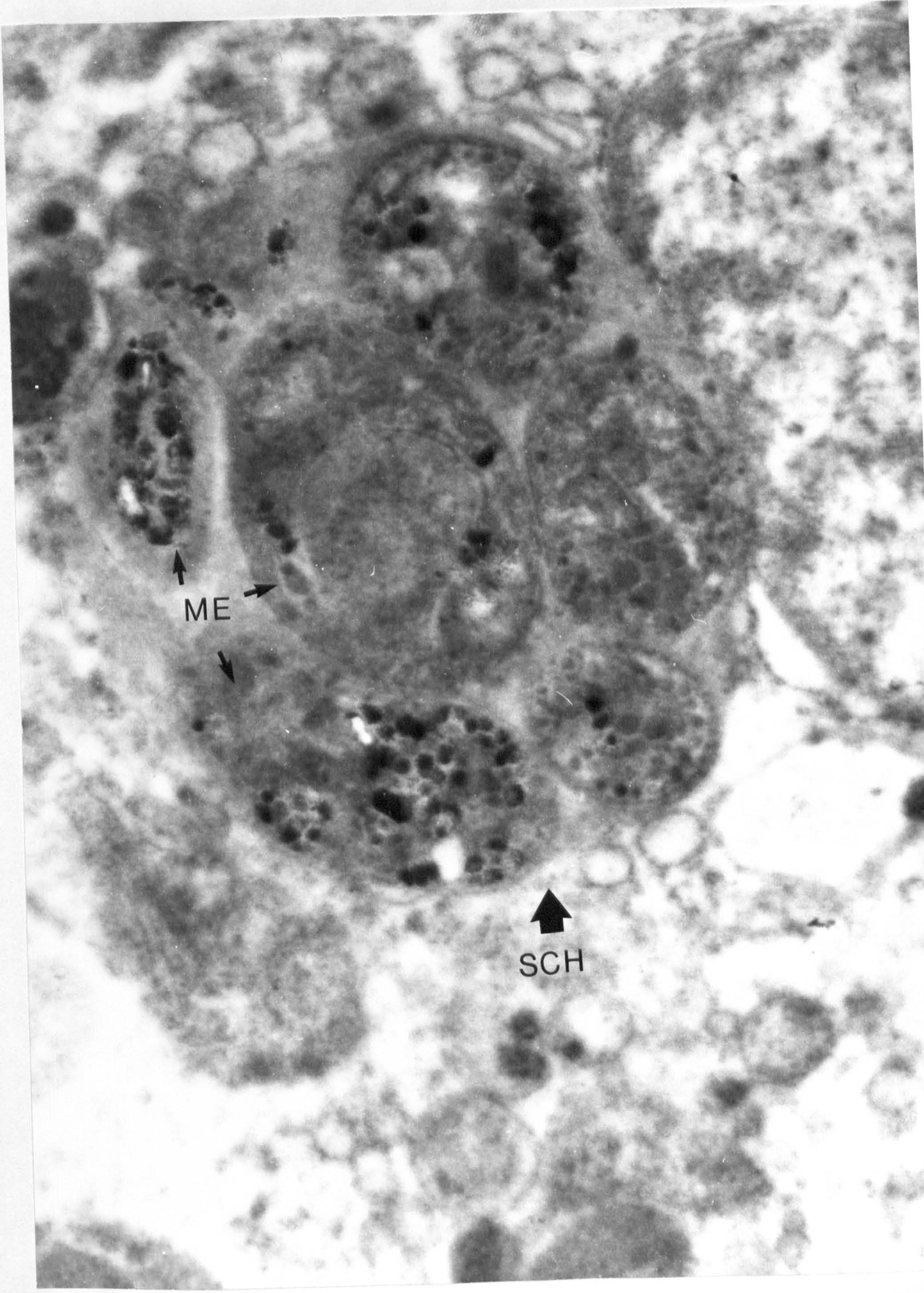

PV
SCH

FIGURE 106.

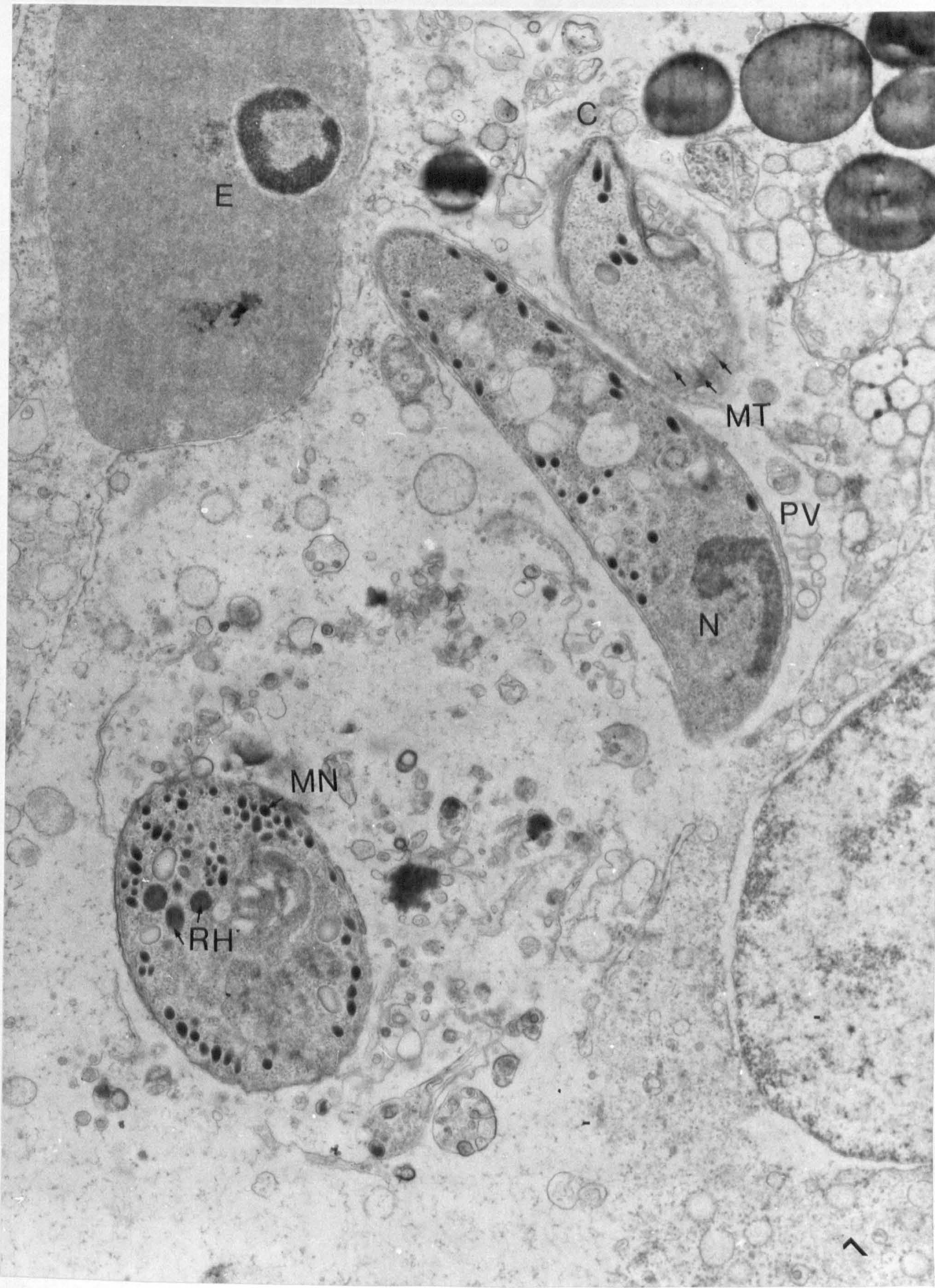
Schizont with 6 merozoites(ME)
x 12,500



ME
SCH

FIGURE 107.

Free merozoites in intracellular space.Numerous micronemes (MN) in one parasite x 7,200



E
C
MT
PV
N
MN
RH

FIGURE 108.

Transverse section of merozoite surrounded by cellular debris.Note well defined nucleus (N) with distinct double nuclear membrane (NM) and clearly visible nuclear pores (NP). An apparently sphaeric refractile body (RB) and what appears to be the "tail-end"(?) of the merozoite(T) can also be observed x 52,000

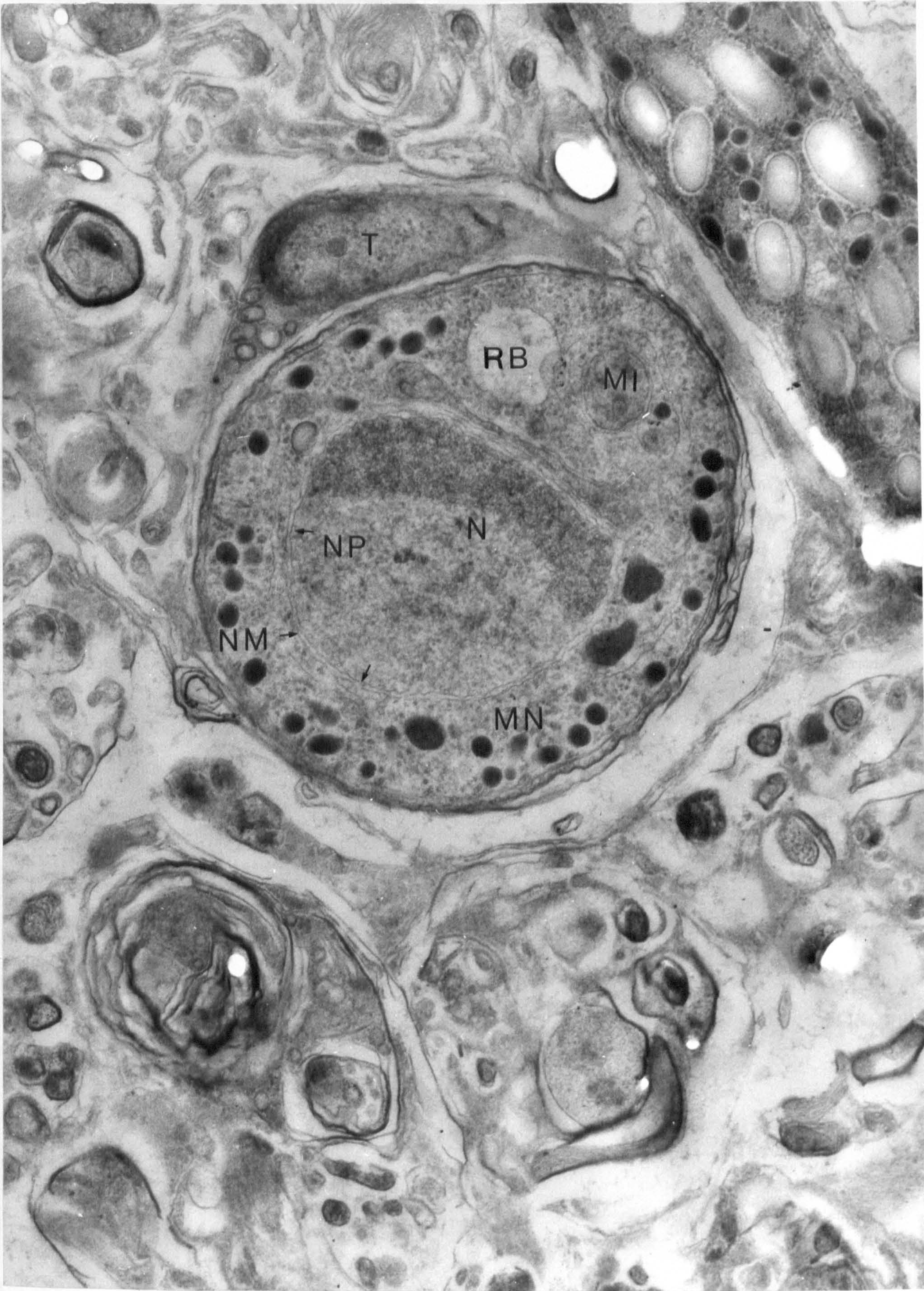

T
RB
MI
N
NP
NM
MN

FIGURE 109.

Intraleucocytic schizont with
2 merozoites and a disintegrated
schizont with remainders of
parasites and cellular debris,
presumably at the outer edge of
a melano-macrophage centre of
the spleen x 3,400

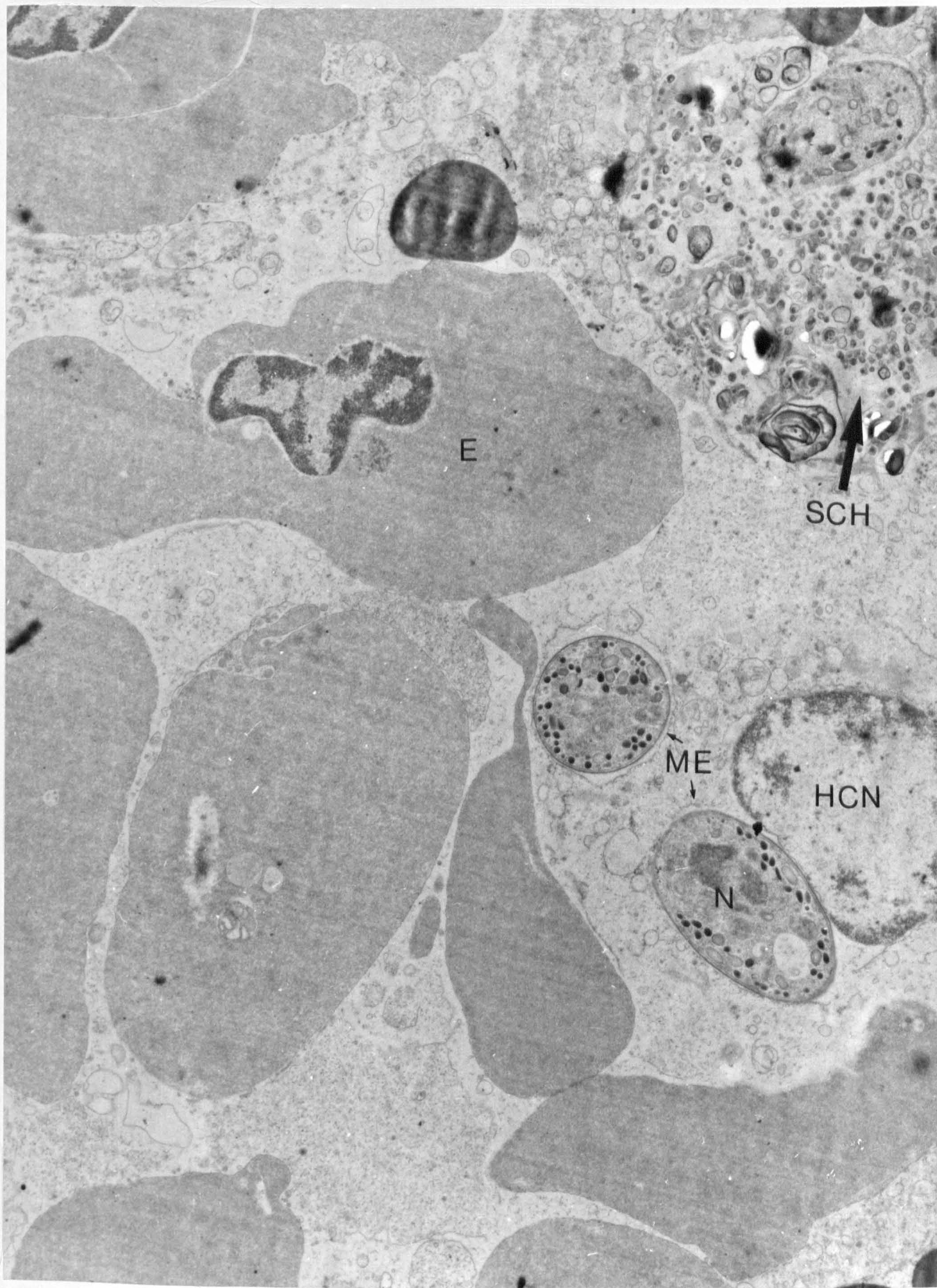

E
SCH
ME
HCN
N

FIGURE 110.

Disintegrated schizont with remainders of intracellular merozoites (SCH) x 2,200

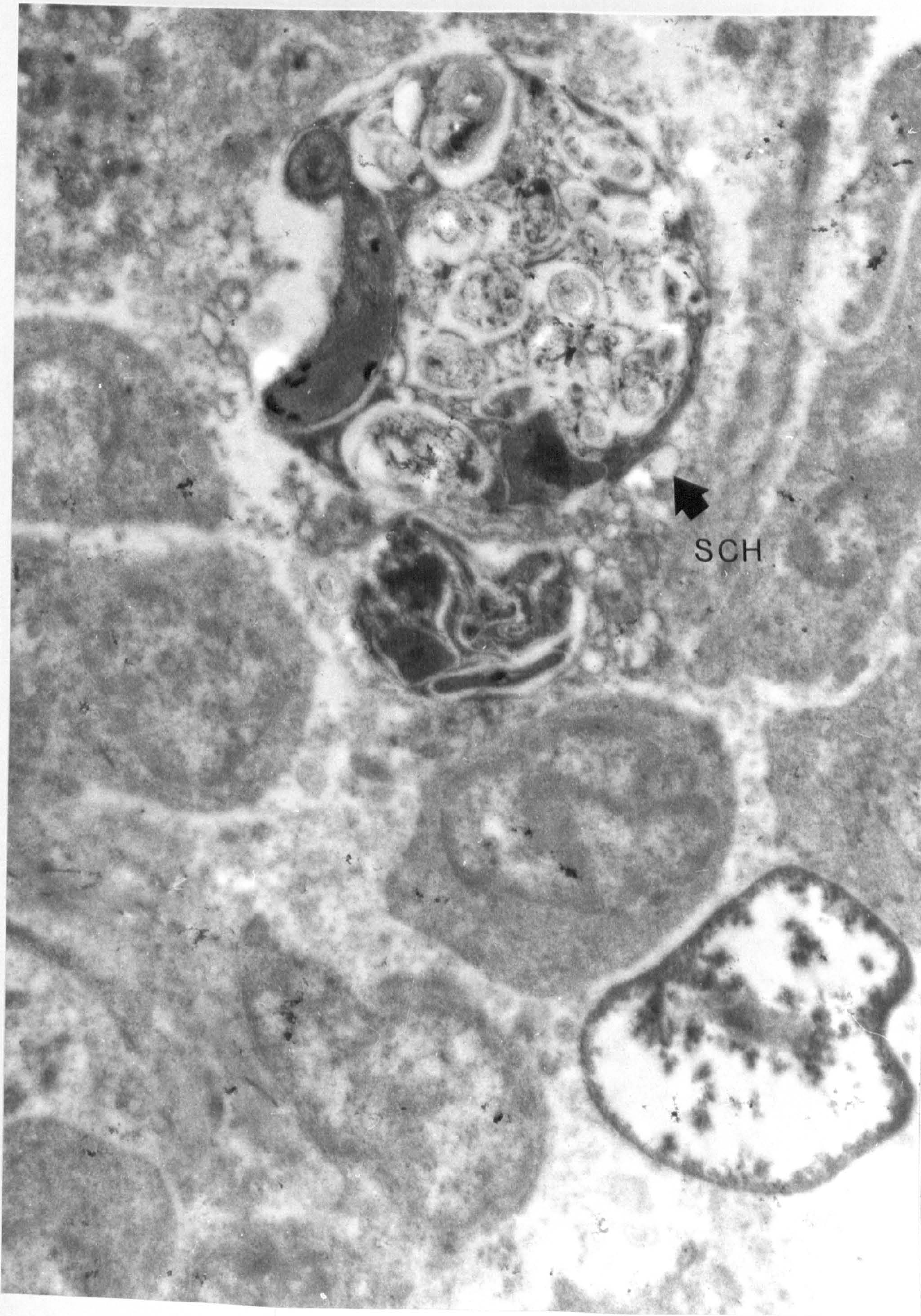

SCH

FIGURE 111.

Several disintegrated schizonts with still clearly recognizable merozoites (ME) in melano-macrophage centre of the spleen x 5,500

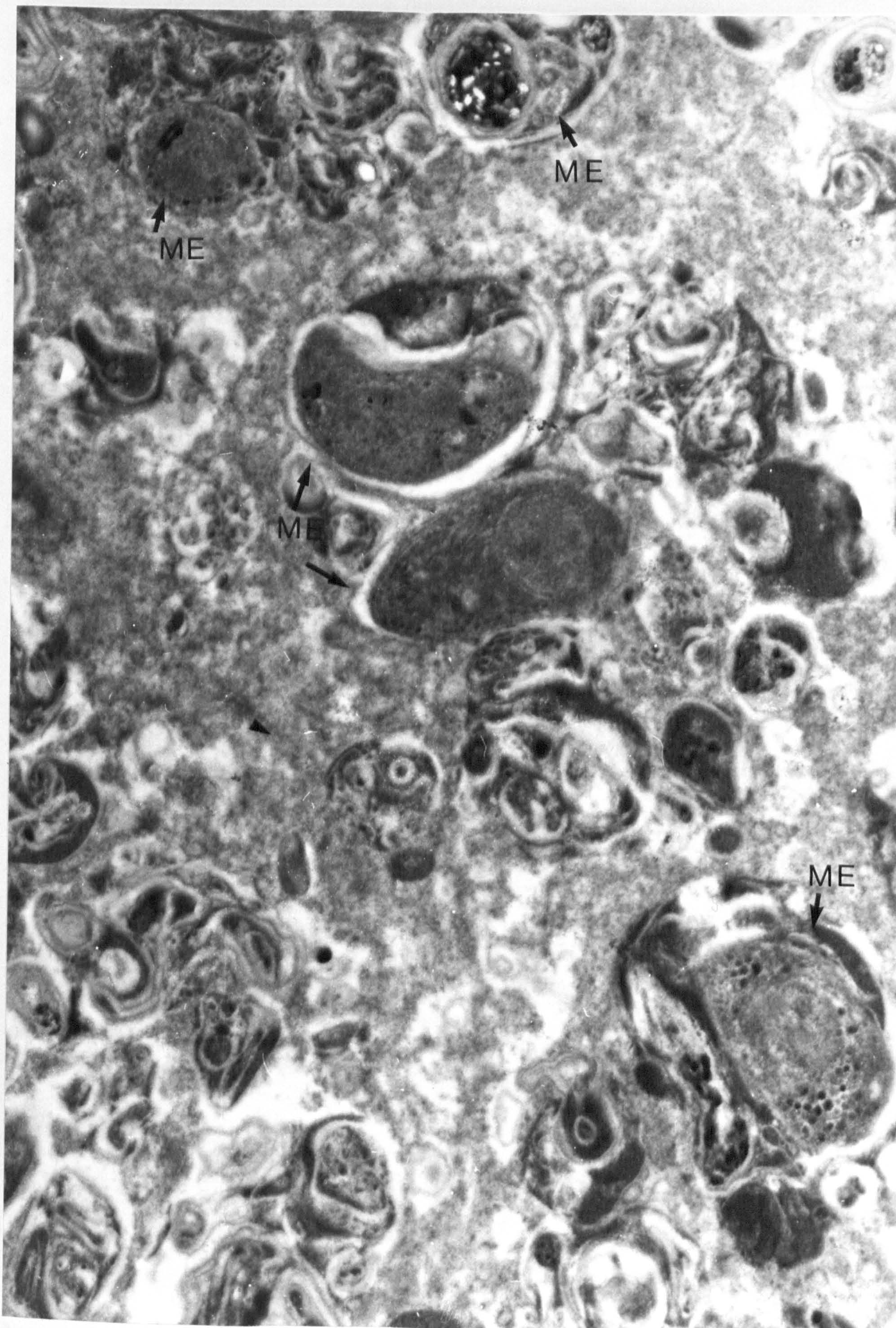
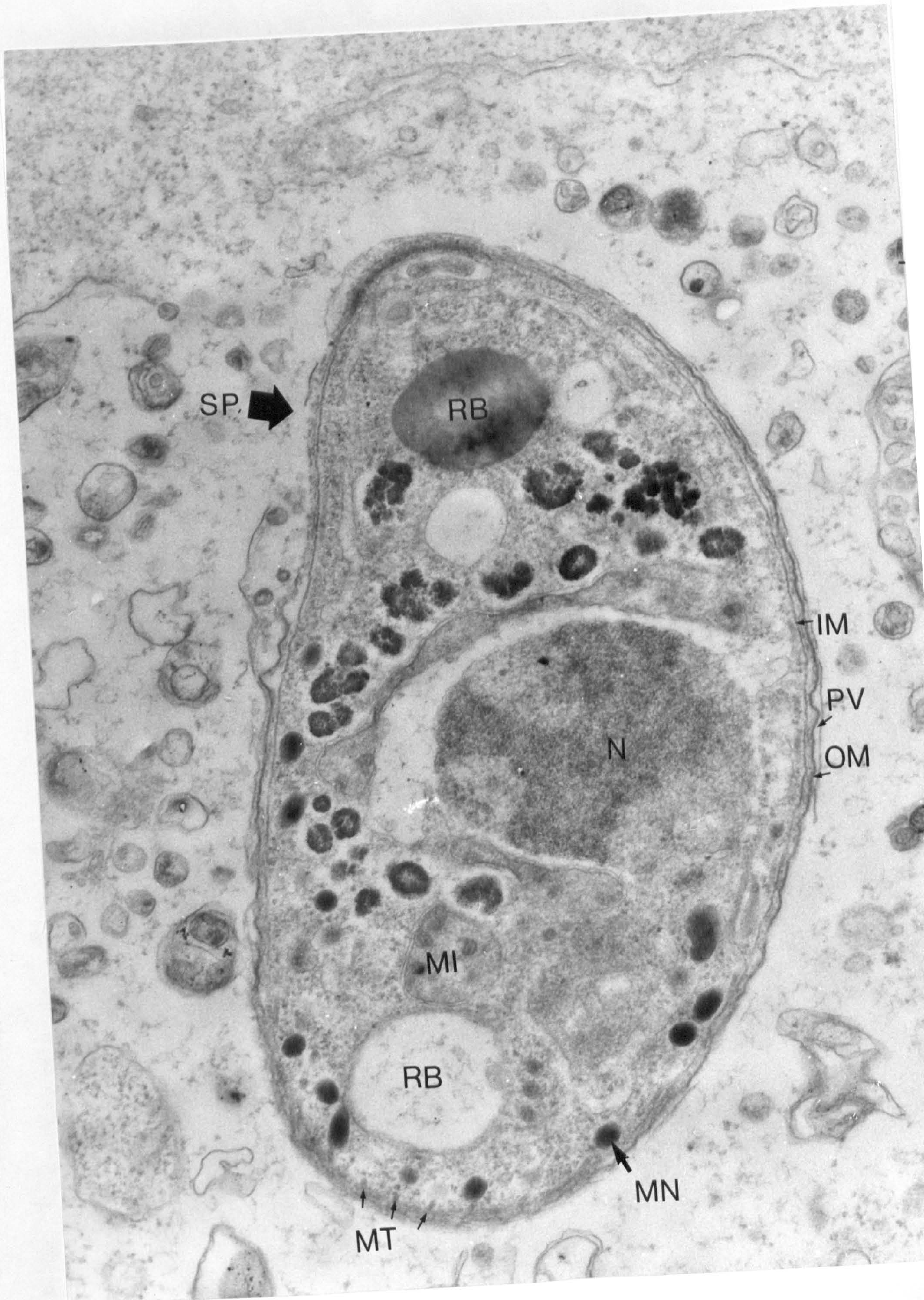

ME
ME
ME
ME

FIGURE 112.

Free parasite with 2 distinct refractile bodies (RB) at opposite poles and considered to be a sporozoite (SP) x 95,000


SP
RB
IM
PV
N
OM
MI
RB
MN
MT

FIGURES 113 - 128.

ULTRASTRUCTURE OF *HAEMOGREGARINA SIMONDI*

FROM *SOLEA SOLEA*

FIGURE 113.

General view of intravascular space in spleen with 2 intra-leucocytic schizonts x 3,400

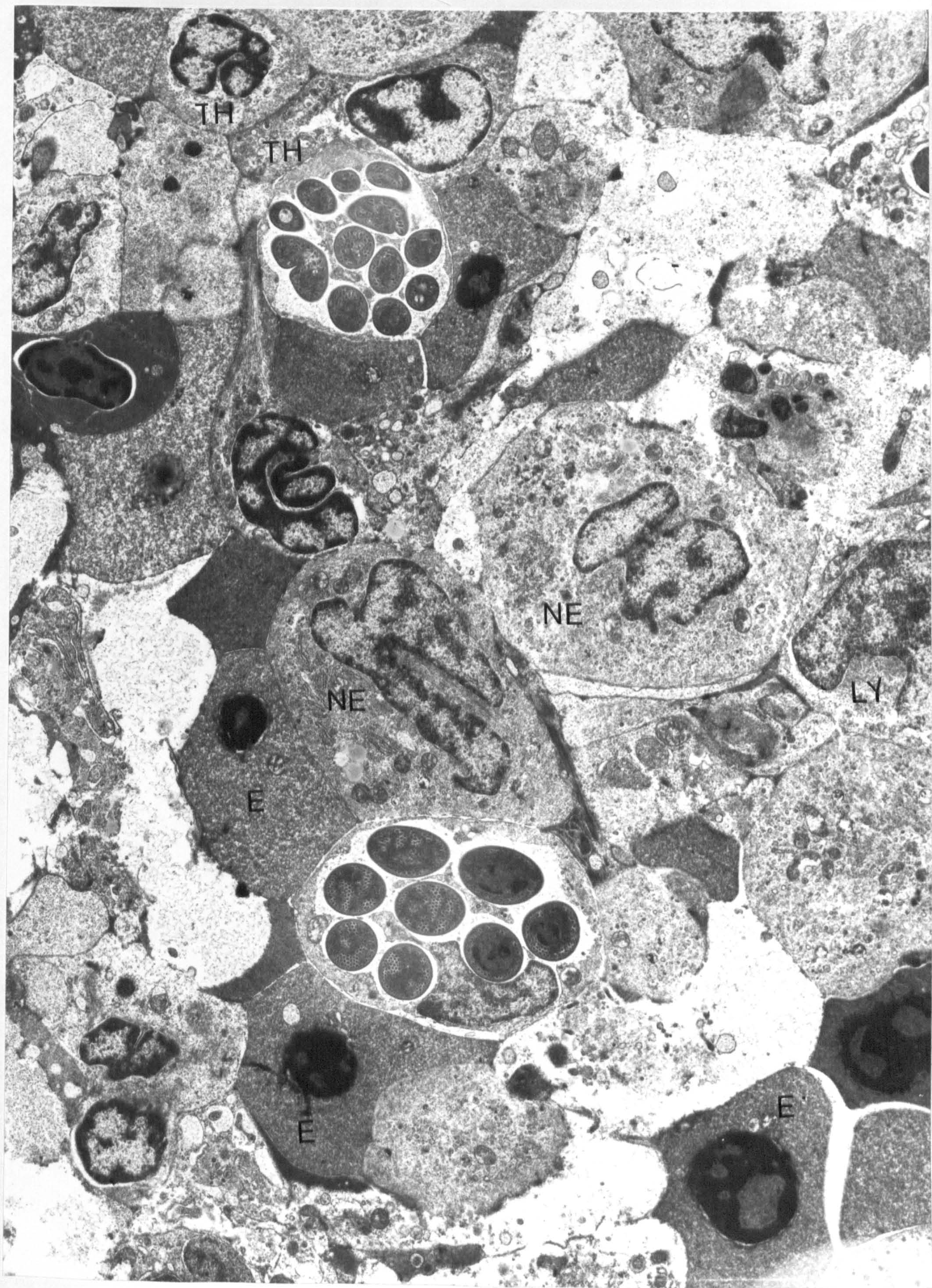

TH
TH
NE
NE
LY
E
E
E

FIGURE 114.

Single intracellular parasite in neutrophil.Probably early merozoite or sporozoite (?) after recent invasion of cell. This is indicated by refractile body(RB) elongate and protruding conoid(C) and the distinct parasitophorous vacuole extended at posterior end of parasite(PV) x 18,000

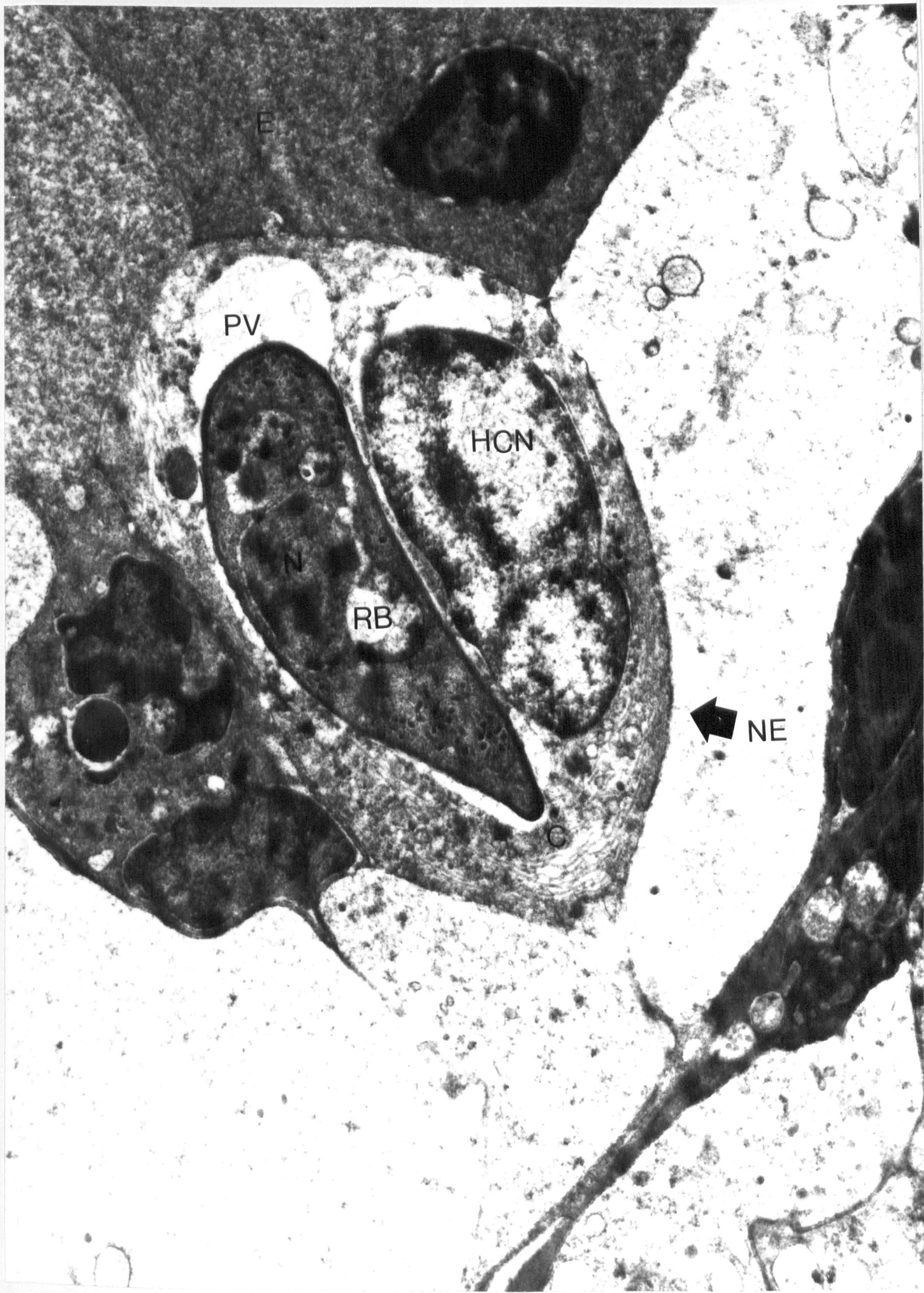

E
PV
HCN
N
RB
C
NE

FIGURE 115.

Schizont of 3 merozoites in a parasitophorous vacuole and refractile bodies(RB).A conoid(C) protruding beyond the polar ring is clearly visible on the right x 18,800

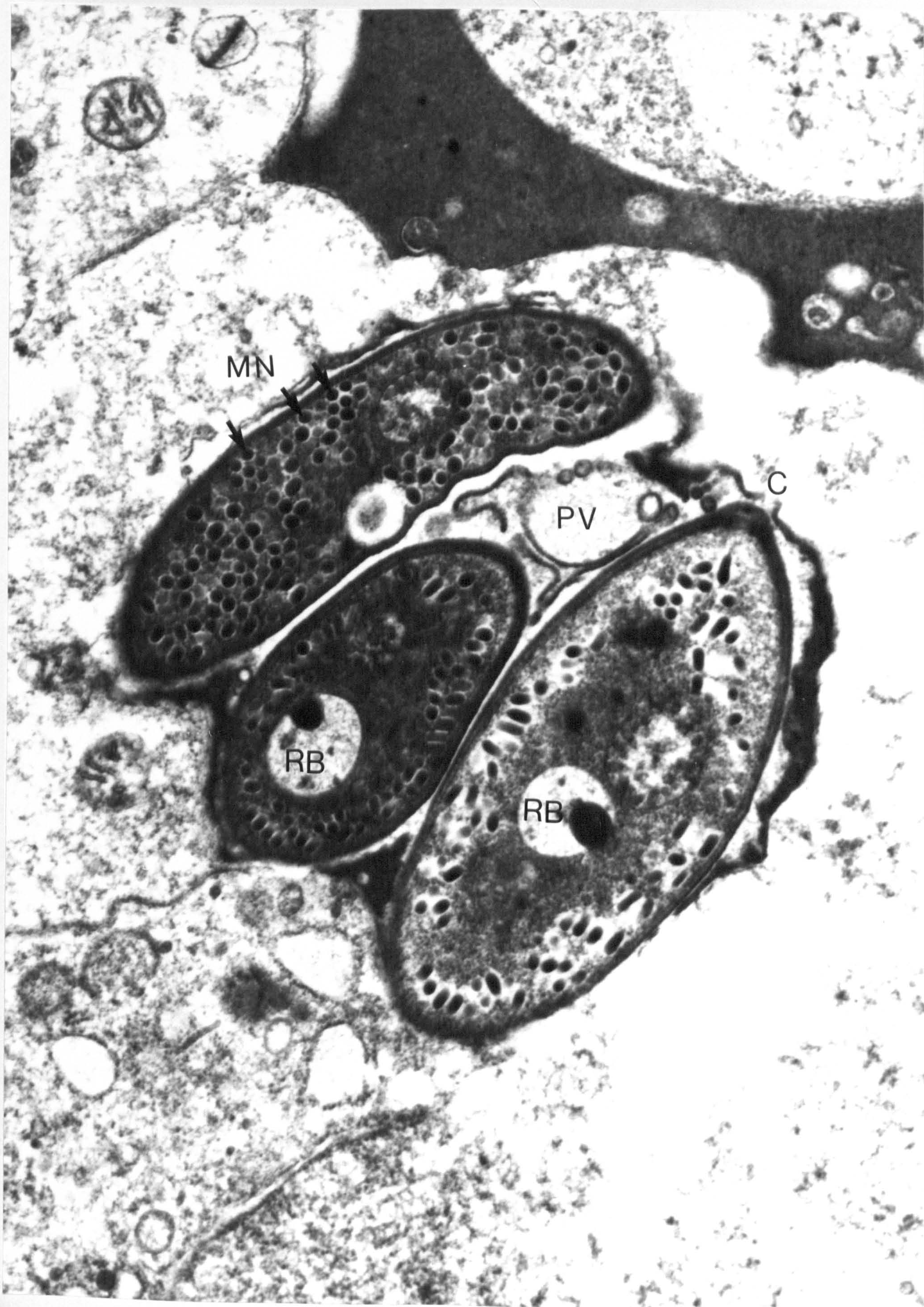

MN
C
PV
RB
RB

FIGURE 116.

Schizont with 6 merozoites
in the intracellular space
x 14,100

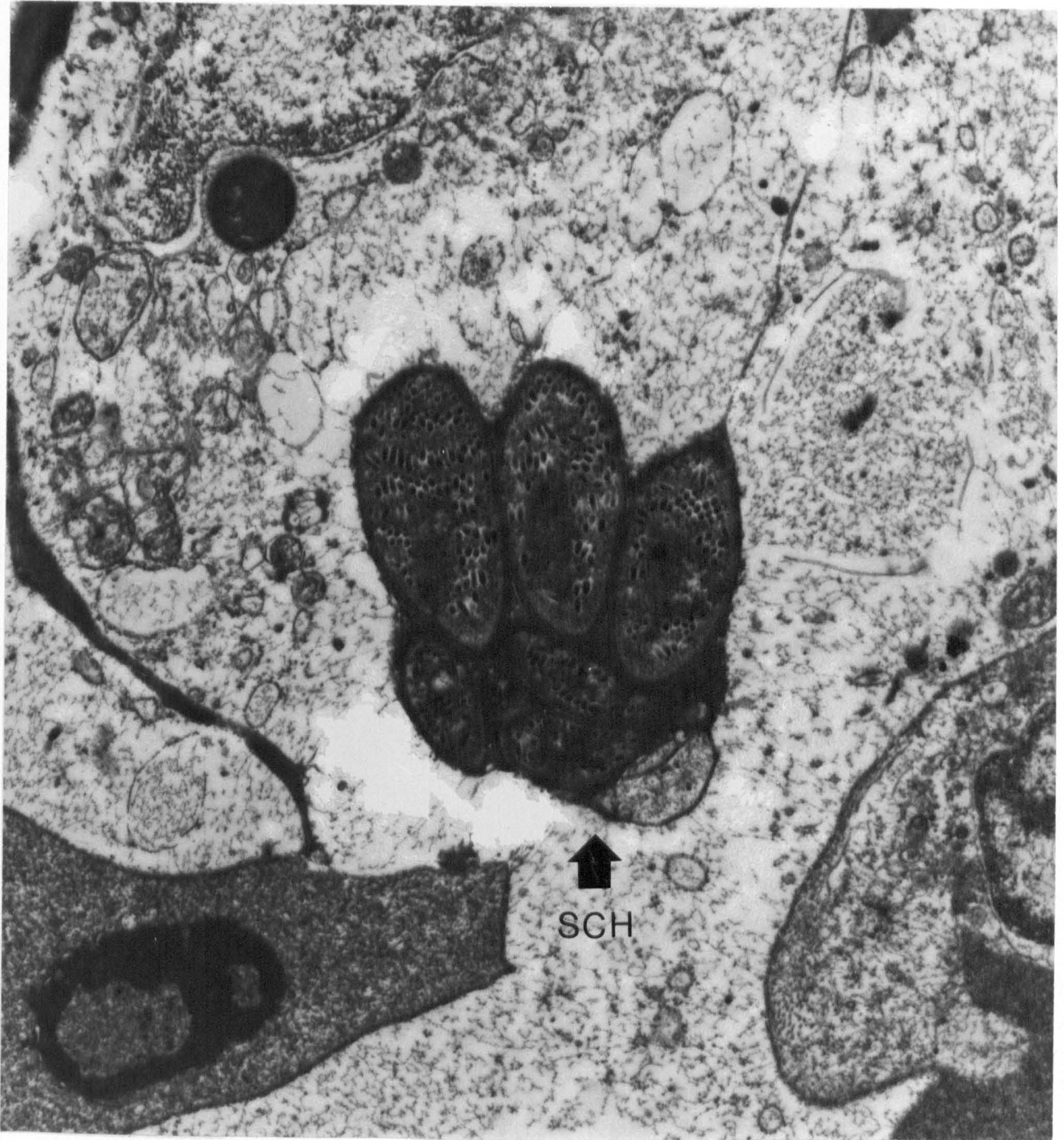

SCH

FIGURE 117.

Intraleucocytic schizont with
8 merozoites in cross-section.
Host cell nucleus appears
compressed to the host cell wall
x 18,800

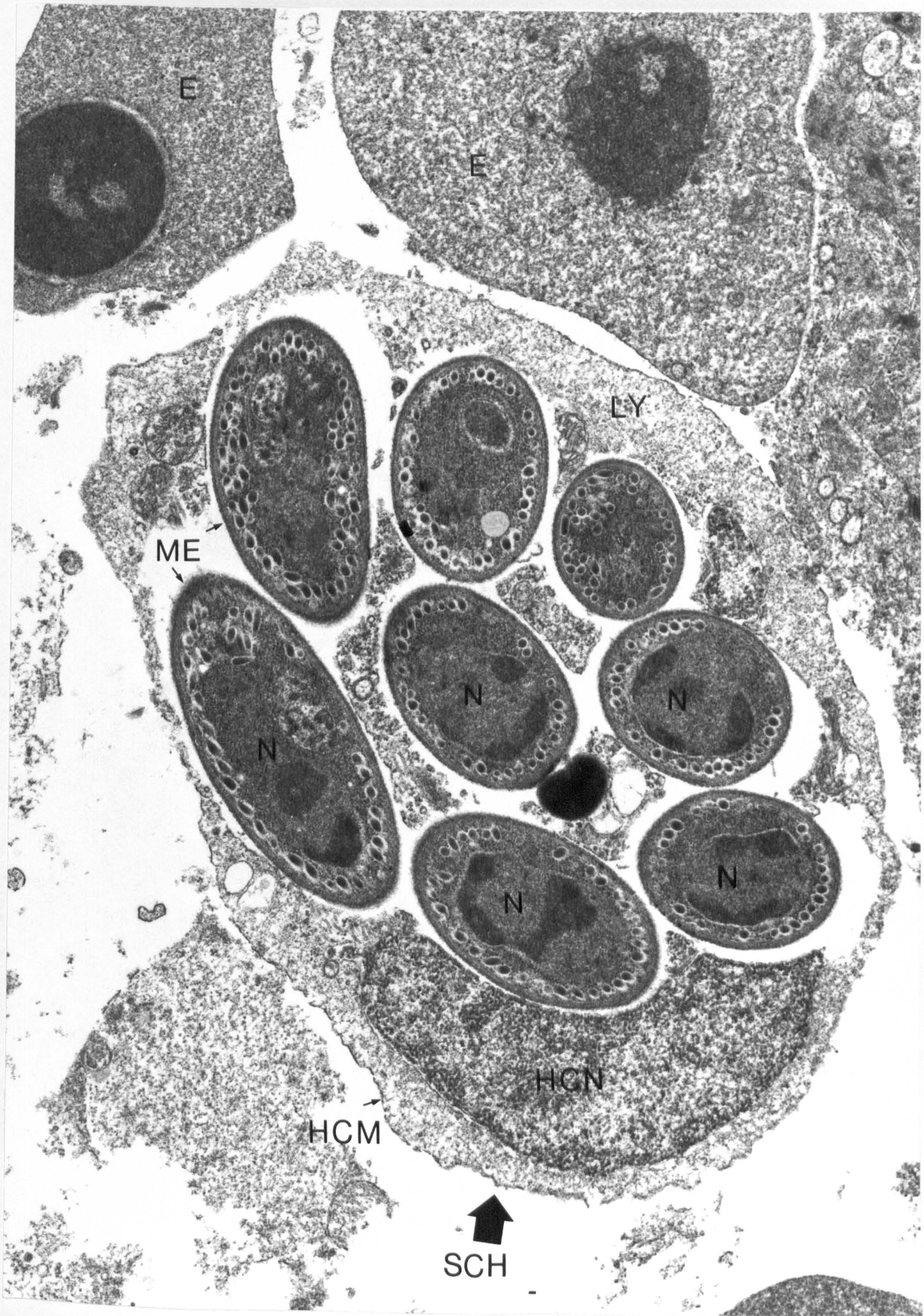

E
E
LY
ME
N
N
N
N
N
HCN
HCM
SCH

FIGURE 118.

Intraleucocytic schizont with
8 merozoites from the blood.The
section has separated the broader
anterior portion of the parasites
containing the nucleus from the
posterior end x 11,200

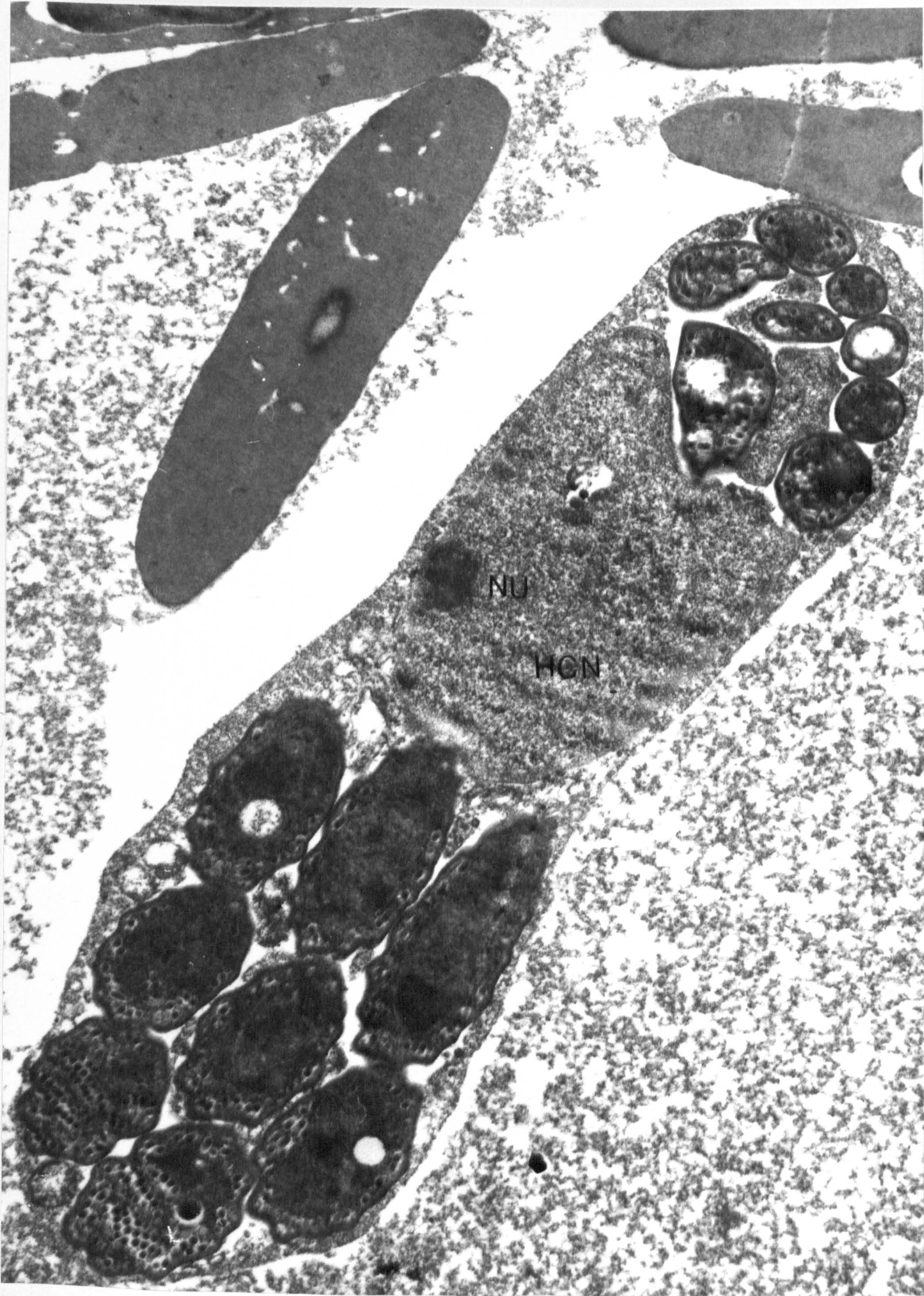

NU
HCN

FIGURE 119.

Intraerythrocytic schizont
from the spleen with 8 merozoites
x 18,800

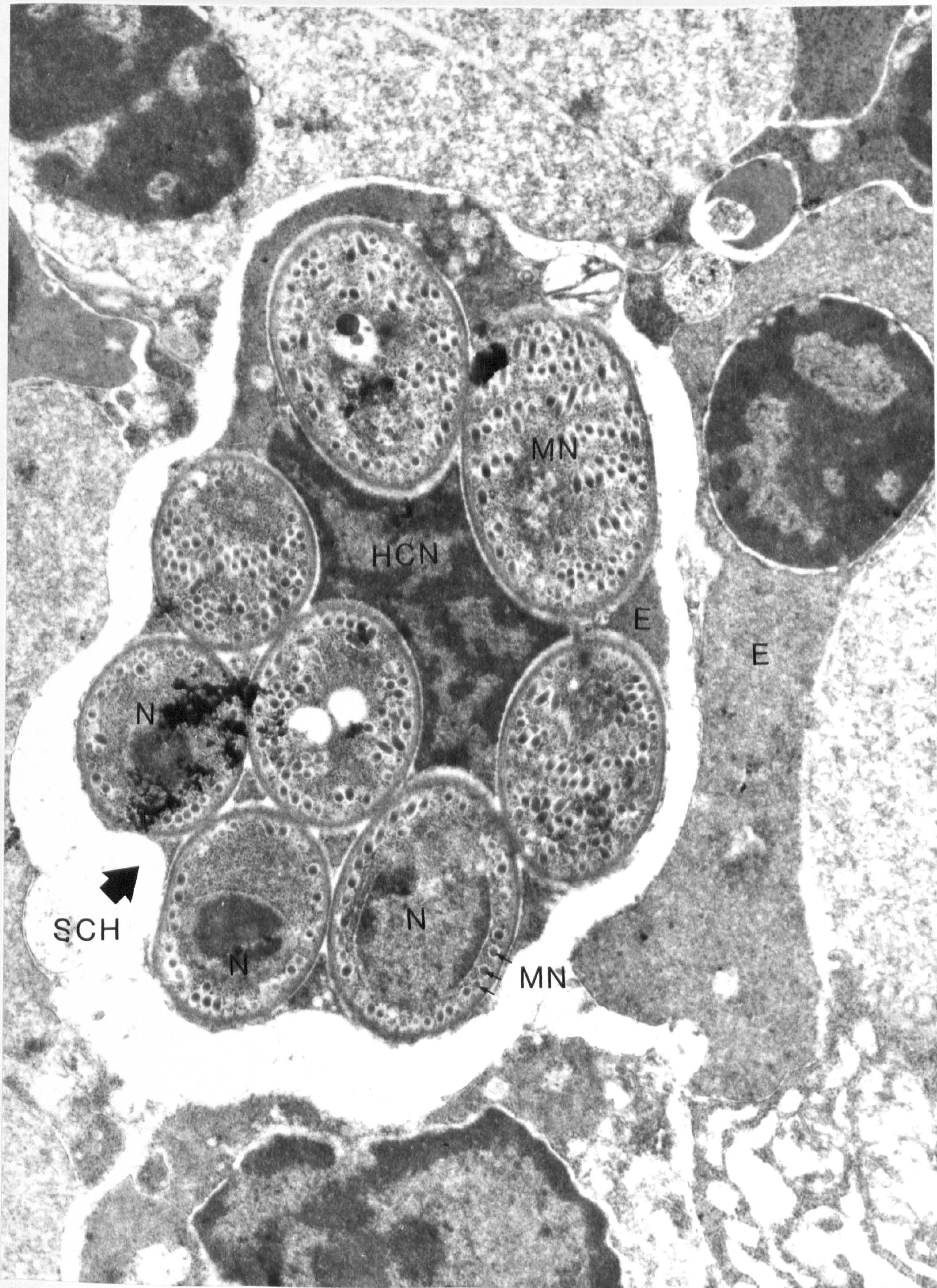

MN
HCN
E
E
N
SCH
N
N
MN

FIGURE 120.

Mature intraerythrocytic schizont.Only the remnants of the host cell nucleus are visible(HCN).The 8 curved merozoites are again sectionned twice x 17,600

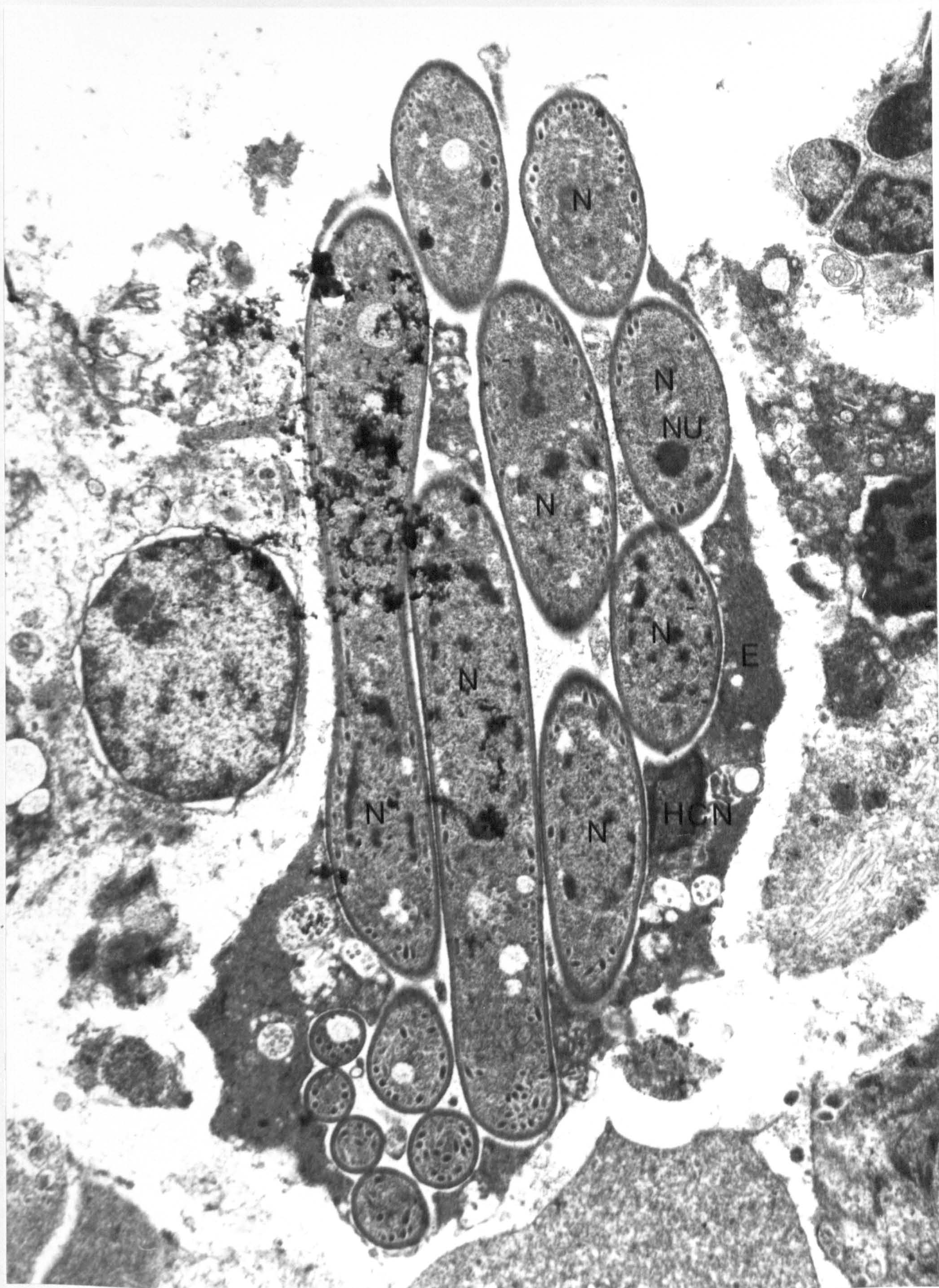

N
N
NU
N
N
E
N
N
HCN
N

FIGURE 121.

Eight free merozoites after being released from a schizont which apparently has disintegrated including the host cell
x 18,800

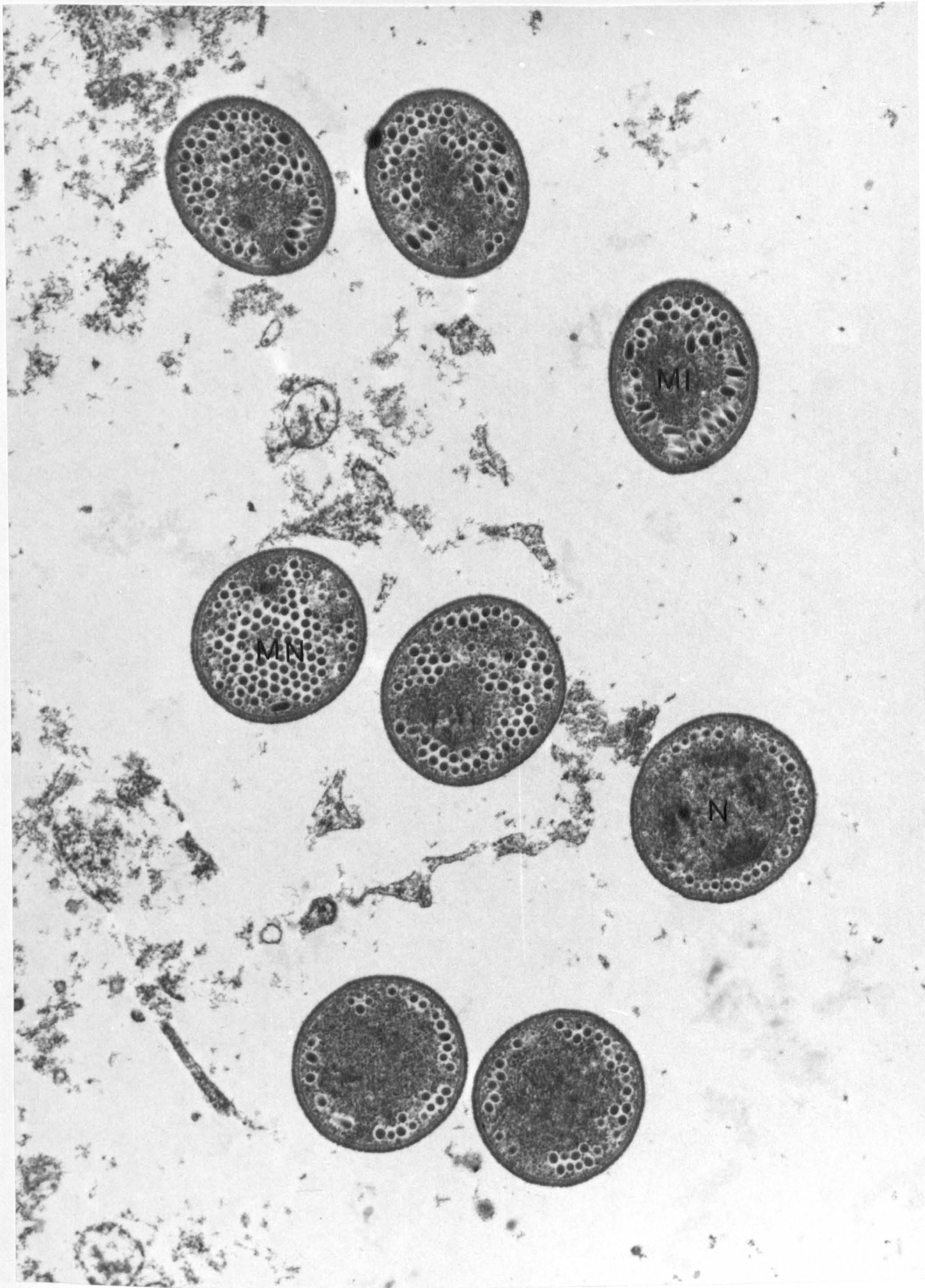

MI
MN
N

FIGURE 122.

Disintegrated schizont
releasing mature merozoites.
A transverse section near the
apical pole shows polar ring(P),
rhoptries(RH) with ductules of
the rhoptries(DRH) and distinct conoid
(C) also seen in the other parasites.
Large numbers of micronemes(MN) and
microtubules (MT) are also seen
x 38,000

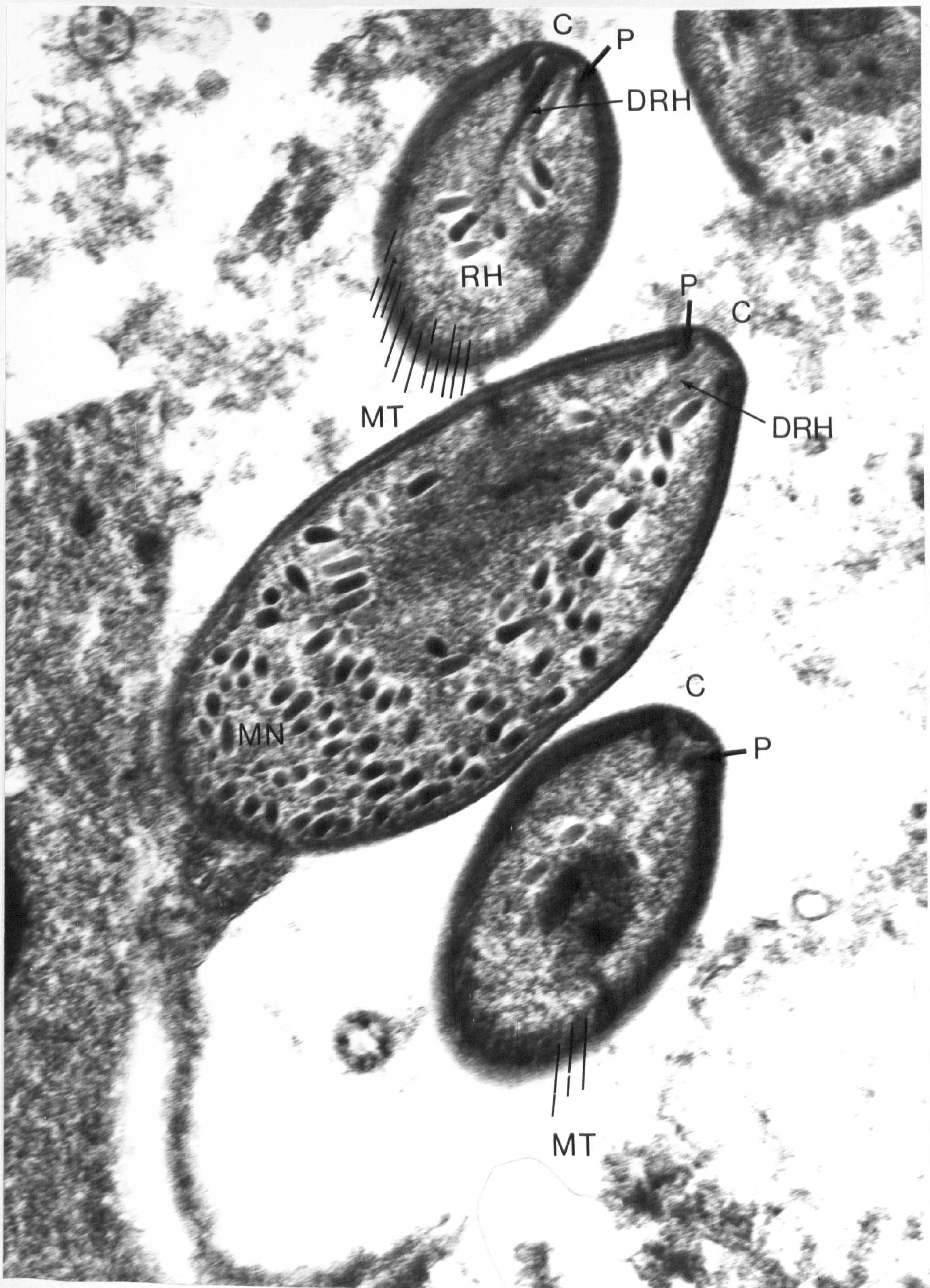

C
P
DRH
RH
MT
P
C
DRH
MN
C
P
MT

FIGURE 123.

Several individual merozoites of a schizont.A tangential section shows the parallel running microtubules (MT),also seen in a transverse section. Another parasite in cross-section shows a characteristic triple unit membrane of the pellicle (OM+MM+IM) x 38,000

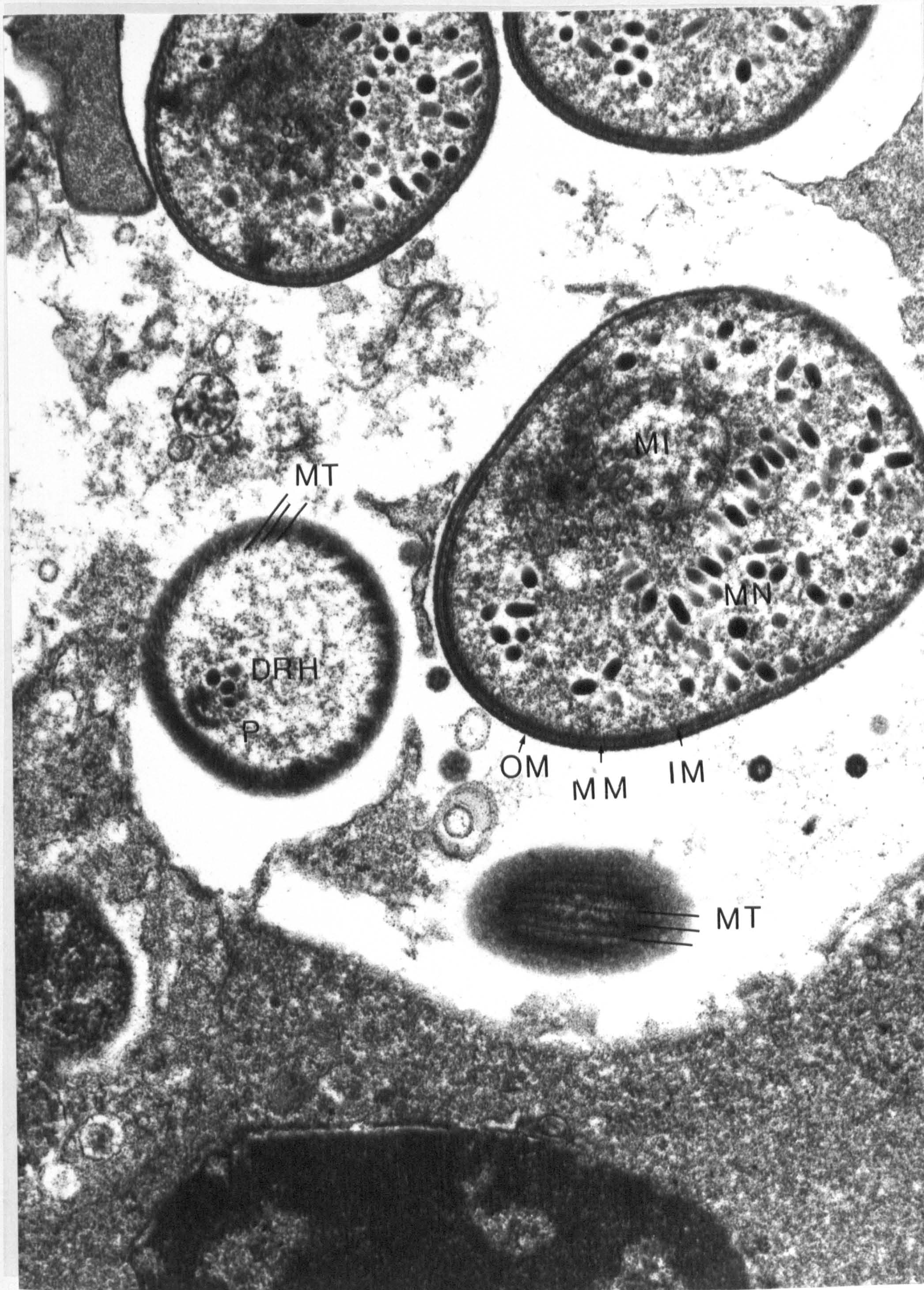

MT
MI
MN
DRH
P
OM
MM
IM
MT

FIGURE 124.

Enlargement of transverse section of merozoite from previous figure to show the 45-60 microtubules (MT), a distinct polar ring (P) and cross-sections through the posterior ends of 3 rhoptries (RH) x 122,360

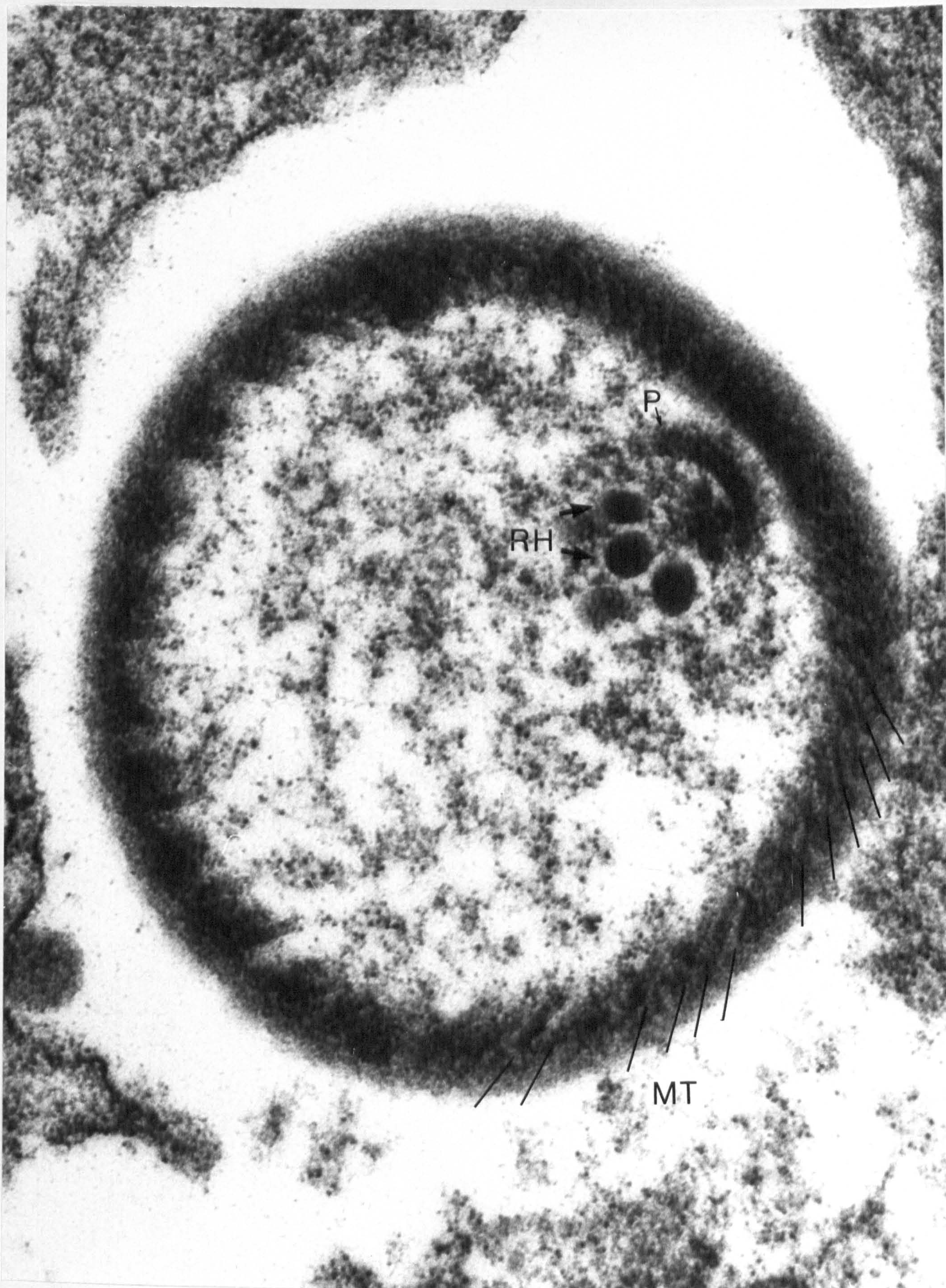

P
RH
MT

FIGURE 125.

Division(?) of merozoite inside an intraleucocytic schizont showing again the triple layered pellicle (OM+MM+IM) x 58,900

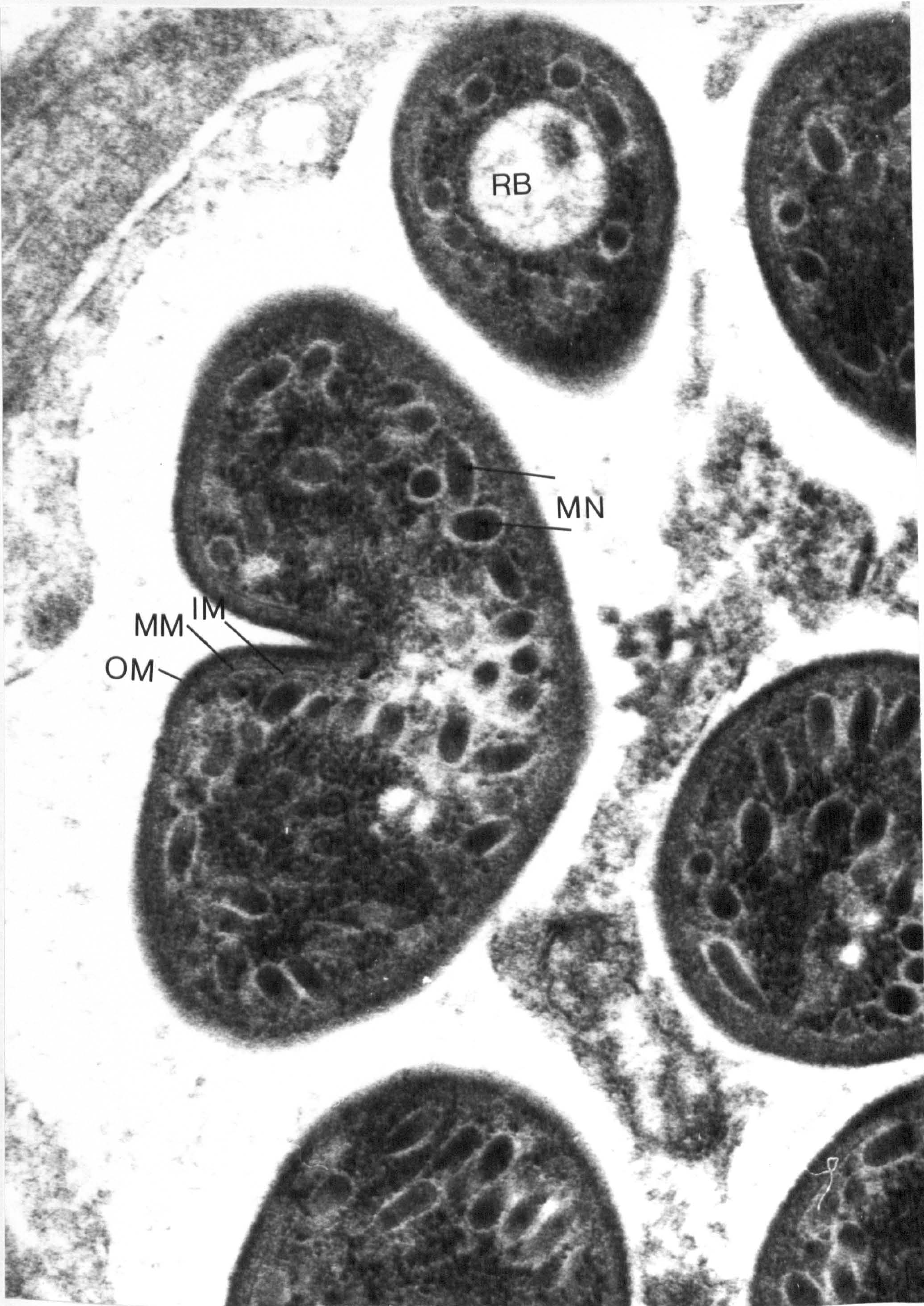

RB
MN
IM
MM
OM

FIGURE 126.

Intraerythrocytic schizont showing from 2-4 mature merozoites in length section.Micronemes are numerous.Also 2-3 paranuclear refractile bodies are visible and small vacuoles or amylopectin granules are found near either pole x 17,600

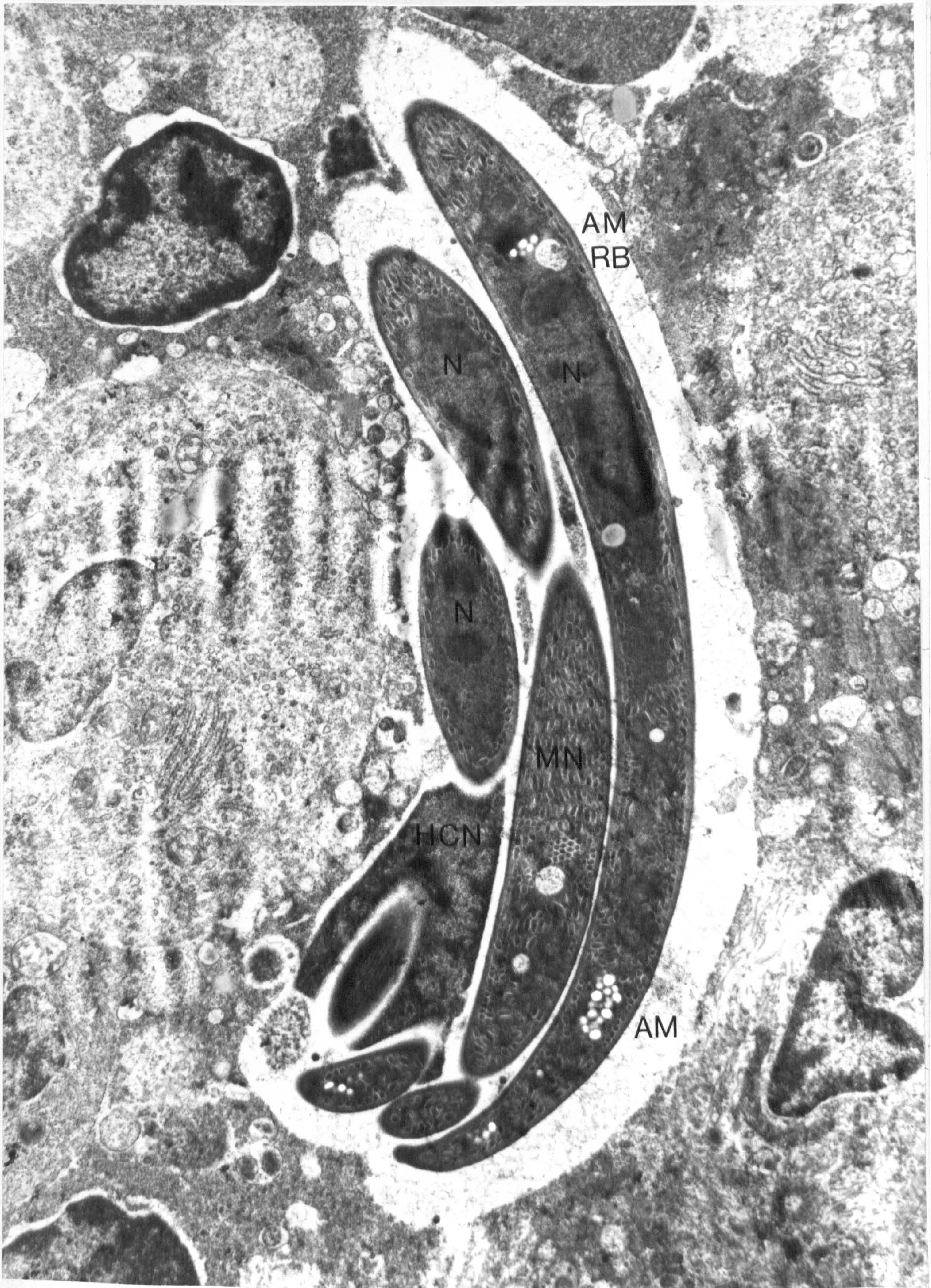

AM
RB
N
N
N
MN
HCN
AM

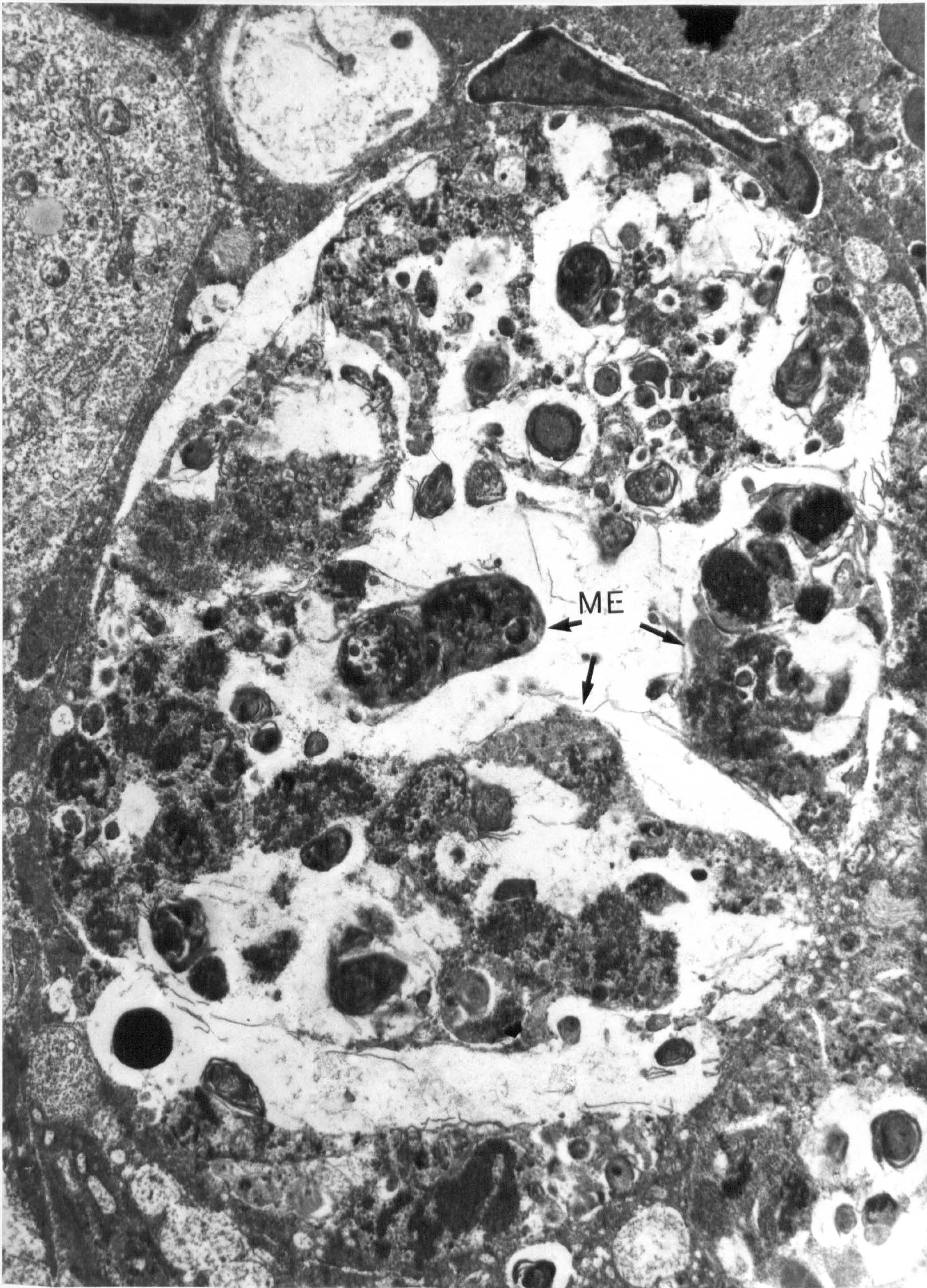
FIGURE 127.

Free banana-shaped organism after rupture of host cell and considered micro-merozoite. A large paranuclear refractile body,numerous micronemes and microtubules extending from the apical pole to the posterior end are clearly visible x 22,000

MN
MT
RB
NM
MI
RB
N
MIG
MT

FIGURE 128.

Disintegrated schizont with cell debris and remainders of merozoites from the melano-macrophage centre (spleen)
x 17,600


ME

FIGURES 129 - 134.

ULTRASTRUCTURAL COMPARISON BETWEEN DIFFERENT DEVELOPMENTAL STAGES OF *H. SACHAI* N.SP. AND *H. SIMONDI*

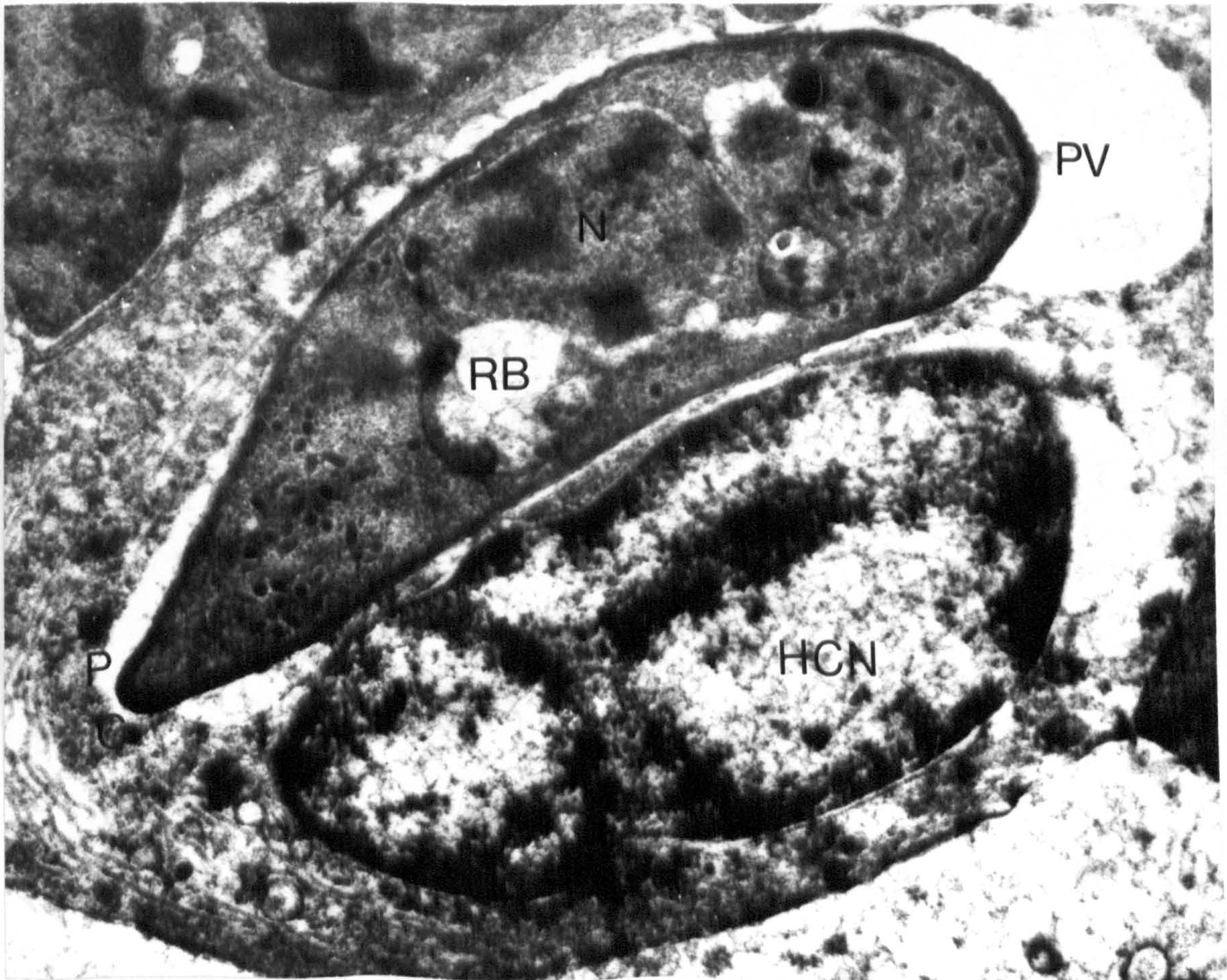
Recently invaded early merozoite

FIGURE 129.

H. sachai n.sp.
x 18,000

FIGURE 130.

H. simondi
x 36,000


PV
N
RB
HCN
P
C

Free merozoites

FIGURE 131.

H. sachai n.sp.
x 23,750

FIGURE 132.

H. simondi
x 11,750

HCN
RH
MN
OM
C
P
V

OM
N
Nu
MN

Intraleucocytic schizonts

FIGURE 133.

H.sachai n.sp.

x 23,750

FIGURE 134.

H.simondi

x 11,750

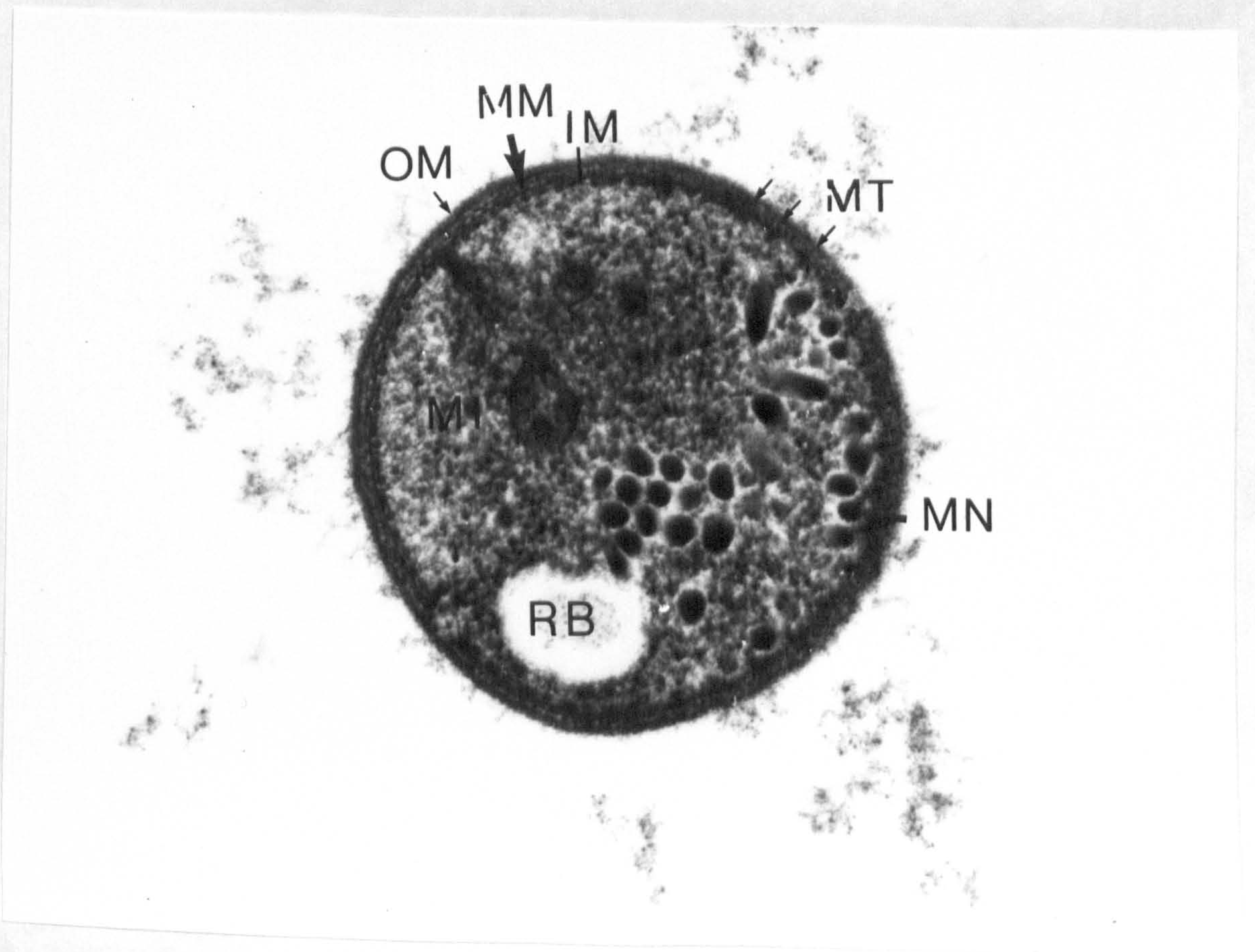
ME
HCM
HC C
HCN
ME
HCM

FIGURE 135.

Developmental stage of H.simondi in the alimentary tract of Hemibdella soleae: Zygote with 3 nuclei in different phases of division x 11,750

FIGURE 136.

Developmental stage of H.simondi in the copepod Lernaeocera sp.: free mature merozoite with refractile body (RB) and pellicle with triple membrane (OM+MM+IM) x 23,750


MM
IM
OM
MT
MI
MN
RB

FIGURES 137 - 138.

TRYPANOSOMA SP. - DEVELOPMENTAL FORMS FROM

THE ALIMENTARY TRACT OF CALLIOBDELLA NODULIFERA

FIGURE 137.

Developmental stage of
trypanosome showing large
mitochondrion(MI),lobed nucleus
(N),shaft of flagellum(F) and
granular cytoplasm(G)
x 2,600

FIGURE 138.

Section of developmental
stage of a trypanosome
from C.nodulifera showing
elongate nucleus(N),rough
endoplasmic reticulum (RER)
and pellicular microtubules(MT)
x 6,500

F
G
CR
N
V
MI

V
NP
G
N
NM
RER
V
MT

GENERAL DISCUSSION AND CONCLUSIONS

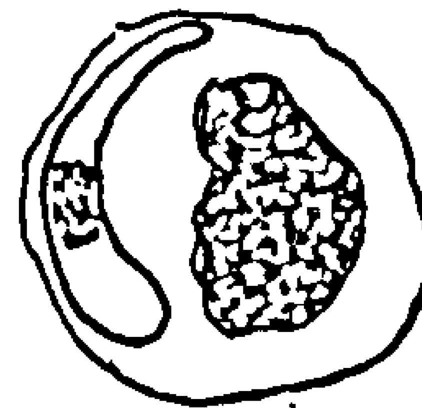
The present study has revealed all of the most significant stages of the life cycle of *Haemogregarina simondi* in its intermediate host the marine piscicolid leech *Hemibdella soleae* and also in its final host the marine teleost *Solea solea* (see Diagram I).

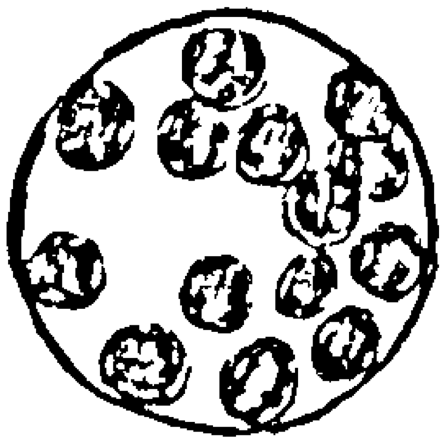
The main sites of schizogonic development in the final host were the leucocytes and erythrocytes of the circulating blood; the exact site for the development of the haemogregarine in the digestive tract of the intermediate host has not yet been determined.

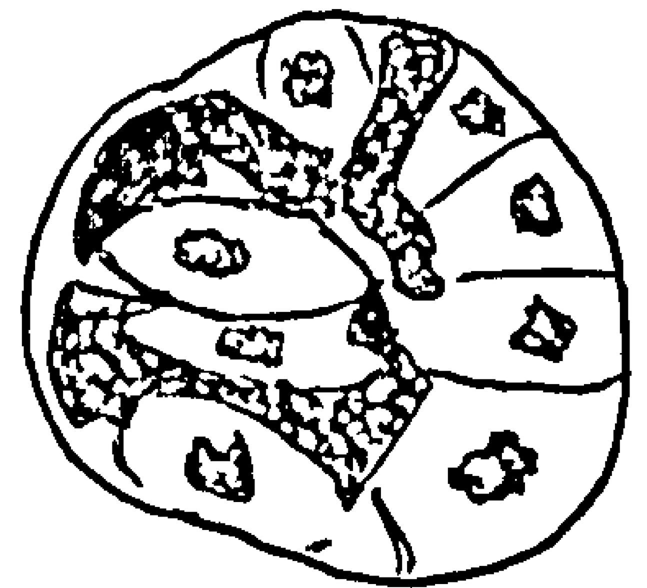
Although syngamy was not observed in the intermediate host, a *zygote* of up to 45.3 by 29.3 micron was seen, which developed into an *oocyst* of up to 248 by 220 micron containing numerous *sporoblasts*. These in turn appeared to leave the oocyst and became *sporocysts* of up to 16.7 by 14.7 micron or more containing an average of 24-26 *sporozoites* of a mean individual size of 9.7 by 0.6 micron.

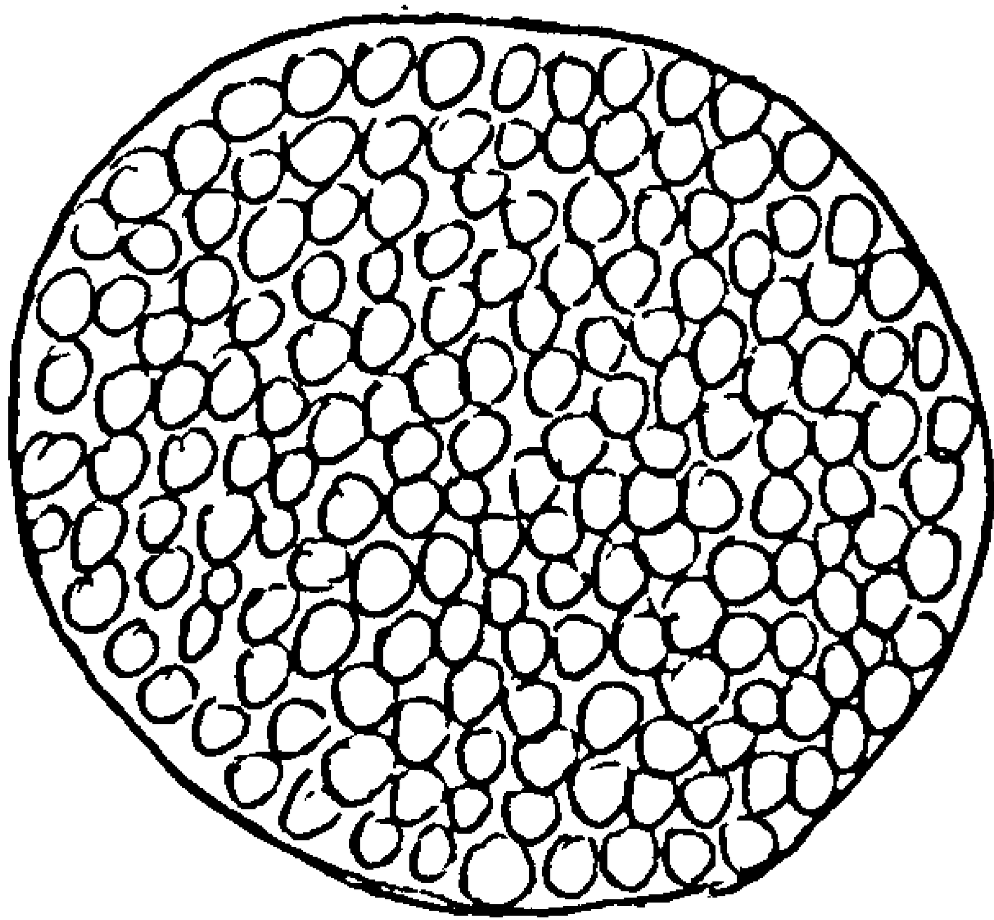
DIAGRAM I

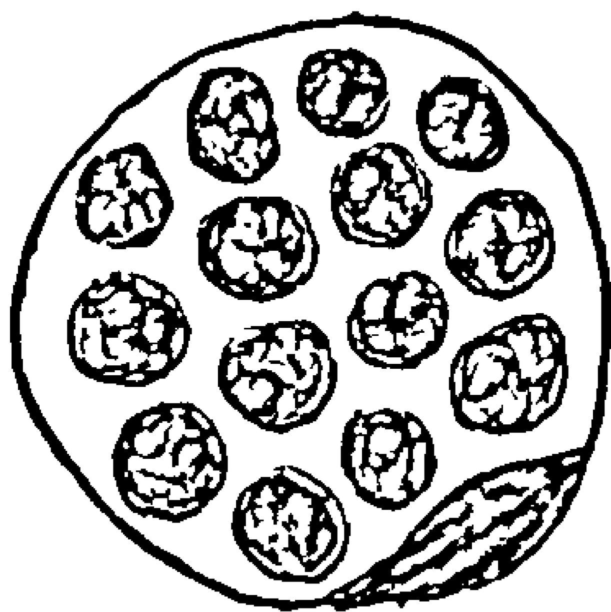
THE PROPOSED LIFE CYCLE OF HAEMOGREGARINA SIMONDI FROM THE BLOOD OF THE MARINE TELEOST SOLEA SOLEA

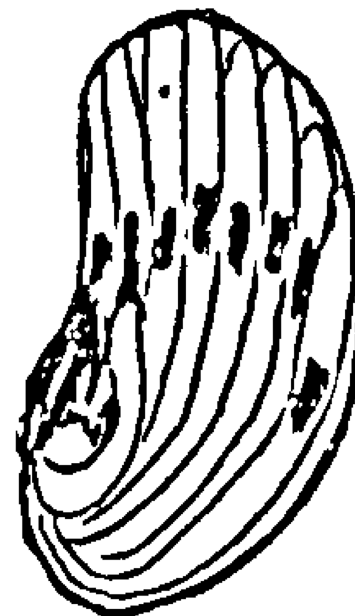


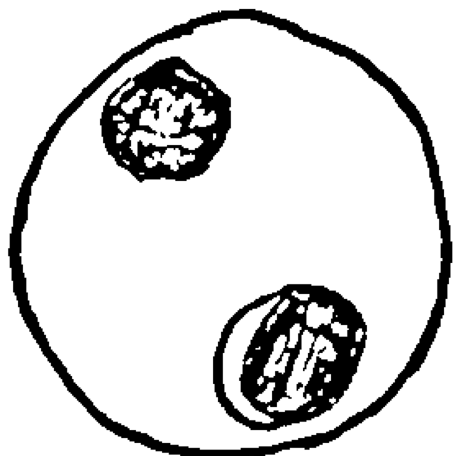


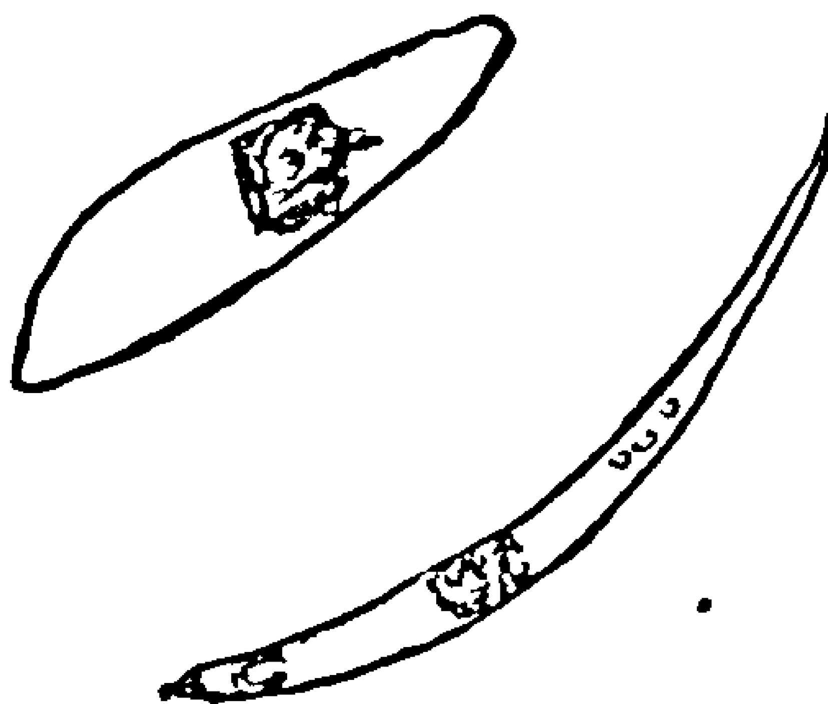












These sporozoites apparently enter the final host via the proboscis of the intermediate host in the course of a blood meal. They immediately enter leucocytes where multiplication takes place and schizonts with up to 8 merozoites are found. The mechanism of cell penetration by the sporozoites was not observed but a proportion of them may also be actively phagocytosed by leucocytes. After the rupture of the intra-leucocytic schizonts the merozoites released enter erythrocytes where a second schizogonic cycle takes place. Again 8 merozoites are produced within these intraerythrocytic schizonts which later apparently differentiate into micro-and macro-gametocytes. This process of differentiation was not observed here. Free micro-gametocytes of an average size of 21.2 by 1.4 micron were frequently encountered in wild and farmed fish. However, the macro-gametocytes were scarce. When found they measured an average of 14.3 by 4.4 micron. These free gametocytes are taken up by the intermediate host with a blood meal and in its alimentary tract they presumably undergo syngamy and propagate a new life cycle.

The life cycle of *Haemogregarina sachai* n.sp. could only be demonstrated completely in its final host.

An intraleucocytic schizogony followed by an intraerythrocytic schizogony was observed as in H.simondi. However, schizonts in leucocytes harboured up to 36 merozoites. An apparent erythrocytic schizogony, which was not however observed, resulted in the production of two micro-gametocytes of 10.9 by 1.1 micron in average and two macro-gametocytes of 5.5 by 2.0 micron in average. The marine leech Calliobdella nodulifera was chosen tentatively as an intermediate host but only a few developmental stages similar to those of H.simondi in Hemibdella soleae were observed in this experimental vector. This was considered suggestive of an assumption that a similar life cycle of H.sachai n.sp. could take place in a suitable marine piscicolid leech.

The present study appears to be the first description of a life cycle for a marine fish haemogregarine to include the stages of development in an intermediate host. Indeed it is only the second time that life cycles of marine fish haemogregarines have been described even in the final host. Laird (1953) described the life cycle of Haemogregarina bigemina in the final host Ericentrus rubrus and was the first worker to define an intraleucocytic schizogony followed by an intraerythrocytic schizogony leading

to the production of a pair of gametocytes. This was later confirmed by Saunders (1958,1964) from a number of marine fish infected with *H.bigemina*.

It is concluded that all haemogregarines of marine fish are likely to undergo a similar development in the blood of their final hosts to that described for *H.bigemina* by Laird (1953) and for *H.simondi* and *H.sachai* n.sp. in the present study. It is further concluded that these haemogregarines are able to develop in an intermediate host such as marine piscicolid leeches but that in the absence of a suitable vector such as marine leeches, other bloodsucking ectoparasites i.e. isopods,copepods or trematodes may play an important role in the cyclical development of at least some haemogregarines of marine fish.

Among the family Haemogregarinidae full descriptions of life cycles have been rarely successfully achieved. Since Reichenow's first account of the life cycle of *Haemogregarina stepanowi* in the intermediate host the freshwater leech *Placobdella catenigera* and in the final host the chelidonid turtle *Emys orbicularis* (1910) only a very few such life cycles have been elucidated for the poikilotherms (Table 17). Most of the life cycles described from the final hosts

TABLE 17

LIFE CYCLES FROM THE FAMILY HAEMOGREGARINIDAE IN POIKILOTHERMS

parasite	intermediate host	final host
Genus HEPATOZOON		
Hepatozoon pettiti[1]	Glossina palpalis	Crocodilus niloticus
Hepatozoon mesnili[2]	Culex fatigans	Gecko verticillatus
Hepatozoon mauritanicum[3]	Hyalomma syriacum	Testudo mauritanica
Hepatozoon breinli[4]	Culex fatigans	Varanus tristis orientalis
Hepatozoon rarefaciens[5]	Culex tarsalis	Drymarchon corais
Hepatozoon argantis[6]	Argas brumpti	Agama mossambica (?)
Genus HAEMOGREGARINA		
Haemogregarina[7] stepanowi	Placobdella catenigera	Emys orbicularis
Haemogregarina[8] nicoriae	Oxybranchus shipleyi	Geoemyda trijuga
Haemogregarina[9] simondi	Hemibdella soleae	Solea solea
Genus KARYOLYSUS		
Karyolysus[10] lacertae	Liponyssus saurarum	Lacerta muralis
Karyolysus sp.[11]	Ophionyssus lacertinus	Lacerta sicula

1= Hoare,1932; 2= Robin,1936; 3= Brumpt,1938; 4= Mackerras,1962
5= Ball,Chao & Telford,1967; 6= Garnham,1954; 7= Reichenow,1910;
8= Robertson,1910; 9= Kirmse,1978; 10= Reichenow,1913,1921;
11= Bergle,1971,1974.

have given as location for the schizogonies the endothelila cells of small capillaries in internal organs (particularly the liver _vide_ the genus _Hepatozoon_!) Ball,Chao and Telford (1967) described stages of _Hepatozoon rarefaciens_ within hepatocytes of the final host the reptile _Drymarchon corais_, similar to those of the genus _Hepatozoon_ in mammals first described by Miller (1909). However,in the case of large schizonts it is often difficult to determine precisely whether they are within blood cells in hepatic capillaries or actually within hepatocytes when studied by light microscopy. Reichenow (1913,1921) claimed to have experimentally shown that when parasites entered the circulation of the liver they were phagocytosed by lymphocytes from where they later invaded the endothelial cells of the vascular system. Schizonts in the present study however were seen exclusively within circulating cells and never inside endothelial cells. This was confirmed by electronmicroscopy. Also Bergle (1974) observed that the developing stages of _Karyolysus_ sp. never occurred within the parenchymatous cells of the internal organs of the final host _Lacerta sicula_ but found them occasionally within endothelial cells in his electron microscopical studies.

It is thus concluded at least on the evidence of the present study that haemogregarines of the genus *Haemogregarina* develop only in cells of the circulating blood and not within parenchymatous cells of internal organs. However, further studies with the aid of the electron microscope are indicated in order to demonstrate this for other genera of the Haemogregarinidae.

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APPENDIX I - V

(containing Tables 1 - 7)

APPENDIX I

Field Questionnaire

B.O.C. International Ltd.-Fish Farming Ltd.Hunterston

Date when disease first observed: October 1976
How many fish involved: 1% of population
What age groups: 1 year +
How many different tanks: 1
How were these tanks stocked: 20 to 60 kg/m^3
Where did fish originate: wild caught North Wales
Water Temperature: 15-17 C PH variability: 7.2-8.1
What feed was used: WFA 7 pellets until Feb.77,then sprats
How often fed per day: 6 - 7 times
How often were tanks cleaned: self cleaning
What experiments were done: pilot scale production trial
Flow rate of sea-water: 15,000 l/p/h
Origin of sea water:ambient or heated effluent
Is sea-water filtered: no
How high is chlorine concentration: less than .02 ppm
Any other chemicals or heavy metals: $FeSO_4$
Normal mortality in tanks: 0.5% per month.
What other diseases prevalent: none
When were Vet.Authorities first notified:not done,only APBU
How many turbot with lesions found to date: 25
Seasonal occurrence: no predominant age: 1 year +
Feeding scheme: increasing pellet size with age
How often are fish handled,graded: monthly since Oct.75
Any particular treatments given: Terramycin
Any drugs incorporated in diet: no
Any other fish species held on premises: trout,dover sole
Any disease in other fish: yes Vibrio
What protection against birds,cats: tank roof
Any observations about sediment(faecal casts): no

APPENDIX I cont.

Any other marine life in tanks: mussels, barnacles

Are hatchery derived fish reared under same conditions as wild turbot: yes

Have diseased fish been isolated: no

How long is the time interval between first observation of lesions and death of fish: not known

How are dead fish disposed off: frozen and despatched with refuse

Have infected fish been marketed: no

Estimated loss in pound sterling: 40

Economic Importance: if it does not increase above 1% incidence it is not likely to be of major significance economically. Although any losses of fish through known causes should guarded against by correct preventive procedures

APPENDIX II

Field Questionnaire

Fitch-Lovell Fish Farming Ltd.Golden Sea,Hunterston

Date when disease first observed: summer 1976
How many fish involved: about 1% visibly
What age groups: 0-1 year group
How many different tanks: 3
How were tanks stocked: -
Where did fish originate: Wales
Water Temperature: 15-19 C PH variability: -
What feed was used: moist pellet
How often fed per day: twice
How often were tanks cleaned: daily
What experiments were done: -
What rate of mixture of water: depending on Temp.
Flow rate of seawater: 200 g/p/m origin: Clyde
Is sea water filtered: no
How high is chlorine concentration: 0.02-0.15 ppm
Any other chemicals or heavy metals: $FeSO_4$
Normal mortality in tanks: one per week
What other diseases prevalent: none,perhaps Vibrio
Temp.of effluent water: 15-27 C Temp.seawater: 6-19 C
When were Vet.Authorities first notified: summer 1976
How many turbot found with lesions to date: circa 150
Seasonal occurrence: none Predominant age: 1 year
Feeding scheme of fish: -
What precautions were undertaken: none-lesions were unnoticed if ventrally
How often are fish handled,graded: sampled monthly
Any particular treatment given: none
Any other drug incorporated in diet: none
Any other fish species held on premises: yes

APPENDIX II cont.

Any diseases in other fish: dover sole"Black spot"

What protection against birds,cats: tank covers

Any observations about sediment(faecal casts): none

Any other marine life in tanks: yes-small fish,weeds, mussels,crabs,barnacles

Are hatchery fish reared under same conditions: yes

Have diseased fish been isolated: yes,where possible

How long is the time interval between first observation of lesions and death of fish: unknown

How are dead fish disposed off: flushed

Have infected fish been marketed: no

Estimated economic loss: -

Economic Importance:

Remerks: One group of fish fed on a dry pellet showed the same visual symptoms earlier than those fed on wet feed and mortality was high.These fish had badly damaged kidneys and most fish had the tumours.

APPENDIX III

W.F.A. (White Fish Authority) at Hunterston

Field Questionnaire

Date when disease first observed?:	June 1977.
How many fish involved? :	50+
What age group? :	adult wild
How many different tanks? :	2
Density of these tanks (stocking rate)? :	35/tank ($11m^3$) 1.3 Sp/m^3 15 kg/tank
Where did fish originate ?:	Portsmouth (Selsey)
Water temperature? : pH-variability	12-15°C
What feed was used?:	*Mytilus* sp. *Arenicula* sp.
How often fed per day? :	3-5 x per week
How often were tanks cleaned? :	Every time after feeding (waste food)
Are tanks disinfected after every cleaning? :	No
What experiments were done, or what purpose of fish? :	Brood stock
Flow rate of seawater? :	$33m^3$/day/tank
Origin of seawater? :	River Clyde
How high chlorine concentration?:	-
Any other chemicals or heavy metals in water? :	No
Normal mortalities in sole tanks?:	-
What other diseases present in these fish? :	-
How many sole died from the parasites? :	2
How many fish found with *Lernaeocera* sp.? : No. per fish :	25 1-22
How many fish found with leeches?: No. per fish :	All → 30

APPENDIX III cont.

Seasonal occurrence? :	Decline of Lernaea in autumn.
What is predominant age group affected? :	Adult
What precautions were undertaken?:	Formalin dip, freshwater dip.
What drugs and at what dilution, what baths were given? :	Dipterex 8ppm. 5 fish treated, apparently all cured.
Did the treatment given have any positive effect on fish? :	Yes
When did fish stop feeding, how long going wihout food? :	End May-End July. Very poor feeding.
How often were fish handled since April 1977? :	Twice - between tanks.
Any other diseases or diseases in other fish? :	-
Any other marine life in tanks observed? :	Occassional
Are hatchery derived sole reared under same conditions as wild caught dover sole? :	Entirely different conditions.
Are diseased fish separated from healthy ones? :	No
When were ectoparasites first observed? :	June
Estimated loss through parasites in pound sterling? :	In comparison with loss through transport neglectable
Economic importance for breeding stock sole? :	Only seasonal, not on hatchery fish.
Have cocoons of leeches been found on particulate sediment of tanks? :	Not observed.
Has an increase in ectoparasites been observed since the time of introduction of the fish to these premises? (i.e. April/May) :	Increase June/July of Lernaeocera.
How and at what depth were these sole caught in the English Channel? :	trammel- net 4 fathoms ½-5 miles offshore.

APPENDIX IV

REPORT ON POSSIBLE CAUSE OF DOVER SOLE MORTALITIES

One Dover sole (Solea solea) originally caught off Portsmouth in April 1977 and introduced to the W.F.A. station at Hunterston thereafter was found in a moribund state on July 7th and transported to the Unit of Aquatic Pathobiology on the same day. The fish was dead on arrival but blood could be drawn successfully and was still good enough to yield satisfactory blood smears; it was not suited for E.M. studies or possible transmission experiments, however. The fish measured 3.4cm in length and 13cm in width and weighed 487g.

A consequent inspection for ectoparasites revealed 1 Hemibdella soleae on the upper surface near the eyes; 5 Entobdella soleae on the lower body surface and 8 Lernaeocera sp. on the gills. At post-mortem examination no abnormalities were observed except for an enlarged and fatty liver. The stomach and intestinal tract were devoid of any food and the total digestive tract measured 49cm. The blood smears, when stained with Giemsa and viewed under high power oil immersion showed a heavy infestation with the blood protozoan Haemogregarina simondi in all stages of development. There was notably an increase in leucocytes and many erythrocytes were destroyed by the action of the numerous schizonts. The leech Hemibdella soleae, the establishment vector for this blood parasite, was not suitable for further investigations. However, smears of all the Lernaeocera sp. revealed numerous free merozoites of this haemogregarine. Other soles from the

same tank were seen with even heavier infestations of both the piscicolid leech *Hemibdella soleae* and *Lernaeocera* sp. It is quite clear that all three parasites act together and certainly contribute to the anaemic condition of the fish. Blood smears of a second sole found dead on July 7th also revealed an astonishingly high infestation with the blood parasite. Under aquaculture conditions this infection can be maintained indefinitely through the presence of so many vectors.

It is, therefore, necessary to do transmission experiments with blood of highly parasitized sole as well as with leeches and copepods. In order to carry out this work, one or two live heavily infested soles should be supplied to the Unit from W.F.A. as well as quantities of live leeches and copepods. The latter two should be kept as cool as possible (refrigeration, shipped on ice) so that the losses due to the high temperature are at a minimum.

Peter Kirmse
Unit of Aquatic Patho-Biology, University of Stirling

14th July, 1977

TABLE 1

MARINE FISHES AND THEIR HAEMATOPROTOZOAN PARASITES

Scientific Name	H	T	Tp	Hh	D
CLASS : AGNATHA					
SUBCLASS : CYCLOSTOMATA					
ORDER : MYXINIFORMES					
Family : Myxinidae (Heptatretidae)					
Heptatretus cirrhatus	X				
CLASS : CHONDRICHTHYES					
SUBCLASS : ELASMOBRANCHII (GNATHOSTOMA)					
ORDER : LAMNIFORMES (GALEOIDEI)					
Family : Scyliorhinidae					
Scyliorhinus caniculus (Linnaeus) {Syn. Scyllium canicula (Cuvier)}		X			
Scyliorhinus stellaris (Linnaeus) {Syn. Scyllium catulus (Müller & Henle)}	X	X			
Family : Carcharhinidae					
Carcharias sp. (Cuvier)	X	X			
Hemiscyllium ocellatum (Bonnaterre)	X	X			
ORDER : SQUALIFORMES (TECTOSPONDYLI)					
Family : Squalidae					
Squalus acanthias Linnaeus	X				
ORDER : RAJIFORMES (BATOIDEI)					
Family : Rajidae					
Raja erinacea Mitchill		X			
Raja nasuta Müller & Henle		X			
Raja undulata (mosaica) Lacépède	X	X			
Raja senta Garman	X				
Raja macrorhynchus (Rafinesque)		X			
Raja clavata Linnaeus		X			
Raja asterias (punctata) (Delaroche)	X	X			

contd/...

Scientific Name	H	T	Tp	Hh	D
Family : Rajidae/contd.					
Raja batis Linnaeus		X			
Raja ocellata Mitchill		X			
Raja oxyrhynchus Linnaeus		X			
Raja capensis Müller & Henle		X			
Raja radiata Donovan	X	X			
Raja microcellata Montagu	X	X			
Psammotics microps		X			
Family : Trygonidae (Dasyatidae)					
Dasyatis americana Hildebrand & Schroeder	X				
ORDER : TORPEDINIFORMES (NARCOBATOIDEI)					
Family : Torpedinidae					
Torpedo marmorata Risso	X				
Torpedo torpedo (Syn. ocellata) (Linnaeus)	X				
CLASS : TELEOSTOMI (OSTEICHTHYES)					
SUBCLASS : ACTINOPTERYGII					
ORDER : CLUPEIFORMES (ISOSPONDYLI)					
SUBORDER : CLUPEOIDEI					
Family : Clupeidae					
Brevoortia tyrannus (Latrobe)	X				
ORDER : SCOPELIFORMES (INIOMI)					
Family : Synodidae (Synodontidae)					
Synodus japonicus (Houttuyn)	X				
Family : Aulopidae					
Aulopus purpurissatus (Richardson)	X	X		X	
ORDER : ANGUILLIFORMES (APODES)					
SUBORDER : ANGUILLOIDEI					

contd/

Scientific Name	H	T	Tp	Hh	D
SUBORDER : ANGUILLOIDEI/contd.					
Family : Anguillidae					
Anguilla anguilla (Linnaeus)	X	X			
Thyrsoidea macrura (Blecker)	X				
Anguilla mauritana Bennett		X			
Anguilla reinhardtii Steindachner		X			
Anguilla bengalensis (Boulenger)		X			
Family : Muraenidae (Echidnidae)					
Gymnothorax funebris Ranzani	X				
ORDER : BELONIFORMES (SYNENTOGNATHI)					
Family : Belonidae					
Strongylura notata (Poey)	X				
Family : Hemiramphidae					
Hemiramphus brasiliensis (Linnaeus)	X				
Hyporhamphus unifasciatus (Ranzani)	X				
ORDER : GADIFORMES (ANACANTHINI)					
Family : Moridae					
Physiculus bachus (Bloch & Schneider)	X	X			
Family : Gadidae					
Gadus morhua callarias (Linnaeus)	X				
Gadus morhua (Linnaeus)	X	X			
Melanogrammus (Gadus) aeglefinus (Linnaeus)	X				
Pollachius virens (Linnaeus)	X				
Urophycis tenuis (Mitchill)	X				
Urophycis chuss (Walbaum)	X				
Pollachius (Gadus) pollachius (Linnaeus)	X				
ORDER : MACRURIFORMES					
Family : Macruridae					

Scientific Name	H	T	Tp	Hh	D
Family : Macruridae/contd.					
Coelorhynchus australis (Richardson)	X	X			
ORDER : CYPRINODONTIFORMES (MICROCYPRINI)					
SUBORDER : CYPRINODONTOIDEI					
Family : Cyprinodontidae					
Fundulus heteroclitus (Linnaeus)					
Fundulus majalis (Walbaum)			X		
ORDER : MUGILIFORMES (PERCESOCES)					
SUBORDER : SPHYRAENOIDEI					
Family : Sphyraenidae					
Sphyraena barracuda (Walbaum)	X	X			
SUBORDER : MUGILOIDEI					
Family : Mugilidae					
Mugil cephalus Linnaeus	X				
Mugil microlepis A. Smith {= Mugil troscheli Schmeltz}	X				
Mugil oligolepis Bleeker	X				
Liza ramada (Risso) {= Mugil capito (Cuvier)}	X				
Mugil brasiliensis Agassiz	X				
Mugil trichodon Poey	X				
Mugil sp.	X				
ORDER : PERCIFORMES (PERCOMORPHI)					
SUBORDER : PERCOIDEI					
Family : Coryphaenidae					
Coryphaena hippurus Linnaeus	X				
Family : Serranidae					
Mycteroperca bonaci (Poey)	X				
Mycteroperca microlepis (Goode & Bean)	X				
Epinephelus adscensionis (Osbeck)	X				

contd./

Scientific Name	H	T	Tp	Hh	D
Family : Serranidae/contd.					
Epinephelus summana (Forskal)	X				
Epinephelus guttatus (Linnaeus)	X				
Epinephelus tauvina (Forskal)	X				
Epinephelus striatus (Bloch)	X				
Epinephelus fuscoguttatus (Forskal)	X				
Epinephelus morio (Valenciennes)	X				
Epinephelus fasciatus (Forskal)	X				
Centropristes striata (Linnaeus)	X				
Ellerkeldia annulata (Günther)	X				
Ellerkeldia semicincta (Cuvier & Valenciennes)	X	X			
Plectropomus maculatum (Bloch)	X				
Variola louti (Forskal)	X				
Cephalopholis miniatus (Forskal)	X			X	
Cephalopholis hemistictus Rüppell	X			X	
Family : Acanthoclinidae					
Acanthoclinus quadridactylus	X				
Family : Malacanthidae					
Malacanthus plumieri (Bloch)	X				
Family : Carangidae					
Seriola falcata Val. {= Zonichthys falcatus (Cuvier & Valenciennes)}	X				
Caranx hippos (Linnaeus)	X				
Caranx crysos (Mitchill)	X				
Caranx bartholomaei Cuvier	X				
Caranx ruber (Bloch)	X				
Seriola dumerili (Risso)	X				
Family : Lutjanidae					
Lutjanus griseus (Linnaeus)	X				
Lutjanus synagris (Linnaeus)	X				
Lutjanus apodus (Walbaum)	X				
Lutjanus bohar (Forskal)	X				
Ocyurus chrysurus (Bloch)	X				
Family : Liognathidae					
Gerres cinereus (Walbaum)	X				
Eucinostomus gula (Cuvier)	X				

Scientific Name	H	T	Tp	Hh	D
Family : Pomadasyidae (Haemulidae)					
Haemulon flavolineatum (Desmarest)	X				
Haemulon plumieri (Lacepede)	X				
Haemulon sciurus (Shaw)	X				
Haemulon album (Cuvier)	X				
Haemulon aurolineatum Cuvier	X				
Family : Sciaenidae					
Bairdiella chrysura (Lacepede)	X				
Menticirrhus littoralis (Holbrook)	X				
Cynoscion nebulosus (Cuvier)	X				
Family : Lethrinidae					
Lethrinus mahsena (Forskal)	X				
Lethrinus nebulosus (Forskal)	X				X
Lethrinus mahsenoides Cuvier & Valenciennes					X
Lethrinus xanthochilus (Klunzinger)				X	
Lethrinus variegatus Valenciennes	X			X	
Family : Sparidae					
Boops {= Box} salpa (Linnaeus)		X			
Calamus bajanado (Bloch & Schneider)	X				
Pagrus laniarus	X				
Lagodon rhomboides (Linnaeus)	X				
Acanthopargus bifasciatus (Forskal)	X				
Argyrops spinifer (Forskal)	X				
Chrysophrys haffara (Forskal)	X				
Dentex argyro		X			
Family : Mullidae					
Upeneus tragula Richardson	X				
Mulloidichthys auriflamma (Forskal)	X				
Family : Cyphosidae					
Kyphosus bigibbus Lacepede	X				
Family : Chaetodontidae (Pomacanthidae)					
Pomacanthus maculosus (Forskal)	X				

Scientific Name	H	T	Tp	Hh	D
<u>Family</u> : Pomacentridae					
Abudefduf saxatilis (Linnaeus)	X				
Parma microlepis	X	X	X		
<u>Family</u> : Labridae					
Thalassoma bifasciatum (Bloch)	X				
Thalassoma purpureum (Forskal)	X				
Lachnolaimus maximus (Walbaum)	X				
Halichoeres bivittatus (Bloch)	X				
Pteragogus opercularis (Peters)	X				
Labrus bergylta Ascanius {= Labrus maculatus (Bloch)}	X				
Cheilinus trilobatus Lacepede	X				
<u>Family</u> : Scaridae					
Scarus ghobban Forskal	X				
Scarus croicensis (Bloch)	X				
Scarus sordidus (Forskal)	X				
Scarus harid (Forskal)	X			X	
Scarus guttatus (Bloch & Schneider)	X				
Sparisoma aurofrenatum (Valenciennes)	X				
Chlorurus sp.	X				
<u>Family</u> : Mugiloididae					
Parapercis hexophthalma (Cuvier)	X				
Parapercis colias	X				
<u>Family</u> : Leptoscopidae					
Leptoscopus macropygus	X				
SUBORDER : BLENNIOIDEI (JUGULARES)					
<u>Family</u> : Blenniidae					
Ericentrus rubrus (Hutton)	X	X			
Blennius velifer Norman {= Blennius trigloides (Valenciennes)}	X				
Coryphoblennius galerita (B. montagui) (Linnaeus)	X				
Blennius pholis Linnaeus	X	X			

contd/...

Scientific Name	H	T	Tp	Hh	D
Family : Blenniidae/contd.					
Blennius gattorugine (Brünnich)	X				
Trypterygion varium (Forster)	X	X			
Trypterygion medium (Günther)	X	X			
Salarias periophthalmus Valenciennes	X				
Blennius cornutus (Lacepede)	X	X			
Notoclinus fenestratus (Forster)	X				
Family : Anarhichadidae					
Anarhichas lupus Linnaeus	X				
Family : Clinidae					
Clinus anguillaris		X			
Clinus perspicillatus	X	X			
Trypterygion rufopileum	X				
Family : Pholidae					
Pholis gunnellus (Linnaeus)	X				
Family : Zoarcidae					
Macrozoarces americanus (Bloch & Schneider)	X				
Zoarces viviparus (Linnaeus)	X				
Zoarces anguilaris	X				
SUBORDER : CALLIONYMOIDEI					
Family : Callionymidae					
Callionymus lyra Linnaeus	X	X			
Family : Acanthuridae					
Ctenochaetus strigosus (Bennett)	X				
Acanthurus sohal (Forskal)	X				
Hepatus olivaceus (Schneider) {= Acanthurus nigricans (Linnaeus)}	X				
SUBORDER : SCOMBROIDEI					
Family : Scombridae					
Scomberomorus regalis (Bloch)	X				
Scomberomorus cavalla (Cuvier)	X				
Auxis thazard (Lacepede)	X				

contd/....

Scientific Name	H	T	Tp	Hh	D
Family : Histiophoridae (Istiophoridae)					
Istiophorus albicans (Latreille) {= Istiophorus americanus (Cuvier & Valenciennes)}	X				
SUBORDER : GOBIOIDEI					
Family : Eleotridae					
Awaous ocellaris (Broussonet)	X				
Stenogobius genivittatus (Valenciennes)	X				
Family : Gobiidae					
Bathygobius soporator (Cuvier)	X				
Amblygobius albimaculatus (Ruppell)	X				
Gobius paganellus (Dijkgraaf)	X				
Gobius cobitis Pallas {= Gobius capito (Moreau)}	X				
Oliverichtus melobesia (Phillipps)	X				
Gobius cruentatus (Gmelin)	X				
Pomatoschistus {= Gobius} minutus (Pallas)	X				
Gobius auratus Risso	X				
Gobius niger Linnaeus	X	X			
Gobius nudiceps		X			
Gobius jozo (Linnaeus)	X				
SUBORDER : COTTOIDEI					
Family : Scorpaenidae					
Scorpaena ustulata (Lowe)	X	X			
Family : Triglidae					
Triglopoms lastarija (Bonnaterre) {= Trigla lineata (Gmelin)}	X				
Trigla lucerna Linnaeus {= Trigla corax (Moreau)}		X			
Family : Hoplichthyidae					
Hoplichthys haswelli	X				

contd/...

Scientific Name	H	T	Tp	Hh	D
Family : Congiopodidae					
Congiopodus leucopaecilus		X			
Family : Cottidae					
Hemitripterus americanus (Gmelin)					X
Myoxocephalus aenaeus (Mitchill)					
Myoxocephalus octodecemspinosus (Mitchill)	X	X		X	
Taurulus (=Cottus) bubalis (Euphrasen)	X	X		X	
Myoxocephalus (=Cottus) scorpius (Linnaeus)	X	X		X	
Artedius fenestralis Jordon & Gilbert	X				
Family : Aponidae :					
Agonus cataphractus					
Family : Cyclopteridae					
Liparis atlanticus (Jordan & Evermann)	X				
ORDER : PLEURONECTIFORMES (HETEROSOMATA)					
SUBORDER : PLEURONECTOIDEI					
Family : Scophthalmidae					
Zeugopterus punctatus (Bloch)	X	X			
Scophthalmus rhombus (Linnaeus)		X			
Scophthalmus maximus (Linnaeus)	X		X	X	
Scophthalmus aquosus (Mitchill)	X				
Family : Bothidae					
Paralichthys dentatus (Linnaeus)	X		X		
Platophrys laterna	X	X			
Family : Pleuronectidae					
Platichthys flesus (Linnaeus)	X	X	X		
Caulopsetta scapha	X	X			
Rhombosolea plebeia (Richardson)	X	X			
Pleuronectes platessa Linnaeus	X	X			

contd/...

Scientific Name	H	T	Tp	Hh	D
Hippoglossoides platessoides (Fabricius)				X	
Liopsetta putnami (Gill)			X		
Pseudopleuronectes americanus (Walbaum)	X		X		
Glyptocephalus cynoglossus (Linnaeus)	X	X		X	
Limanda limanda (Linnaeus)	X	.X			
Limanda ferruginea (Storer)		X			
Limanda platessoides					
Microstomus kitt (Walbaum)	X				
Family : Soleidae					
Solea solea (Linnaeus)	X	X		X	
Solea lutea (Bonaparte)	X				
Solea monochir (Bonaparte)		X			
Trinectes maculatus (Bloch & Schneider)	X	X			
ORDER : TETRODONTIFORMES (PLECTOGNATHI)					
SUBORDER : BALISTOIDEI					
Family : Balistidae					
Balistes capriscus Gmelin	X	X			
Balistes vetula Linnaeus	X				
SUBORDER : TETRODONTOIDEI					
Family : Tetrodontidae					
Sphaeroides maculatus (Bloch & Schneider)	X				
Tetraodon hispidus Linnaeus	X				
ORDER : GOBIESOCIFORMES (XENOPTERI)					
Family : Gobiesocidae					
ORDER : LOPHIIFORMES					
Family : Lophiiformidae					
Lophius americanus Valenciennes	X				

TABLE 2

TRYPANOSOMES OF MARINE FISH

Scientific Name	Fish Host	Reference
T. scyllii	Scyllium canicula Scyllium stellare	Laveran & Mesnil, 1901.
T. rajae	Raja punctata	Laveran & Mesnil, 1902.
T. soleae	Solea solea	Laveran & Mesnil, 1902, Lebailly, 1906. Henry, 1910.
T. granulosum var. magnum	Anguilla anguilla	Laveran & Mesnil, 1902.
T. granulosum var. minimum	Anguilla anguilla	Laveran & Mesnil, 1902.
T. platessae	Pleuronectes platessa	Lebailly, 1904.
T. flesi	Platychthys flesus	Lebailly, 1904.
T. laternae	Platophrys laterna	Lebailly, 1904.
T. bothi	Scophthalmus rhombus	Lebailly, 1905.
T. limandae	Limanda limanda	Brumpt & Lebailly, 1904.
T. callionymi	Callionymus lyra	Brumpt & Lebailly, 1904.
T. cotti	Cottus bubalis	Brumpt & Lebailly, 1904.
T. gobii	Gobius niger	Brumpt & Lebailly, 1904.
T. delagei	Blennius pholis	Brumpt & Lebailly, 1904.
T. carchariasi	Carcharias sp.	Laveran, 1908.
T. giganteum	Raja oxyrhynchus	Neumann, 1909.
T. variabile	Raja asterias	Neumann, 1909.
T. scorpaenae	Scorpaena ustulata	Neumann, 1909.
T. triglae	Trigla corax	Neumann, 1909.
T. zeugopteri	Zeugopterus punctatus	Henry, 1910.
T. anguillicola	Anguilla mauritanica Anguilla bengalensis Anguilla reinhardtii	Johnston & Cleland, 1910.
T. dorhni	Solea monochir	Yakimoff (cit. Laveran & Mesnil, 1912).
T. capigobii	Gobius nudiceps	Fantham, 1919.
T. nudigobii	Gobius nudiceps	Fantham, 1919.

contd/...

Scientific Name	Fish Host	Reference
Trypanosoma sp.	Agonus cataphractus	Henry, 1919.
Trypanosoma sp.	Raja capensis Box salpa Dentex argyro	Fantham, 1919.
T. pulchra	Gilbertia semicincta	Mackerras & Mackerras, 1925.
T. aulopi	Aulopus purpurissatus	Mackerras & Mackerras, 1925.
T. murmanensis	Gadus morhua	Nikitin, 1927.
T. blenniclini	Blennius cornutus Clinus anguillaris	Fantham, 1930.
T. myoxocephali	Myoxocephalus octo-decemspinosus	Fantham, Porter & Richardson,1942.
T. marplatensis	Psammotics microps	Bacigalupo & De la Plaza, 1948.
T. heptatreti	Heptatretus cirrhatus	Laird, 1948.
T. gargantua	Raja nasuta	Laird, 1951.
T. coelorhynchi	Coelorhynchus australis Physiculus bachus	Laird, 1951.
T. caulopsettae	Caulopsetta scapha	Laird, 1951.
T. tripterygium	Tripterygion varium Tripterygion medium	Laird, 1951
T. congiopodi	Congiopodus leucopaecilus	Laird, 1951.
T. parapercis	Parapercis colias	Laird, 1951.
Trypanosoma sp.	Sphyraena barracuda	Saunders, 1958.
T. balistes	Balistes capriscus	Saunders, 1958.
Trypanosoma sp.	Glyptocephalus cynoglossus Gadus morhua	So, 1972.

TABLE 3

TRYPANOPLASMS OF MARINE AND FRESHWATER FISH

Part I : Marine Fishes

Scientific Names	Fish Hosts	Reference
Tp.parmae	Parma microlepis	Mackerras&Mackerras, 1925
Tp.flesi	Platichthys flesus	Nowicki,1940
Tp.newingtoniensis	Pseudopleuronectes americanus	Bullock,1953
Tp.bullocki	Pseudopleuronectes americanus; Liopsetta putnami; Fundulus heteroclitus; Fundulus majalis	Strout,1964; Laird & Bullock, 1969
Trypanoplasma sp.	Scophthalmus maximus	Kirmse,1975

Part II : Freshwater Fishes

Scientific Names	Fish Hosts	Reference
Tp.borreli	Leuciscus erythrophthalmus; Cyprinus carpio; Catostomus commersonii; Salmo gairdneri; Oncorhynchus tshawytscha; Salmo trutta; Oncorhynchus kisutch; Catostomus snyderi; Cottus sp.	Laveran & Mesnil,1902 Keysselitz,1906 Mavor,1915 Wales & Wolf,1955
Tp.cyprini	Cyprinus carpio Carassius auratus	Plehn,1903

TABLE 3 cont.

TRYPANOPLASMS OF MARINE AND FRESHWATER FISH

Scientific Names	Fish Hosts	Reference
Tp.varium	Cobitis barbatula	Léger,1904
Trypanoplasma sp.	Phoxinus laevis	Léger,1904; Laveran & Mesnil,1907
Tp.guernei	Cottus gobio	Brumpt,1905
Tp.barbi	Barbus fluviatilis	Brumpt,1906
Tp.abramidis	Abramis brama	Brumpt,1906
Tp.truttae	Salmo fario	Brumpt,1906
Trypanoplasma sp.	Labeo macrostoma	Rodhain,1907
Tp.gurneyorum	Esox lucius	Minchin,1909; Narotzky,1914; Laird,1961
	Coregonus clupeaformis Salvelinus namaycush	Laird,1961
Tp.keysselitzi	Tinca tinca	Minchin,1909
Tp.congeri	Conger conger	Elmhirst & Martin,1910
Tp.clariae	Clarias macrocephalus	Mathis & Léger,1911
Trypanoplasma sp.	Monopterus javanensis	Mathis & Léger,1911
Tp.valentini	Salmo fario	Gauthier,1920
Trypanoplasma sp.	Misgurnus anguillicaudatus	Tanabe,1924
Trypanoplasma ninae kohl-yakimov	Silurus glaris	Yakimoff,1925
Tp.salmositica	Oncorhynchus kisutch	Katz,1951
Tp.lynchi	Cottus rhotheus Cottus aleuticus	Katz,1951

Table 4

HAEMOGREGARINES OF MARINE FISH

Scientific Name	Fish Host	Reference
H. simondi	Solea solea	Laveran and Mesnil, 1901; Lebailly, 1904; Henry, 1910.
H. bigemina*	Blennius pholis	Laveran and Mesnil, 1901.
H. delagei	Raja mosaica Raja punctata	Laveran and Mesnil, 1902.
	Raja microcellata	Robertson, 1906.
	Raja radiata Raja senta	So, 1972.
H. cotti	Cottus bubalis	Brumpt and Lebailly, 1904 Henry, 1910.
H. blanchardi	Gobius niger	Brumpt and Lebailly, 1904
H. gobii	Gobius niger	Brumpt and Lebailly, 1904
H. platessae	Pleuronectes platessae	Lebailly, 1904; Robertson, 1906; Henry, 1910.
	Microstomus kitt	Noble, 1957.
	Pseudo-pleuronectes americanus	Fantham, Porter and Richardson, 1942; Laird and Bullock, 1969.
	Paralichthys dentatus	Bullock, 1958; Laird and Bullock, 1969.
	Glyptocephalus cynoglossus	So, 1972.
	Scophthalmus aquosus	So, 1972.
	Trinectes maculatus	Laird and Morgan, 1973.
H. flesi	Pleuronectes flesus	Lebailly, 1904; Robertson, 1906.
H. laternae	Platophrys laterna	Lebailly, 1904.
H. bothi	Bothus rhombus	Lebailly, 1905.
H. lignieresi	Anguilla anguilla	Laveran, 1906.
H. bettencourti	Anguilla anguilla	Franca, 1908.

Scientific Name	Fish Host	Reference
H. torpedinis	Torpedo ocellaris	Neumann, 1909.
H. polypartita	Gobius paganellus	Neumann, 1909.
H. minuta	Gobius minutus	Neumann, 1909.
H. scorpaenae	Scorpaena scrofa	Neumann, 1909.
H. clavata	Solea lutea	Neumann, 1909; Kohl-Yakimoff and Yakimoff, 1915.
H. rovingnensis	Trigla lineata	Minchin and Woodcock, 1910.
H. cotti scorpii	Cottus scorpius	Henry, 1910.
H. zeugopteri	Zeugopterus punctatus	Henry, 1910.
H. labri	Labrus maculatus	Henry, 1910.
H. binucleata	Callionymus lyra	Henry, 1910.
H. gadi pollachii	Gadus pollachius	Henry, 1910.
H. anarhichadis	Anarhichas lupus	Henry, 1912.
H. aeglifini	Gadus aeglifinus	Henry, 1913.
	Gadus morhua	Fantham, Porter & Richardson, 1942.
Haemogregarina sp.	Urophycis chuss	Mavor, 1915.
H. yakimovi-kohl	Gobius capito	Kohl-Yakimoff and Yakimoff, 1915.
H. wladimirovi	Gobius cruentatus	Kohl-Yakimoff and Yakimoff, 1915.
H. hartochi	Gobius aurantus	Kohl-Yakimoff and Yakimoff, 1915.
H. marzinowskii	Gobius jozo	Kohl-Yakimoff and Yakimoff, 1915.
H. lobianci	Torpedo marmorata	Kohl-Yakimoff and Yakimoff, 1915.
H. londoni	Blennius trigloides	Kohl-Yakimoff and Yakimoff, 1915.

Scientific Name	Fish Host	References
Haemogregarina sp.	Pagrus laniarus Mugil capito	Fantham, 1919.
H. mugili	Mugil brasiliensis	Carini, 1932
	Awaous ocellaris	Laird, 1958.
	Stenogobius genivittatus	Laird, 1958.
	Mugil sp.	Laird, 1958,
	Mugil cephalus	Saunders, 1964.
H. thyrsoideae	Thyrsoidea macrurus	De Mello and Vales, 1936.
H. myoxocephali	Myoxocephalus octodecemspinosus	Fantham, Porter and Richardson, 1942; So, 1972.
H. urophycis	Urophycis tenuis	Fantham, Porter and Richardson, 1942.
Haemogregarina sp.	Centropristis atriatus	Fantham, Porter and Richardson, 1942.
H. salariasi	Salarias periophthalmus	Laird, 1951.
H. coelorhynchi	Coelorhynchus australis Physiculus bachus	Laird, 1952.
H. hoplichthys	Hoplichthys haswelli	Laird, 1952.
H. acanthoclini	Acanthoclinus quadridactylus	Laird, 1953.
H. leptoscopi	Leptoscopus macropygus	Laird, 1952.
Haemogregarina sp.	Cynoscion nebulosus	Saunders, 1954.
H. achiri	Achirus fasciatus	Saunders, 1955.
Haemogregarina sp.	Mugil trichodon	Saunders, 1958.
Haemogregarina sp.	Sphaeroides maculatus	Bullock, 1958.

Scientific Name	Fish Host	Reference
H. dasyatis	Dasyatis americana	Saunders, 1958.
H. irkalukpiki	Salvelinus alpinus	Laird, 1961.
H. brevoortiae	Brevoortia tyrannus	Saunders, 1964.
Haemogregarina sp.	Lophius americanus	Bridges, Pedro and Laird, 1975.
H. quadrigemina	Callionymus lyra	Brumpt and Lebailly, 1904
H. callionymi	Callionymus lyra	Brumpt and Lebailly, 1904

* All further host records for H. bigemina are found in Table 5

TABLE 5

GEOGRAPHICAL DISTRIBUTION AND FISH HOSTS FOR HAEMOGREGARINA BIGEMINA WITH SPECIAL REFERENCE TO THE INCIDENCE OF INFECTION

Fish Hosts	Incidence	Location	Reference
Blennius pholis Blennius gattorugine		France	Laveran & Mesnil, 1901.
Blennius pholis Blennius montagui		Italy	Neumann, 1909.
Blennius pholis Blennius gattorugine		England	Henry, 1913.
Zoarces viviparus		England	Bentham, 1917.
Zoarces anguillaris		Canada	Fantham, Porter & Richardson, 1942.
Oliverichtus molobesia	1/1	New Zealand	Laird, 1953.
Ericentrus rubrus	16/20		
Tripterygion varium	2/5		
Tripterygion medium	1/36		
Notoclinus fenestratus	1/1		
Menticirrhus littoralis	2/46	U.S.A.	Saunders, 1955.
Centropristes striatus			Bullock, 1958.
Strongylura notata	2/52	Bahamas	Saunders, 1958.
Caranx crysos	1/16		
Eucinostomus gula	2/46		
Lutianus apodus	2/46		
Lutianus synagris	1/45		
Ocyurus chrysurus	1/15		
Haemulon sciurus	3/45		
Haemulon album	1/46		
Calamus bajanado	1/45		
Abudefduf saxatilis	1/43		
Thalassoma bifasciatum	4/48		
Mugil trichodon	1/54		
Sphyraena barracuda	1/6		

contd/...

Fish Hosts	Incidence	Location	Reference
Bathygobius soporator	3/42	Bahamas	Saunders, 1958.
Malacanthus plumieri	1/1		
Balistes vetula	1/45		
Gymnothorax funebris	1/1	U.S.A.	Saunders, 1958a
Scomberomorus regalis	1/27		
Scomberomorus cavalla	1/48		
Istiophorus americanus	2/4		
Caranx hippos	1/3		
Seriola dumerili	8/34		
Coryphaena hippurus	1/45		
Mycteroperca bonaci	8/70		
Mycteroperca microlepis	2/12		
Epinephelus adscensionis	4/10		
Epinephelus guttatus	1/13		
Epinephelus striatus	1/9		
Epinephelus morio	10/103		
Lutianus griseus	17/90		
Haemulon flavolineatum	2/3		
Haemulon plumieri	5/45		
Lachnolaimus maximus	1/13		
Balistes capriscus	3/10		
Clinus perspicillatus	1/8	South Pacific	Laird, 1958.
Tripterygion rufopileum	2/2		
Hyporhamphus unifasciatus	1/45	Bermuda	Saunders, 1959.
Caranx ruber	2/46		
Zonichthys falcatus	1/4		
Halichoeres bivittata	1/48		
Synodus japonicus	3/6	Red Sea/ Africa	Saunders, 1960.
Parapercis hexophthalma	4/12		
Plectropomus maculatus	6/27		

contd/...

Fish Hosts	Incidence	Location	Reference
Cephalopholis miniatus	5/59	Red Sea/ Africa	Saunders, 1960.
Cephalopholis hemistictus	8/44		
Variola louti	9/24		
Epinephelus fasciatus	5/52		
Epinephelus fuscoguttatus	7/51		
Epinephelus taurina	4/10		
Epinephelus summana	5/50		
Lutianus bohar	4/9		
Lethrinus mahsena	6/50		
Lethrinus nebulosus	8/54		
Lethrinus variegatus	6/37		
Acanthopargus bifasciatus	1/4		
Argyrops spinifer	8/50		
Chrysophrys haffara	3/14		
Ctenochaetus strigosus	2/10		
Kyphosus bigibbus	16/52		
Upeneus tragula	1/1		
Mulloidichthys auriflamma	3/20		
Pomacanthus maculosus	1/2		
Thalassoma purpureum	3/15		
Pteragogus opercularis	5/51		
Cheilinus trilobatus	2/33		
Scarus sordidus	6/39		
Amblygobius albimaculatus	1/1		
Artedius fenestralis		Canada	Laird, 1961 b.
Strongylura notatus	2/39	U.S.A.	Saunders, 1964.
Lagodon rhomboides	2/60		
Gerres cinereus	1/25		
Bairdella chrysura	2/50		
Menticirrhus littoralis	1/12		

contd/...

Fish Hosts	Incidence	Location	Reference
Hemiramphus brasiliensis	1/1	Puerto Rico	Saunders, 1966.
Caranx bartholomaei	1/9		
Caranx hippos	1/10		
Haemulon aurolineatum	2/40		
Scarus croicensis	2/44		
Sparisoma aurofrenatum	1/16		
Auxis thazard	1/1		

TABLE 6 a

HAEMOHORMIDIUM (Henry, 1910) IN MARINE AND FRESHWATER POIKILOTHERMES

Scientific Names	Host/Habitat	Reference/Locality
Hh. cotti	fish/marine	Henry,1910 / U.K.
Hh. aulopi	fish/marine	Mackerras&Mackerras, 1925 / Australia
Hh. jahni	newt/freshwater	Nigrelli,1929 / USA
Hh. mariae	fish/freshwater	Hoare,1930 / Africa
Hh.rubrimarensis	fish/marine	Saunders,1960/Egypt
Hh. stableri	frog/freshwater	Schmittner&McGhee, 1961 / USA
Hh. tetragonis	fish/freshwater	Becker&Katz,1965/USA
Haemohormidium sp.	fish/marine	Laird&Bullock,1969/ Canada
Hh. ophicephali	fish/freshwater	Misra,Haldar & Chakravarty,1969/India
Hh. terranovae	fish/marine	So,1972 / Canada
Hh. beckeri	fish/marine	So,1972 / Canada

TABLE 6 b

DACTYLOSOMA (Labbé, 1894) IN MARINE AND FRESHWATER POIKILOTHERMES

Scientific Names	Host/Habitat	Reference/Locality
D. ranarum	frog/freshwater	Lankester,1882 / U.K.
D. splendens	frog/freshwater	Labbé, 1894 / France
D. sylvatica	frog/freshwater	Fantham,Porter & Richardson,1942/Canada
D. tritonis	newt/freshwater	Fantham, 1905 / U.K.
D. jahni	newt/freshwater	Nigrelli,1929 / USA
D. mariae	fish/freshwater	Hoare,1930 / Africa Baker,1960 / Africa
D. salvelini	fish/freshwater	Fantham,Porter & Richardson,1942/Canada
D. lethrinorum	fish/marine	Saunders,1960 /Egypt

TABLE 7 a

BLOODSAMPLES COLLECTED FROM MARINE FISH

OF THE ATLANTIC COAST OF FRANCE

Part I : Winter 1975/76

Common Names	Scientific Names	Numbers collected
SOLE	Solea solea (L.)	12
DORADE ROYALE	Sparus aurata (L.)	5
DORADE ROUSSEAU	Pagellus bogaraveo (Brunnich)	4
DORADE ROSÉ	Pagellus centrodontus (Gunther)	4
DORADE GRISET	Spondyliosoma cantharus (L.)	6
LIEU JAUNE	Pollachius pollachius (L.)	3
MAQUERAU	Scomber scombrus (L.)	2
TACAUD	Trisopterus luscus (L.)	5
CHINCHARD	Trachurus trachurus (L.)	3
CONGRE	Conger conger (L.)	4
BAR	Dicentrarchus labrax (L.)	10
COLIN	Pollachius virens (L.)	4
TURBOT	Scophthalmus maximus (L.)	9
GRONDIN	Trigla sp.	2
LIMANDE	Limanda limanda (L.)	1
ROUGET	Mullus surmuletus (L.)	2
ST.PIERRE.	Zeus faber (L.)	2
RAIE	Raja sp.	4
MERLUCHON	Merluccius merluccius (L.) juv.	2
	Total	84

TABLE 7 b

BLOODSAMPLES COLLECTED FROM MARINE FISH

OF THE ATLANTIC COAST OF FRANCE

Part II : Summer 1976

Common Names	Scientific Names	Numbers collected
LANGUE D'AVOCAT	Dicologoglossa cuneata (Moreau)	48
MULET	Mugil sp.	40
DORADE ROUSSEAU	Pagellus bogaraveo (Brunnich)	31
DORADE GRISET	Spondyliosoma cantharus (L.)	17
DORADE ROSE	Pagellus centrodontus (Gunther)	46
DORADE ROYALE	Sparus aurata (L.)	26
TRUITE DE MER	Salmo trutta (L.)	7
SOLE PERDRIX	Microchirus variegatus (Donovan)	8
SOLE	Solea solea (L.)	241
RASCASSE	Scorpaena atlantica (L.)	15
THON ROUGE	Thunnus thynnus (L.)	49
THON BLANC	Thunnus alalunga (Gmelin)	33
BARBUE	Scophthalmus rhombus (L.)	47
SAUMON	Salmo salar (L.)	24
CHINCHARD	Trachurus trachurus (L.)	9
ANGUILLE	Anguilla anguilla (L.)	215
TACAUD	Trisopterus luscus (L.)	8
GRONDIN	Trigla sp.	30
SEBASTE	Sebastes sp.	50
SARDINE	Sardina pilchardus (Walbaum)	34
MAQUERAU	Scomber scombrus (L.)	22
RAIE	Raja sp.	8
EPERLAN	Osmerus eperlanus (L.)	17
CONGRE	Conger conger (L.)	24
CABILLAUD	Gadus morhua (L.)	15
BAR	Dicentrarchus labrax (L.)	32
PLIE	Pleuronectes platessa (L.)	10
TURBOT	Scophthalmus maximus (L.)	20
GRANDE VIVE	Trachinus draco (L.)	34
LOTTE	Lophius piscatorius (L.)	16
VIELLE	Labrus bergylta (Ascanius)	17
ROUGET	Mullus surmuletus (L.)	31
ST.PIERRE	Zeus faber (L.)	18
COLIN	Pollachius virens (L.)	18
MERLU	Merluccius merluccius (L.)	11
MERLAN	Merlangius merlangus (L.)	8
OMBRINE	Umbrina cirrosa (L.)	9
MERLUCHON	Merluccius merluccius (L.) juv.	8
	Total :	1,288