

AN INVESTIGATION INTO CERTAIN ASPECTS OF THE BIOLOGY OF
MALAMEBA LOCUSTAE, A PROTOZOAN PARASITE OF LOCUSTS

by

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I hereby declare that this thesis is my own work
and has not been submitted in whole or in part for a
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An investigation into certain aspects of the biology of Malameba locustae, a protozoan parasite of locusts.

INTRODUCTION.

A. General Introduction.

This study was initiated on discovering that a large proportion of locusts in the colony being maintained by the Locust Research Unit of the University of the Witwatersrand, were parasitised by an amoeba. As this infection was causing a high percentage mortality among the locusts, aspects of the biology of the amoeba were studied with a view to eliminating the disease from the colony.

Three species of locusts are maintained by the Research Unit - Locusta migratoria, Locustana pardalina and Schistocerca gregaria. They are housed in cages 15 ins. by 10 ins. by 10 ins. within an insectary in which the temperature is controlled to approximately 80°F. and the relative humidity to approximately 60%. The colony is fed and cleaned daily and the numbers of locusts fluctuate between 3 and 6 thousand individuals.

The parasitic amoeba was first found in the colony by Dr. D.J. Nolte in the malpighian tubes of locusts which were being dissected in the course of cytogenetic studies.

A search of the literature showed that the amoeba was first described in 1936 by two American workers, R.L. King and A.B. Taylor, who found it in the malpighian tubes of laboratory bred grasshoppers. They named this amoeba Malpighamoeba locustae. In 1937, after comparing the parasite with other members of the genus Malpighamoeba, Taylor and King erected a new genus Malameba with Malpighamoeba locustae as its type species.

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As well as naming the amoeba, King and Taylor worked on various aspects of its biology. They described the morphology of the trophozoite and cyst and their distribution in the host and also the symptoms occurring in the infected grasshopper. One of their experiments showed to what extent Malameba locustae was capable of parasitizing various species of grasshopper. Details of the life history of the parasite were also described. Finally the incidence of the amoeba in natural populations was investigated and an attempt was made to raise this incidence by distributing cysts of Malameba locustae in the field.

The parasite was studied again in 1958 by two South African workers, Professor Bush and one of his students M.G. Bingham, both of the University of Natal, Pietermaritzburg. This investigation was undertaken at the request of Mr. A. Lea, Chief Locust Officer, Department of Agriculture, who had observed cysts of a parasite in the malpighian tubes of large numbers of the brown locust, Locustana pardalina (Walk), during the 1953 - 1954 outbreak of this insect.

Bingham described the trophozoites and cysts occurring in the brown locust and compared them with Malameba locustae as described by Taylor and King. The work also dealt with the internal distribution of the parasite and the effect on the host. An unsuccessful attempt was made to infect two other species of grasshoppers with cysts of the amoeba.

From 1958 to 1962 Malameba locustae was investigated by H.E. Prinsloo of the Department of Locust Control, who made a more detailed study of the morphology of the parasite and certain aspects of its life history and effect on the brown locust, Locustana pardalina. Prinsloo investigated the distribution and morphology of the Feulgen positive bodies found in both the cysts and trophozoites of Malameba locustae.

In studying the effect of the parasite on the brown locust, Prinsloo investigated the influence of the infection on the incidence of diapause eggs,

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the influence on the bacterial flora of the female locust and the effect of the amoebae on the malpighian tubes. Finally an attempt was made to eradicate the infection from Locustana pardalina by testing the amoebicidal effect of a number of chemicals on the cysts of Malameba locustae, in vitro.

B. Morphology of Malameba locustae.

1. Size.

Previous workers have measured the size of the trophozoites and cysts of Malameba locustae and these results are summarised in the following table:

Table 1.

Author.	Trophozoites. Greatest diameter.	Cysts. (microns)			
		Length.	Mean.	Breadth.	Mean.
Taylor & King. (1936)	5-10 microns.	8.5-10.0	9.6	4.6-6.2	5.5
Bingham. (1958)	7 microns.	10.0	-	6.0	-
Prinsloo. (1962)	4-14 microns.	12.0	-	8.0	-

Unfortunately, none of these authors mentioned how many individuals were measured in each case or the methods used to measure the amoeba. This makes it difficult to compare the results obtained by the various workers. The present study was made in an attempt to obtain more accurate measurements of the parasite.

2. Structure.

King and Taylor (1936), Bingham (1958) and Prinsloo (1962) all gave descriptions of the structure of Malameba locustae. These studies were made made using the light microscope.

On trying to confirm the observations of these workers, I found that the light microscope did not yield any new information and decided, therefore, to investigate the ultrastructure of the parasite using the electron microscope.

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The findings of the various workers are summarised as follows:

Trophozoite:

King and Taylor described the trophozoite as being uninucleate and having hyaline cytoplasm containing 8 to 30 highly refractile granules in the living animal. They reported that locomotion took place by means of wavelike contractions of hemi-spherical pseudopodia and occasionally by means of filose pseudopoda. In fixed material the cytoplasm was found to be finely granular and the nucleus possessed a prominent karyosome with a little diffuse chromatin peripherally situated.

Bingham found the trophozoite to have a centrally placed nucleus and the cytoplasm to contain large numbers of highly refractile granules showing Brownian movement. He did not, however, observe any locomotion in the trophozoites that he studied.

Prinsloo described the trophozoites as circular and possessing refractile granules in the cytoplasm. No active locomotion was seen although half-round pseudopodia were sometimes observed. No contractile vacuoles were found. The nuclei were seen to have a well marked karyosome and in some cases two nuclei were present.

Cysts:

In their original paper, King and Taylor found the cysts to be uninucleate, oval and with a circular cross-section. The cyst wall was found to be thick and hyaline and the living cytoplasm to contain refractile granules similar to those found in the trophozoite. The nucleus usually had a small karyosome and peripheral chromatin. They found also that the cyst often had a prominent vacuole and one or two rod-like inclusions in the cytoplasm.

/ Bingham

Bingham also described the cysts as having oval, highly refractile walls, with a centrally placed nucleus. He pointed out that newly formed cysts are very granular, whereas older cysts found in the faeces seldom had more than two conspicuous granules.

Prinsloo found the cysts to be uninucleate, thick walled and possessing a number of refractile granules in the cytoplasm.

C. Life History of *Malameba locustae*.

Little is known about the life history of *M. locustae*. Taylor and King (1936 and 1937) described the transmission from host to host as being by ingestion of the cyst with the food. They gave the time from primary ingestion until cysts appeared in the malpighian tubes as being 14 to 18 days in *Melanoplus differentialis* and in several other species of grasshopper. Taylor and King went on to describe the presence of trophozoites in the cells of the lumen of the midgut and gastric caecae. They also observed that there was a build-up of the infection in the malpighian tubes resulting ultimately in large numbers of cysts being voided in the faeces.

Bingham (1958) confirmed most of Taylor and King's work but could not find any trophozoites in the gut lumen cells.

Prinsloo (1962) did not add any further information about the life history of the parasite in the host.

In the present study an attempt was made to find out more about the life history of the parasite. The investigation was carried out with the following objectives in view:

- (a) The factors required for the eclosion of the cyst.
- (b) The subsequent fate of the parasite until cysts are voided in the faeces.

/ D.

D. A method for concentrating the cysts of *Malameba locustae* from locust faeces.

In order to investigate the biology of *Malameba locustae* it was found necessary to obtain large numbers of cysts of the parasite. A method for obtaining large quantities of cysts was therefore developed with the following objects in view:

Firstly, as a supply of cysts for studies on the morphology and life history of the amoeba.

Secondly, as a source of cysts to test ways of eradicating the amoeba from the laboratory population of locusts, and finally, to obtain large numbers of cysts for the possible infection of field populations of locusts.

Of the previous workers, Prinsloo (unpublished 1963) was the only one who attempted to make a concentrate of cysts. The method used was to dissect out the malpighian tubes from the infected locust, grind them up and then separate out the cysts by filtering them through cotton wool and centrifuging. The resultant liquid containing large numbers of cysts was used by Prinsloo in her work on the hatching of cysts.

To obtain greater numbers of cysts it was decided to use the faeces of the infected animals as a source of cysts. The use of faeces gave the added advantage of allowing infected animals to continue producing large numbers of cysts.

E. The Control of *Malameba locustae* in laboratory populations of locusts.

The control of the amoeba can be undertaken at any stage of its life history. This means that control can be attempted either when the parasite is in the host or when it is outside the host in the infective stage. In this study it was decided to tackle the problem of eliminating the infective stage of the amoeba, as the eradication of this stage would be an important step toward freeing the locusts of the parasite.

The problem can be approached in several ways: Firstly, a large proportion of the cysts can be eliminated by maintaining clean laboratory conditions. This requires the constant removal of faeces from the locust cages. Although the cages have perforated floors which allow a large

/ proportion

proportion of the faeces to fall out of the cage, a fair amount of faecal matter remains inside. Steps must be taken to remove this remaining source of infection and, ideally, the cages should be cleaned twice daily, morning and evening. The laboratory should also be kept as clean as possible by using a vacuum cleaner to remove all dust and locust debris. The maintenance of such a routine requires the services of enough efficient manpower.

The second approach is the direct attack on the cysts themselves using physical and chemical methods to -

- (a) kill the cysts;
- (b) prevent the development of the cysts; and
- (c) cause the cysts to hatch outside of the host.

In the present study methods (a) and (c) were investigated ((c) being dealt with in the section on the life history of the parasite). Part (b), the prevention of the development of the cysts, was investigated by Prinsloo (unpublished report 1963) who tested a number of chemicals in an attempt to suppress the hatching of the cysts.

The ideal method of killing cysts (and also trophozoites) would be one that -

- (a) at low concentrations acting for a short time is capable of killing a large percentage of the cysts;
- (b) does not have any effect on any stage of the locust;
- (c) is easy to apply and retains its action for a long time.

MATERIALS

MATERIALS AND METHODS.

In order to check the measurements obtained using the camera lucida, a number of cysts were measured in the following way:

A. Morphology of *Malameba locustae*.

Slide preparations of cysts in locust saline were photographed through

(1) Size:

A micrometer slide was also photographed at the same magnification. Enlargements were then made from the negatives and the size of the cysts and divisions of the micrometer scale were measured directly from the photographs using vernier slide calipers. (Plate 1 and Table 4). The head and end of the abdomen of an infected locust were cut off with scissors and the entire gut removed by gripping the foregut with forceps and pulling anteriorly. A number of malpighian tubes were then dissected from the gut with fine forceps and placed in a drop of locust saline (Stall, 1961) on a slide. These tubes were cut into small pieces with fine pointed scissors, thus freeing as much of their contents as possible. A coverslip placed on the drop of saline and tubes completed the preparation.

The slide was studied under the microscope using a X 43 objective and a X 15 eyepiece lens. The outline of each trophozoite or cyst in a sample was drawn using a camera lucida. In order to obtain a

representative sample, the slide was moved from right to left and all trophozoites or cysts entering the field were drawn. After drawing a

sufficient number, the slide was removed and a micrometer slide inserted in its place. The divisions of the scale on this slide were marked on paper, using the same setting of the camera lucida, and then measured. The mean of these measurements was calculated, thus enabling the total magnification to be found for each sample.

The measurements of the drawings of the trophozoites and cysts were made with vernier slide calipers and these measurements converted to microns using the total magnification obtained from the micrometer slide (Tables 2 and 3).

As the trophozoites are only roughly circular in shape it was decided to measure their greatest "diameter" and to use this as the indication of their size. The cysts, on the other hand, are regular in shape and so both their length and breadth were easily measured.

In order to check the measurements obtained using the camera lucida, a number of cysts were measured in the following way:

Slide preparations of cysts in locust saline were photographed through a Leitz microscope with "plano" optics, using a Leitz plate camera attachment. A micrometer slide was also photographed at the same magnification. Enlargements were then made from the negatives and the size of the cysts and divisions of the micrometer scale were measured directly from the photographs using vernier slide calipers. (Plate 1 and Table 4).

A further series of measurements were made to determine if the osmotic pressure had any effect on the size of the trophozoites or cysts.

Although a number of these stains were successful in the trophozoites, Malpighian tubes from an adult male, Locusta migratoria, were placed in solutions of different osmotic pressures. Three solutions were used: distilled water, normal locust saline and a saturated salt solution. In the distilled water and saturated salt solution, no trophozoites were seen and so measurements could only be made of cysts. (Table 5).

were made by dissecting malpighian tubes from the infected locusts,

placed them in locust saline on a slide and cutting the tubes into small pieces.

(2) Structure:

Light Microscope.

In an attempt to stain living parasites, the following stains, dissolved in small quantities of locust saline, were added to fresh preparations of trophozoites and cysts:-

body cavity was then opened dorsally and the malpighian tubes dissected out with fine forceps. The tubes were placed directly into a 1% solution of osmium tetroxide in veronal acetate by Alizanrot order to find the best fixation time, malpighian tubes from the same locust were fixed for varying lengths of time, i.e. 2 min., 5 min., 15 min., 30 min. and 60 min. It was found that 60 min. fixation gave the best results and so

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Alizarin	Indigo carmine.	
Aurantia	Erithrosine.	
Azure B.	Nile Blue Sulphate.	X
Azo Carmine B	Crystal Violet.	X
Azo Carmine G	Celestin Blue.	
Acid fuchsin.	Fast Green.	X
Aniline blue.	Methyl green.	X
Thionine.	Methyl violet.	X
Toluidin blue.	Phloxine.	
Neutral red.	Naphthol yellow.	

Although a number of these stains were successful in the trophozoites and cysts (those stains marked with an X) there was no differentiation of any structure.

The best results were obtained with fresh preparations of the trophozoites and the cysts in locust saline. These fresh preparations were made by dissecting malpighian tubes from the infected locusts, placing them in locust saline on a slide and cutting the tubes into small pieces. Cysts which had been separated from the faeces of infected locusts were treated in various ways in an attempt to discover the factors responsible for eclosion.

Electron Microscope.

Locusts were anaesthetized by cooling them to 0° C. with ice. The body cavity was then opened dorsally and the malpighian tubes dissected out with fine forceps. The tubes were placed directly into a 1% solution of osmium tetroxide in veronal acetate buffer, pH 7.3. In order to find the best fixation time, malpighian tubes from the same locust were fixed for varying lengths of time, i.e. 2 min., 5 min., 15 min., 30 min. and 60 min. It was found that 60 min. fixation gave the best results and so

/ this

this time was used in all subsequent fixations. After the tissue had been fixed it was washed in veronal acetate buffer and then dehydrated in an ascending series of methyl alcohol and embedded in a 1 : 4 mixture of methyl and butyl methacrylates at 55 - 60° C. Thin sections approximately 250Å) of the embedded tissue were cut on a Porter Blum ultra-microtome using glass knives. The sections were supported on formvar coated copper grids and studied using a Siemens Electron microscope, Elmiscope I.

Dried cysts, extracted from locust faeces, were fixed, embedded and sectioned in the same manner.

All the **electron** micrographs were taken on roll film and enlargements made on Agfa paper using a Leitz enlarger.

B. Life history of *Malameba locustae*.

(a) Eclosion of the cysts.

Cysts which had been separated from the faeces of infected locusts were treated in various ways in an attempt to discover the factors responsible for eclosion.

The first treatment consisted of placing cysts in buffer solutions of varying hydrogen ion concentrations (pH) and examining them after one hour to see how many had hatched. The buffer solution used was a citric acid/phosphate mixture (Biol. Laboratory data, pp.73) and values of pH used in the treatment were 2, 3, 4, 5, 6, 7, 8 and 9. A control was run by placing cysts into a locust saline solution of pH 7.9. This treatment was repeated a total of three times (at room temperature).

/ The

The second treatment consisted of placing cysts in solutions of extracts of various parts of the alimentary system of L. migratoria adults and then examining them after one hour. The extracts were made by dissecting out the relevant part of twenty locusts, then homogenising them in a pH 6 buffer solution, followed by filtration at 0° C. The parts of the locust used were - salivary gland, foregut, caecae plus midgut, malpighian tubes and lastly, hindgut. A control was run by placing cysts in pH 6 buffer solution. The whole treatment was repeated three times (at room temperature).

(b) Life History.

In order to investigate the progress of the disease in the locust, transverse serial sections were made of the gut of each instar of L. migratoria, i.e. 1st to 5th instars and the adult.

The gut, including the malpighian tubes was removed by cutting off the head and end of the abdomen and pulling out the entire alimentary tract anteriorly. This was then placed in Bouins fixative and fixed for 12 hours. After washing, dehydration, clearing and embedding in paraffin wax, transverse serial sections, 10 microns thick were cut and stained with Hansen's haematoxylin and eosin. The sections were mounted in D.P.X. and examined under a compound microscope.

Observations were also made on the malpighian tubes of each instar of L. migratoria, S. gregaria and L. pardalina by removing them from the locust and placing them in a drop of locust saline on a slide.

Faeces from each instar of L. migratoria, S. gregaria and L. pardalina were examined by macerating them with needles in a drop of water on a slide and observing the presence or absence of cysts under a compound microscope. (A quick method of determining whether adults are infected).

C. A method for concentrating the cysts of *Malameba locustae*.

The method consists basically of:

- collecting faeces;
- blending the faeces with water;
- sieving off the larger components;
- filtering to eliminate most of the smaller components of the faeces;
- centrifuging and washing to concentrate and clean the cysts;
- and
- finally drying the cysts and storing them for future use.

The details of the method are:

- (a) The faeces were collected from the cages of the three species of locust bred in the laboratory (*Locusta migratoria*, *Locustana pardalina* and *Schistocerca gregaria*). The faeces of 5th stage hoppers and the adult locusts were used, as it is in these stages that cyst production is at a maximum.
- (b) 500 ml. of water was placed into a Waring blender and the machine switched on. A handful of faeces (approximately 40 gms.) was then slowly added, followed by a further 200 ml. water. The mixture was blended for 5 minutes. (Plate 2 (a) and (b)).
- (c) The resulting thick liquid was poured through a sieve with size 10 mesh and the residue washed through with water to make up the total filtrate to approximately 2 litres.

This filtrate was then passed through a series of increasingly finer sieves of mesh sizes 20, 30, 40 and 50. (Plate 2 (c)).

/ (d)

- (d) The liquid was next filtered through cotton wool and the volume again made up to approximately 2 litres by washing water through the cotton wool. (Plate 3 (d)).
- (e) After filtering, the liquid containing the cysts was centrifuged (800 ml. at a time) at a speed of 1,000 r.p.m. for 3 minutes. The liquid above the cysts was poured off and water added to wash the cysts. A further centrifuging followed. (Plate 3 (e)).
- (f) The cysts, plus a small quantity of the liquid were poured into shallow petri dishes and allowed to dry in the sun. The dried cysts in powder form were stored in specimen tubes. (Plate 3 (f)).

D. Methods for the control of *Malameba locustae* in laboratory populations of locusts.

The first problem that arose was how to tell whether a cyst was alive or dead. Three solutions present themselves; the first being to see if the cyst will hatch in a suitable medium; the second is to use a suitable dye that will be absorbed to a different degree by living and dead cysts, and the third to see if the cyst is capable of infecting an uninfected locust. For convenience, the second method was investigated.

A number of stains were tested by dissolving a small quantity of the stain in locust saline and applying this to a number of preparations, namely, fresh preparations of cysts from malpighian tubes, cyst extract from faeces, cyst extract which had been boiled in water for 30 minutes and cyst extract which had been in 70% alcohol for one week. Of the stains tested, methyl violet proved to be the most successful.

/ When

When fresh preparations of cysts from malpighian tubes were stained with methyl violet, an average of 8% of the cysts took up the stain completely while the rest remained unstained or only very slightly stained. The number of stained cysts was higher in the cyst extract preparation and about 15% of the cysts took up the stain. In the preparations of cysts that have been boiled or soaked in alcohol, 100% of the cysts were strongly stained. This indicated that only dead cysts were stained by the methyl violet. Absolute proof of this could only be obtained if an efficient method were available for hatching cysts when it would be possible to test the stained and unstained cysts to see whether or not they could hatch. Such a method is not at present available and so the assumption was made, from the indirect evidence of the uptake of the stain in the various preparations, that only dead cysts were stained by the methyl violet.

Cysts, in the form of cyst extract, were subjected to a number of treatments using physical and chemical agents. Samples of these cysts were then taken and stained with methyl violet. The percentage of cysts taking up the stain was found by counting the proportion of stained and unstained cysts.

The details of each treatment will be given in the results of this section.

/ RESULTS.

RESULTS.

A. Morphology of Malameba locustae.

1. Size.

The sizes of trophozoites and cysts are given in Tables 2 and 3 respectively, all measurements by camera lucida having been made in locust saline.

Table 2.

Species, age, sex.	Number in the sample.	Diameter in microns.		
		Smallest.	Largest.	Mean.
<u>Locusta migratoria.</u> Adult, female.	50	7.7	16.9	11.3
<u>L. migratoria.</u> Adult, female.	50	7.0	13.4	10.2
<u>L. migratoria.</u> Adult, female.	50	7.0	14.6	9.8
<u>L. migratoria.</u> Adult, male.	50	6.8	14.6	10.1
<u>Locustana pardalina.</u> Adult, male.	20	6.9	11.2	9.2
<u>Schistocerca gregaria.</u> Adult, male.	30	6.9	13.1	9.5
Combined Results.	250	6.8	16.9	10.2

Table 3.

Species, age, sex.	Number in the sample.	Length (microns)			Breadth (microns)		
		Smallest.	Largest.	Mean.	Smallest.	Largest.	Mean.
<u>Locusta migratoria.</u> Adult, male.	50	12.1	17.6	14.0	6.85	9.76	8.27
<u>L. migratoria.</u> Adult, female.	100	11.9	17.6	13.8	6.75	10.9	8.55
<u>Locustana pardalina.</u> Adult, male.	30	11.4	13.8	12.9	6.18	9.19	7.89
<u>Schistocerca gregaria.</u> Adult, female.	30	12.0	15.4	13.2	6.99	8.70	7.89
Combined Results.	210	11.4	17.6	13.5	6.18	10.9	8.15

In Table 4 the sizes of cysts are given as measured by photography (Plate 1), and in Table 5 measurements are given under different osmotic pressures.

Table 4.

Species, age, sex.	Preparation.	Number in the sample.	Length (microns)			Breadth (microns)		
			Smallest	Largest	Mean	Smallest	Largest	Mean
<u>Locusta migratoria.</u> Adult, female.	Fresh cysts in saline.	50	10.4	14.0	12.5	6.82	8.53	7.74
	Old cysts in water.	50	11.4	15.5	13.4	7.23	9.15	8.28
Combined Results.		100	10.4	15.5	13.0	6.82	9.15	8.01

Table 5.

All measurements made on cysts from an adult male Locusta migratoria.

Solution containing cysts.	Number in the sample.	Length (microns)			Breadth (microns)		
		Smallest	Largest	Mean	Smallest	Largest	Mean.
Normal saline.	50	12.13	17.64	14.0	6.85	9.76	8.27
Distilled water.	50	10.95	15.43	13.5	7.32	9.45	8.19
Saturated saline.	50	11.18	15.67	13.2	6.85	8.90	7.95

(2) Structure.

Light Microscope.

Trophozoites.

In fresh preparations of amoebae (Plate 1), the trophozoites appeared as roughly circular bodies with a thin outer wall and containing a number of refractile granules. The trophozoites presented varying appearances and those different aspects were classified roughly as follows:

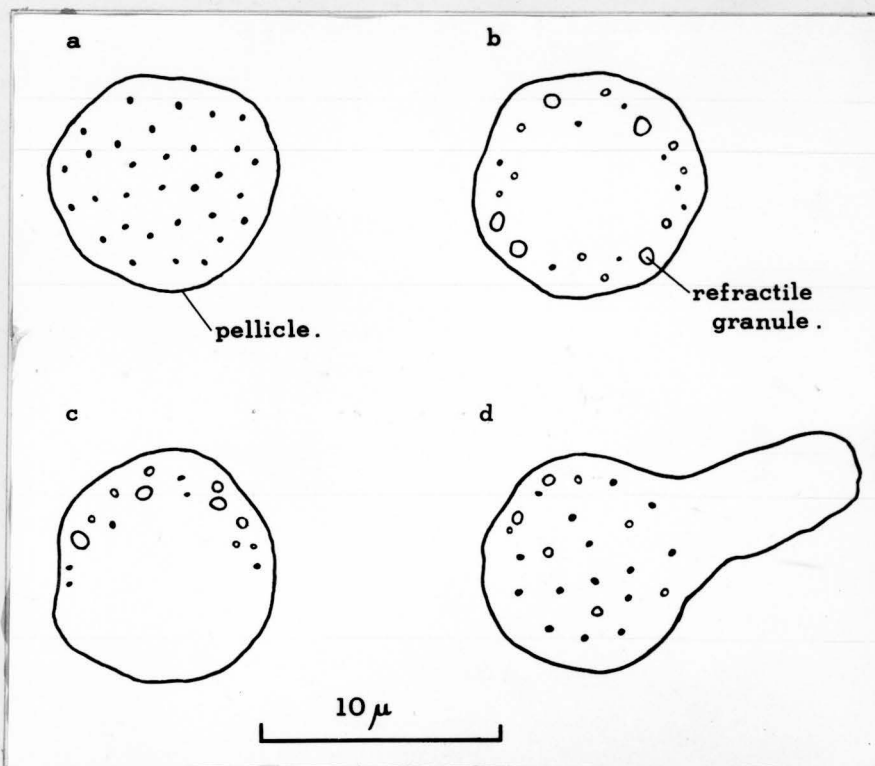
- (1) In this type the shape of the trophozoite is roughly circular and the cytoplasm contains a number of refractile granules, distributed evenly throughout the cytoplasm.
Fig. 1 (a).
- (2) In these trophozoites the refractile granules are distributed round the periphery. Fig. 1 (b).
- (3) The refractile granules of this type are found in an arc at the periphery of the trophozoite. Fig. 1 (c).
- (4) In this last kind, the refractile granules are evenly distributed but the outer wall of the trophozoite has distended to form an elongated projection.
Fig. 1 (d).

/ Single

Single nuclei were sometimes seen, but in all the preparations examined no locomotion, contractile vacuoles or pseudopodia were observed.

Figure 1.

Different Forms of Trophozoites.



Cysts.

Cysts seen in fresh preparations were oval in shape and had thick walls. The cytoplasm of the cyst often contained refractile granules. The arrangement of the granules in the cysts was as follows:

1. In which the granules are very small and evenly distributed throughout the cytoplasm. Fig. 2 (a).
2. In this case the granules are of medium size and fairly evenly distributed. Fig. 2 (b).

/ 3.

Cysts that had been voided in the faeces and that are dry presented a different appearance to those seen in fresh preparations. The dry cysts had either no granules or one to three medium-sized

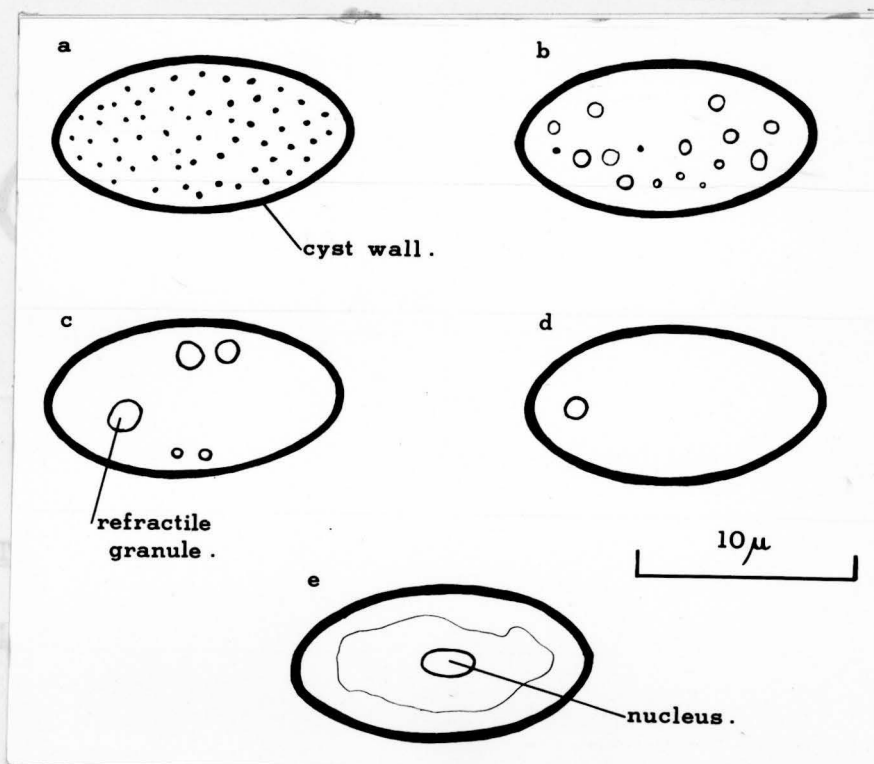
granules. In these cysts there is only one medium-sized granule situated at one end. Fig. 2 (d). No nucleus

occasionally a cyst is seen containing a central nucleus with no granules visible. Fig. 2 (e).

Cysts as seen in the faeces.

Figure 2.

Cysts as seen in the malpighian tubes
of the locust.



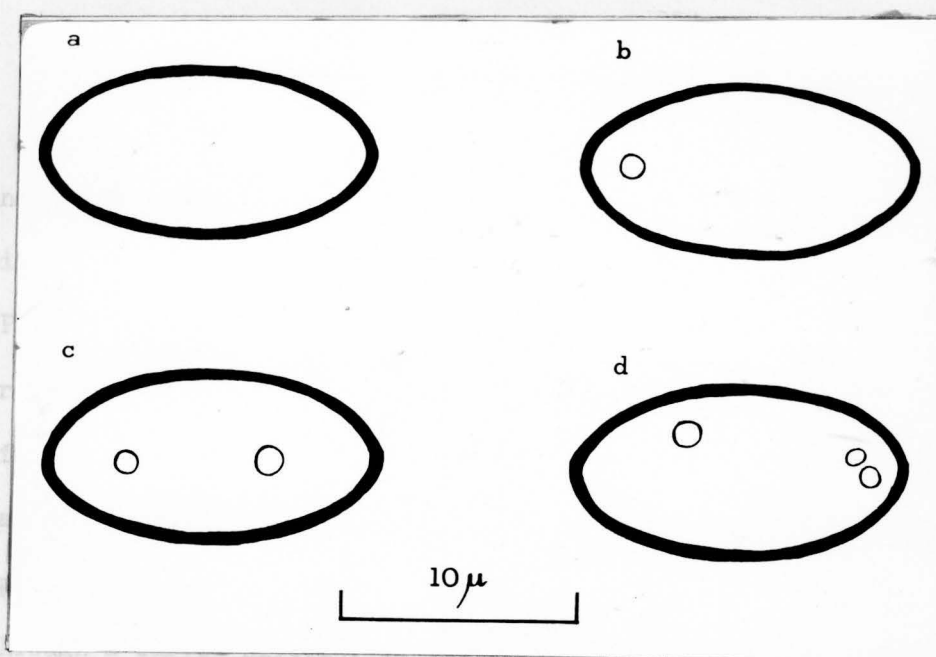
the cysts to the malpighian tube of the host.

The trophozoites are always situated close to the wall of the tube and many of them have the pellicle in close contact with the microvilli of the tube cells. (See Plate 4 (b)).

Cysts that had been voided in the faeces and that are dry presented a different appearance to those seen in fresh preparations. The dry cysts had either no granules or one to three medium-sized granules usually situated at the ends of the cyst. Figs. 3 (a) to (d). No nuclei were seen in these cysts:

Figure 3.

Cysts as seen in the faeces.



A number of cytoplasmic vesicles are uniformly distributed throughout

ELECTRON MICROSCOPE. (ates 6a; 8a; 9, etc.).

Locality of the parasite.

Plates 4 (a) and (b) show the relationship of the trophozoites and the cysts to the malpighian tube of the host.

The trophozoites are always situated close to the wall of the tube and many of them have the pellicle in close contact with the microvilli of the tube cells. (See Plate 4 (b)).

A number of cysts are found at, or near, the wall of the malpighian tube (Plate 4), but the great majority are found in the lumen of the tube, away from the walls.

Trophozoite.

(a) The cell surface.

The trophozoite is bounded by a distinct membrane.
(Plates 5a, b; 7a, b; 8, 9, 10).

(b) Cell inclusions.

The nucleus is oval in shape (having a mean length of 2.0 microns and a mean breadth of 1.5 microns) and is large in relation to the total size of the trophozoite. It is bounded by a double nuclear membrane. (Plates 5a; 6a, b; 7a, b; 8b etc.). A nucleolus is sometimes present and chromatin, peripherally situated, can also be seen in some of the nuclei. (Plates 5a; 6b; 7a, b; 10a, b.). The nuclear contents have a finely granular appearance. (Plate 6). A number of trophozoites appear to have two nuclei. (Plate 10). This may indicate a section through a curved nucleus, or the two nuclei may indicate a dividing cell.

A number of cytoplasmic vesicles are uniformly distributed throughout the cytoplasm. (Plates 6a; 8a; 9, etc.).

Mitochondria are present in the trophozoites and their number varies from 2 to 11. The diameter of the mitochondria varies from 0.4 microns to 1.0 microns. The cristae are not very numerous and appear as small rounded spheres occurring close to the wall of the mitochondria. (Plates 6a, 7b, etc.).

/ In

In some of the trophozoites there are bodies similar to the chromatoid bodies described by Barker (Barker and Deutsch, 1958) and these are dark with a lattice-like appearance. (Plates 5, 8 and 10).

A number of small vesicles are visible. (Plate 15b).

At high magnifications the cytoplasm of the trophozoite appears granular. (Plates 5b; 6b 7b).

The chromatoid bodies are granular in appearance and very prominent, with as many as 17 present. (Plate 16b).

Intermediate stages.

As well as trophozoites and fully formed cysts (as found in the faeces), a number of intermediate stages were seen. These forms were classified into -

(a) trophozoites beginning to encyst;

(b) newly formed cysts.

(a) Encysting trophozoites.

The cell surface appears the same as in the trophozoite. The nuclear material is visible although the nuclear membrane is no longer present. Mitochondria are present and they have a large number of cristae. Chromatoid bodies are present in greater numbers and are larger than those in the trophozoite. They have a prominent appearance due to the presence of a surrounding light coloured area. (Plates 11, 12).

Table 6.

(b) Newly formed cysts.

In newly formed cysts the main body of the cyst is surrounded by a membrane with as many as 10 layers. (Plates 14a; 16a; 17b; 18a). The surface of the new cyst is slightly scalloped. (Plate 13b).

The nuclear and nucleolar material are sometimes visible but the nuclear membrane is not visible. (Plate 14).

A number of small vesicles are visible. (Plate 15b).

Mitochondria are rarely seen.

The chromatoid bodies are granular in appearance and very prominent, with as many as 17 present. (Plate 16b).

At high magnification the cytoplasm appears highly granular.

Cysts.

The fully formed cyst is surrounded by a many-layered membrane. (Plate 20).

The pellicle of the cyst appears to be relatively thick and is sometimes visible as a double structure. (Plate 19b).

The interior of the cyst shows very little differentiation and apart from a few circular bodies, consists of a granular mass of cytoplasm. (Plates 18 to 20).

B. Life history of *Malameba locustae*.

(a) Eclosion of cysts.

Table 6.

The effect of varying pH on the eclosion of cysts of *Malameba locustae*.

Three repetitions gave the same results.

<u>pH</u>	<u>Effect on the cysts.</u>
2.0	-
3.0	-
4.0	-

/ 5.0

Effect on the cysts.
5.0 A few cysts hatched (1%).

6.0 A number of small vesicles are visible. (Plate 15b).
7.0 A few more cysts hatched (3%).
Mitochondria are rarely seen.

7.9 (control) A few cysts hatched (2%).
8.0 The chromatoid bodies are granular in appearance and very prominent,
with as many as 17 present. (Plate 16b).

At high magnification the cytoplasm appears highly granular.

unaffected.

Cysts.

Table 7.

The fully formed cyst is surrounded by a many-layered membrane.
(Plate 20).

The pellicle of the cyst appears to be relatively thick and is
sometimes visible as a double structure. (Plate 19b).

Extract.

Effect on the cysts.

8.1 The interior of the cyst shows very little differentiation and
apart from a few circular bodies, consists of a granular mass of cytoplasm.
(Plates 18 to 20).
A few more cysts hatched. (10%).

B. Life history of *Malameba locustae*.

(a) Eclosion of cysts.

Table 6.

As in Table 6, the majority of the cysts remained unaffected by
the treatments.
The effect of varying pH on the eclosion of cysts of *Malameba locustae*.

Three repetitions gave the same results.

(b) Life history.

pH	Effect on the cysts.
----	----------------------

2.0	-
-----	---

3.0	-
-----	---

4.0	-
-----	---

/ 1st

/ 5.0

pH.	Effect on the cysts.
5.0	A few cysts hatched (1%).
6.0	A few more cysts hatched (4%).
7.0	A few more cysts hatched (3%).
7.9 (control)	A few cysts hatched (2%).
8.0	A few cysts hatched (1%).
9.0	-

In all of the solutions, the majority of the cysts remained unaffected.

Table 7.

The effect of various extracts of Locusta migratoria on the cysts of Malameba locustae.

Three repetitions gave the same results.

Extract.	Effect on the cysts.
Salivary gland.	A few cysts hatched. (5%).
Foregut.	- (1%).
Midgut + caecae.	A few more cysts hatched. (10%).
Malpighian tubes.	- (2%).
Hindgut.	- (1%).
pH 6 buffer (control).	- (2%).

As in Table 6, the majority of the cysts remained unaffected by the treatments.

Locusta migratoria, Locustana pardalina and Schistocerca gregaria showed a similar picture.

(b) Life history.

On examining the serial sections of the guts of the instars of Locusta migratoria, the following was found:

In L. migratoria and L. pardalina, the sequence of events appeared very similar while in Schistocerca gregaria it was slightly different.

/ 1st

/ The

1st instar. *Findings* No trophozoites or cysts seen.

2nd instar. *L.M., L.P.* Trophozoites seen in the gastric caecae and in the midgut between the peritrophic membrane and the gut lumen cells. No cysts seen.

3rd instar. *L.M., L.P.* Trophozoites seen in the caecae and midgut as in the second instar. In addition, trophozoites seen in fair numbers in some of the anterior malpighian tubes.

4th instar. *L.M., L.P. and S.G.* Trophozoites seen in the caecae and midgut as in the second instar. Trophozoites also seen in large numbers in some of the anterior and posterior malpighian tubes. Trophozoites and cysts in large numbers and equal proportions seen in some of the anterior and a few of the posterior malpighian tubes. *seen in both anterior*

5th instar. Trophozoites in small numbers together with large numbers of cysts seen in nearly all the anterior and in over half of the posterior malpighian tubes. Cysts seen in large numbers in the hindgut.

Adult. Trophozoites in small numbers together with large numbers of cysts in all the anterior and posterior malpighian tubes. Cysts seen in large numbers in the hindgut.

Examination of fresh malpighian tubes of the various instars of Locusta migratoria, Locustana pardalina and Schistocerca gregaria showed a similar picture.

In L. migratoria and L. pardalina, the sequence of events appeared very similar while in Schistocerca gregaria it was slightly different.

/ The

The findings were as follows:

1st instar L.m., L.p., S.g. No trophozoites or cysts seen.
 2nd instar L.m., L.p. No trophozoites or cysts seen.
 3rd instar L.m., L.p. and
 2nd instar S.g. A few trophozoites seen in the anterior
 and posterior malpighian tubes.

C. A method for concentrating the cysts from the faeces.
 No cysts seen.

4th instar L.m., L.p. and
 3rd instar S.g. Specimens in this category gave varying
 results. In some, a fair number of
 trophozoites were seen in the anterior
 tubes and only a few in the posterior
 tubes. In others large numbers of
 trophozoites were seen in both anterior
 and posterior tubes.
 In (a) it can be seen that the larger particles have been eliminated
 by the extraction process and that only cysts and smaller particles remain.
 The production of faeces by locusts depends on the numbers present at any one time.
 The figures for the production of faeces by adults and 5th stage hoppers over a four-day period are as follows:-

5th instar L.m., L.p. and
 4th instar S.g. Few trophozoites and large numbers of
 cysts seen in all anterior and most of
 the posterior tubes.

Adult L.m., L.p., and
 5th and Adult S.g. Few trophozoites and large numbers of
 cysts seen in all anterior and posterior
 malpighian tubes.

Day.	No. of Cages.	No. of Locusts.	Weight of Faeces/ Locust (gr.).	Mean Weight of Faeces/ Locust (gr.).
2	19			
3	18			0.09
4	19	1,361	111	0.08
MEAN			/ Daily	0.10

Daily examination of faeces of all the instars of Locusta migratoria, Locustana pardalina and Schistocerca gregaria showed that large numbers of cysts first appear in the faeces during the 5th instar of L. migratoria, the 5th instar of L. pardalina and the 4th instar of S. gregaria.

C. A method for concentrating the cysts of *Malameba locustae* from locust faeces.

Plate 21 shows (a) cysts as they occur in the faeces of an infected locust and (b) cysts prepared by the extraction process.

In (a) it can be seen that the faeces consist of the remains of the grasses eaten by the locust and these are relatively large in size as compared with the cysts. Also occurring in the faeces are much smaller particles, the identity of which has not been determined.

In (b) it is seen that all the larger particles have been eliminated by the extraction process and that only cysts and smaller particles remain.

The production of faeces by locusts in the Locust Research Unit depends on the numbers present at any one time. The amount of faeces which can be used for cyst extraction is further limited to the number of adults and 5th stage hoppers in the population. The figures for faeces production by adults and 5th stage hoppers over a four-day period are as follows:-

Table 8.

Day.	No. of Cages.	No. of Locusts.	Weight of Faeces (gm.)	Mean Weight of faeces/Locust (gm.).
1	22	1,325	170	0.13
2	19	1,269	102	0.08
3	18	909	85	0.09
4	19	1,381	111	0.08
MEAN			117	0.10

/These

These results show that one locust produces an average of 0.10 gm. faeces per day or alternatively, that 1 gm. faeces per day requires 10 locusts.

In order to obtain some idea of (a) the weight of cysts voided from one locust during one day, (b) the possible output of cyst extract obtainable from the Locust Research Unit per day, and (c) the number of locusts required to produce 1 gm. of cyst extract per day, the faeces and extract were weighed.

The results were:

Table 9.

Weight of Faeces.	Weight of Extract.	Weight of Extract from 1 gm. Faeces.	Weight of Faeces for 1 gm. Cyst Extract.
27 gm.	0.33 gm.	0.012 gm.	82 gm.
42 gm.	0.53 gm.	0.013 gm.	79 gm.
41 gm.	0.50 gm.	0.012 gm.	82 gm.
49 gm.	0.51 gm.	0.010 gm.	85 gm.
MEAN		0.012 gm.	82 gm.

i.e. 1 gm. of faeces produces an average 0.012 gm. of cyst extract or 82 gm. of faeces are required to produce 1 gm. cyst extract.

As one locust produces an average of 0.10 gm. faeces per day, then the mean weight of cysts from that amount of faeces is 0.0012 gm. per day.

As it requires 82 gm. faeces/day to produce 1 gm. of extract and one locust produces 0.10 gm. faeces/day, it will require 820 locusts to produce 1 gm. cyst extract/day.

The average output of faeces containing cysts from the Locust Unit over the four-day period was 117 gm./day and so this could be expected to yield 1.43 gm. cyst extract per day.

/ The

D. The results can be summarised as follows: populations of locusts.

Physical treatments:

One locust gives a mean weight of 0.10 gm. faeces/day and

(a) this yields 0.0012 gm. cyst extract/day.

One gram cyst extract requires a mean weight of 82 gm.

faeces/day or the total faeces from 820 locusts from one

day.

An attempt was made to find out how many cysts there were in 1 gm. of cyst extract. The method used was as follows: 10 mgm. of cyst extract was blended in 500 ml. water. 1 ml. of this mixture was placed in a Sedgewick-Rafter counting cell and a count made of the cysts. This process was repeated five times and the mean value for the number of cysts in 1 ml. of the mixture was 5,462 cysts, thus in 500 ml. there must have been 2,731,000 cysts which is also the number in 10 mg. Therefore, in 1 gm. of cyst extract there should be approximately 2,731,000,000 cysts. One locust should therefore void approximately 22,758 cysts per day.

Time.	Temperatures in °C.							
	50°	60°	65°	70°	75°	80°	90°	Boiling.
1 min.	10	20	32	62	95	100	100	100
5 min.	18	31	46	89	100	100	100	100
10 min.	17	31	49	100	100	100	100	100
15 min.	17	29	56	100	100	100	100	100

The figures in the above columns are percentage mortality.

(b) The effect of ultra violet light on cysts.

Small quantities of cyst extract were placed on slides and spread to form a thin layer. These slides were placed 15 inches from a Westinghouse Sterilamp WL-15 ultra violet lamp and left for varying times. Samples of the cysts were stained with methyl violet and the percentage mortality calculated. Control slides were covered with thick card to exclude all ultra violet light. The results in Table 11 are the mean of two repetitions.

/ Table

D. The control of *Malameba locustae* in laboratory populations of locusts.

Physical treatments.

(a) The effect of high temperatures on the cysts.

A quantity of cyst extract was placed in a specimen tube. The tube was placed in a water bath at the required temperature and a small quantity of water of the same temperature placed in the tubes to cover the cysts. When a suitable time had elapsed the tube was removed from the bath and immediately filled with water at room temperature in order to reduce the temperature rapidly. A control tube was kept at room temperature for the same period of time. A sample of the cyst material was then taken and the percentage of dead cysts calculated. The results in Table 10 are the mean of three repetitions.

Table 10.

Effect of high temperatures on cysts.

Time.	Temperatures in °C.								Control.
	50°	60°	65°	70°	75°	80°	90°	Boiling.	
1 min.	10	20	32	62	95	100	100	100	15
5 min.	18	31	46	89	100	100	100	100	17
10 min.	17	31	49	100	100	100	100	100	15
15 min.	17	29	56	100	100	100	100	100	18

The figures in the above columns are percentage mortality.

(b) The effect of ultra violet light on cysts.

Small quantities of cyst extract were placed on slides and spread to form a thin layer. These slides were placed 15 inches from a Westinghouse Sterilamp WL-15 ultra violet lamp and left for varying times. Samples of the cysts were stained with methyl violet and the percentage mortality calculated. Control slides were covered with thick card to exclude all ultra violet light. The results in Table 11 are the mean of two repetitions.

/ Table

Table 11.

The effect of ultra violet on cysts.

Results are the mean of three repetitions.

Time.	Ultra Violet % Mortality.	Control % Mortality.
5 min.	30	10
10 min.	39	18
15 min.	43	14
20 min.	38	14
25 min.	39	17
30 min.	44	15
35 min.	39	20
60 min.	34	11
120 min.	34	15
180 min.	30	14

Chemical Treatments.

Concentration of the Alcohol.

A number of chemicals were used in an attempt to kill the cysts.

In each treatment cyst extract was placed in the liquid containing the chemical of known concentration and left for a suitable time interval.

When the treatment was completed the cysts were washed several times with water to remove all traces of the chemical used in the test. Samples of the cysts were stained with methyl violet and the percentage mortality calculated. Controls were provided by letting cyst extract stand in water for the same time interval as the experimental sample.

The results of the treatments are as follows:-

/ Acetic

Acetic Acid.

Cresol (Jeyes Fluid).

Table 12.

Results are the mean of three repetitions.

Time.	Concentration of the Acetic Acid.				
	100%	90%	50%	10%	Control.
1 Min.	80	97	72	56	10
5 Min.	100	100	97	64	11
10 Min.	100	100	97	67	20
15 Min.	100	100	100	71	14

The figures in the above columns are percentage mortality.

Ethyl Alcohol.

Figures in the above columns are % mortality.

Table 13.

Chlorocresol.

Results are the mean of three repetitions.

Cysts were placed in a saturated solution of chlorocresol (1 gm./250 ml.

Time.	Concentration of the Alcohol.				
	100%	90%	50%	10%	Control.
1 Min.	18	24	18	35	12
5 Min.	22	34	20	46	14
10 Min.	11	75	26	56	17
15 Min.	10	78	25	34	16

Cysts were placed in concentrated formalin (40% formaldehyde in water) and

The figures in the above columns are percentage mortality.

in 90% formalin for 15 minutes.

% mortality in 100% formalin 32.

% mortality in 90% formalin 30.

% mortality in control / Cresol 20.

/ Phenol

Cresol (Jeyes Fluid).

Table 14.

Results are the mean of two repetitions.

Time.	% of Cresol in Water.			
	100%	50%	10%	Control.
1 min.	13	9	12	10
5 min.	9	11	8	9
10 min.	14	11	15	12
15 min.	15	11	17	11

Figures in the above columns are % mortality.

Chlorocresol.

Cysts were placed in a saturated solution of chlorocresol (1 gm./250 ml. water) and left for 15 minutes.

% mortality in chlorocresol 18.

% mortality in control 15.

Formalin.

Cysts were placed in concentrated formalin (40% formaldehyde in water) and in 90% formalin for 15 minutes.

% mortality in 100% formalin 32.

% mortality in 90% formalin 30.

% mortality in control 20.

/ Phenol

Phenol (Carbolic Acid).

DISCUSSION

Table 15.

These results are the mean of three repetitions.

1. Size.

Time.	Concentration of saturated solution of Phenol.								
	Pure Phenol	100%	90%	80%	70%	60%	50%	10%	Control.
1 min.	95	100	97	89	64	30	23	31	16
5 min.	100	100	100	98	87	60	28	26	12
10 min.	100	100	100	100	100	91	43	14	20
15 min.	100	100	100	100	100	98	56	17	18

the trophozoite varies from 7 to 8 microns, a difference of 12.5% and the mean value is 7.6 microns. Figures in the above columns are percentage mortality. 9.6 to 12.0 and 5.5 to 8.0 microns, differences of 20% and 31% respectively.

In the present work the size of the trophozoites was also found to vary considerably, i.e. ***** a difference of 55% (Table 2). The variation in the length and breadth of the cyst (Table 3) was 11.4 to 17.6 and 6.18 to 10.9 microns, respectively, differences of 42% and 45%. (It is interesting to note the close correlation in the variation of the length and breadth of the cysts. This is to be expected when one considers the regularity of the shape of the cyst). The mean values of the length and breadth of the cyst vary from 12.9 to 14.0 and 7.89 to 8.55 microns, i.e. differences of 8% and 7.7%.

DISCUSSION

On looking at Tables 1, 2 and 3, it is seen that the measurements in the present work are generally higher than those of the previous workers, both for the trophozoites and the cysts. The present measurements are nearer in values to those of Prinsloo than to those of Bingham and Taylor and King.

/ If

DISCUSSION.

A. MORPHOLOGY OF MALAMEBA LOCUSTAE.

1. Size.

From Table 1, it can be seen that the measurements obtained by the previous workers vary within fairly large limits, i.e. between 4 and 14 microns in the diameter of the trophozoites and 8.5 to 12.0 and 4.6 to 8.0 microns in the length and breadth of the cysts. This represents a difference of 71% in the diameter of the trophozoites and 29% and 43% in the length and breadth of the cyst. The mean values of the measurements obtained by each worker vary to a less marked degree. The mean diameter of the trophozoite varies from 7 to 8 microns, a difference of 12.5% and the mean values for the length and breadth of the cyst vary from 9.6 to 12.0 and 5.5 to 8.0 microns, differences of 20% and 31% respectively.

In the present work the size of the trophozoites was also found to vary considerably, i.e. from 6.8 to 16.9 microns, a difference of 59%.

(Table 2). The variation in the length and breadth of the cyst

(Table 3) was 11.4 to 17.6 and 6.18 to 10.9 microns, respectively,

differences of 42% and 45%. (It is interesting to note the close correlation in the variation of the length and breadth of the cysts. This is to be expected when one considers the regularity of the shape of the cyst). The mean values of the length and breadth of the cyst vary from 12.9 to 14.0 and 7.89 to 8.55 microns, i.e. differences of 8% and 7.7%.

On looking at Tables 1, 2 and 3, it is seen that the measurements in the present work are generally higher than those of the previous workers, both for the trophozoites and the cysts. The present measurements are nearer in values to those of Prinsloo than to those of Bingham and Taylor and King.

/ If

If the mean values of the previous workers (Table 1) are combined, the average value for the diameter of the trophozoite is 7.5 microns and the average values for the length and breadth of the cyst are 10.5 and 6.5 microns. When the mean values in the present study are combined (Tables 2 and 3), the average diameter of the trophozoite is 10.2 microns and the average length and breadth of the cyst is 13.5 and 8.2 microns, respectively.

On comparing these combined mean values it is found that, in the trophozoite, the differences between the previous and present values for the diameter are 2.7 microns, representing a difference of 27%. The difference between the values for the length and breadth of the cyst are 3.0 and 1.7 microns, differences of 23% and 21%.

The differences in the above results could be accounted for by one or more of the following factors.

(a) Experimental methods.

As mentioned in the Introduction, the previous workers did not give full details as to the methods they used in making the measurements. Such information would have been invaluable in comparing the various results. From the present work it can be seen that the number of individuals in a sample may affect the mean value. In Table 2, the smallest number in a sample is 20 and the mean value for the diameter in this case is the lowest for the series. This effect is again seen in Table 3 where the smaller sample gives a lower value for the mean.

Another factor which has a definite effect on the size of the trophozoite is the osmotic pressure of the surrounding medium. (Table 5). When this medium has a very low and a very high osmotic pressure, the trophozoites disappear and cannot be found in the preparation.

It is reasonable to argue that smaller changes would also have an effect on the size of the trophozoite. (This could be tested experimentally). The cysts on the other hand, seem to be unaffected by drastic changes in the osmotic pressure as, on calculating the standard error of the differences between the means, no significant differences were found in the sizes of the cysts in the three solutions. This resistance of the cyst to changes in the external medium might account for the measurements of the various workers varying to a less marked degree in the cyst than in the trophozoite.

(b) Experimental error.

This can be attributed to the following:

- (i) Microscope error which arises if the microscope does not have "Plano" optics. Drawings made at the edge of the field differ slightly in size to those made in the centre of the field when conventional lenses are used. The error can be avoided by using "Plano" optics and by drawing objects in the centre of the field of view.
- (ii) Drawing error, which arises from two causes, the first being the relative sharpness of the pencil used and the second being the human error in tracing the object using the camera lucida. This error can be overcome by keeping the pencil sharp and by using the photographic method. (Table 4).

The results obtained from cysts measured by this method give the mean value of the length and breadth of the cyst as 13.0 and 8.0 microns respectively. These values correspond closely to the mean values obtained using the camera lucida, which gave

/ and

/ values

values of, length 13.5 microns and breadth 8.15 microns.

(Table 3). This close correspondence indicates that 'drawing error' is not a large source of error.

(iii) Calibration error occurs as a result of slight differences of thickness between the calibration slide and the preparation

slide. This means that when the micrometer slide is inserted, the microscope has to be refocussed slightly.

(iv) Measuring error arises in the measuring of the drawings using the slide calipers.

The summation of these errors could account for the differences in measurements obtained by the various workers.

(c) Actual differences.

It is thought that the actual differences in the size of the parasite might account for the difference in size between the form of Malameba locustae found in South Africa and that measured by Taylor and King in America. The difference in the measurements of the amoeba found in the three species of locusts is thought to be due to differences in experimental method and error, rather than to actual differences in the size of the parasite.

It is interesting to note (Tables 2 and 3) that the size of the amoeba does not vary to any extent between the three species of locust studied or between male and female locusts of the same species.

In conclusion, the present series of measurements gives higher values for the size of Malameba locustae than previously reported.

The differences between these values and those obtained by Prinsloo

/ and

and Bingham are attributed to a combination of differences in the techniques used to measure the amoeba. Those differences between the present values and those obtained by Taylor and King, are attributed to actual size differences in the parasite.

2. Structure.

Light Microscope.

The results of the present investigation confirmed certain of the previous workers' observations.

In the trophozoite, nucleus and refractile granules were observed. Pseudopodia or locomotion, however, were not seen as reported by Taylor and King, and by Prinsloo. The form of the trophozoite as described in the results (Fig. 1(d)) is not regarded as having a pseudopodium, as the pellicle only appears to be distended and contains no cytoplasm. It is thought that this distention is an artifact caused by osmotic pressure. The various arrangements and numbers of granules in the cytoplasm of the trophozoites might represent various stages in their life histories but there is no evidence to support this hypothesis.

In the cyst, a nucleus, thick walls and refractile granules, as recorded by Taylor and King, Bingham, and Prinsloo, were observed. Differences in cysts as found in the malpighian tubes and the faeces, which were mentioned by Bingham, were also seen. (Figs. 2 and 3). This reduction in the number of granules suggests a decrease in activity possibly related to the survival of the cyst over long periods in adverse conditions.

/ Electron

Electron Microscope.

Far greater detail is observed in using the electron microscope than when studying the parasite under the light microscope. The fine structure of both the trophozoite and the cyst is clearly seen. From the photographs it is also possible to follow the morphological changes that take place when the trophozoite develops into the cyst. Although these changes indicate related physiological events, the various biochemical processes cannot be elucidated by electron microscopy alone as it requires further histochemical and biochemical analysis.

Low power observations show the trophozoites situated close to the wall of the malpighian tube while the cysts lie in the lumen of the tube. The cysts, therefore, are in a more advantageous position to be moved down the tube and voided whereas the trophozoites are more able to maintain their position in the tube by virtue of their close contact with the tube wall. (The electron micrographs do not help in showing by what mechanism the trophozoites maintain themselves in this position).

In the trophozoite the electron micrographs confirm the presence of a thin outer wall and nucleus. The granules seen under the light microscope might be equivalent to the mitochondria seen in the electron micrographs.

During the change from trophozoite to cyst, the following sequence of events occurs: In the trophozoite the nuclear membrane disappears and the mitochondria develop more cristae while the chromatoid bodies are present in greater numbers and are prominent. This is followed by the appearance of the many-layered membrane, characteristic of the fully

/ formed

/ Under

formed cyst, as well as a reduction in the number of mitochondria and the prominent nature of the chromatoid bodies.

(a) The final stage of encystment is reached with the complete development of the many-layered outer membrane and thick pellicle plus the disappearance of nucleus, mitochondria and chromatoid bodies.

The disappearance of the nuclear membrane could indicate a stage of mitosis or meiosis. However, no further evidence as to whether or not this is so, is available from the electron micrographs.

The early increased development of the mitochondria might mean that there is an increased utilization of energy which could be associated with either the development of the chromatoid bodies or, together with these bodies, be concerned in the production of the many-layered outer membrane. This increase in mitochondrial development might also be associated with the storage of cytoplasmic reserves preceding encystment. (Van Wagtendonk, 1955).

The exact function of the chromatoid bodies is uncertain. Barker & Deutsch (1958) showed, by cytochemical methods, that the chromatoid bodies of Entamoeba invadens contain a high RNA content with some unspecified protein. The electron micrographs of the present study show that the chromatoid bodies are visible until the last stages of encystment and persist after the disappearance of the nucleus and mitochondria. This fact does not, however, bring us closer to finding out their function.

On completion of the outer membrane, cytoplasmic activity drops to a low level and the cyst enters a period of quiescence.

/ Under

/ In

In the present study, hydrogen concentration and presence of
Under the light microscope a few granules were observed in the
mature cysts which were not found when using the electron microscope.

The reason for this is not understood but might have been due to either

- (a) thin sections studied not including one of these granules or
- (b) the granules not being fixed or stained with osmium. It is thought
that the former explanation is the more likely one and that the granules
might be homologous with the chromatoid bodies seen in the younger
cysts.

In order to complete any morphological study of Malameba locustae
it is necessary to obtain information of the process on the eclosion of
the cyst. As an efficient method for initiating this process was not
discovered, it was impossible to study the ultra-structure of the
parasite during this phase of its life history.

The cytoplasmic structures found in Malameba locustae do not differ
from those in other Rhizopoda (Pitelka, 1963) and the amoeba has a full
complement of the organelles necessary for its continued existence.

B. LIFE HISTORY OF MALAMEBA LOCUSTAE.

When the cysts of Malameba locusta are ingested by the locust they
must eclose within a certain time or otherwise be passed through the
alimentary tract. This time must be within the time taken for food to
pass completely through the gut. In Locusta migratoria this is one hour
at 32°C. and in Schistocerca gregaria the average time is 1.5 hours.
(Uvarov, B. 1948).

The actual factors which initiate eclosion in Malameba locustae
are not known. Van Wagtendonk (1955) reviewed the known factors causing
excystment in protozoa and these include osmotic pressure, food and oxygen
concentration and the enzymatic dissolution of the cyst wall.

In the present study, hydrogen concentration and presence of gut enzymes were investigated as possible initiators of eclosion of the cyst. The substances used proved largely ineffective in causing the cysts to hatch. There were slight indications, however, as to the possible requisites for eclosion. In the buffer solutions alone the range of pH causing cysts to hatch was from 6 - 7.9. This corresponds to the range of pH found in the alimentary tract of several species of locusts and grasshoppers. (Uvarov, 1948). The best hatching obtained occurred in a pH 6.0 together with enzymes from the midgut plus caecae, and slightly less hatching occurred with a pH of 6.0 and enzymes from the salivary glands.

Although the gut extracts contained the necessary ingredients, the quantities and conditions required for eclosion were not achieved in the present investigation. A more detailed approach using separate enzymes as reported occurring in the alimentary canal of various species of locusts and grasshoppers (Uvarov, 1948) and at their optimum working conditions, might yield more promising results. A start could be made with those found in the salivary glands, caecae and midgut.

That the cysts hatch in or before the gastric caecae is strengthened by the observation, in the serial sections, of trophozoites in the gastric caecae of Locusta migratoria. The sequence of events, as observed in the sectioned material, was interpreted as follows:

- (a) After being ingested, the cysts hatch in or before reaching the gastric caecae - as indicated by the presence of trophozoites in the gastric caecae of the second instar.

/ (b)

(b) Trophozoites pass from the gastric caecae down the midgut by way of the space between the gut lumen cells and the peritrophic membrane - this was observed in the second instar. Taylor and King (1937) reported having seen trophozoites occurring in the cells of the gut lumen. This was not observed by Bingham or in the present investigation.

(c) From the midgut the trophozoites pass into and up the anterior malpighian tubes - the picture as found in the third instar. It is not known how the trophozoites manage to enter the malpighian tubes against the flow of excretory material. This flow, however,

might not be constant but be sporadic in nature.

(d) The trophozoites multiply in the anterior tubes and invade the posterior malpighian tubes where they also increase in numbers - as observed in the fourth instar.

(e) Cysts are formed as soon as the number of trophozoites in the malpighian tubes is large. The exact density of trophozoites and the factors initiating cyst formation are not known - fourth instar.

(f) Large numbers of cysts are formed and fill the malpighian tubes. They are also voided in large quantities in the faeces. From an examination of the faeces, cysts appear to start being voided in any numbers in the fifth instar of Locusta migratoria and Locustana pardalina and in the fourth instar of Schistocerca gregaria, a duration of approximately twenty-two days from the emergence of the first instar.

(g) After a period of time the cysts are ingested by another host. It is thought that the cysts remain viable for a long time but no work has been done on this aspect.

Examination of the fresh malpighian tubes from the three species of locusts largely confirmed the findings in the malpighian tubes of the serial sections. The increase in the trophozoites and cyst formation took place one instar earlier in Schistocerca gregaria, although the end result is the same.

Prinsloo (1962) has suggested that the cyst is able to hatch and give rise to trophozoites within the malpighian tubes. This has not been observed and the morphological evidence points to the cysts having to leave the host before their development can be completed.

C. A METHOD FOR CONCENTRATING CYSTS OF M. LOCUSTA.

Plate 21 shows that the method of extracting cysts developed in this study is largely successful. The bulk of the faecal material has been eliminated leaving cysts and particles smaller than cysts. For the purposes of the study, i.e. aspects of the life history of the parasite, the cyst extract has proved entirely satisfactory.

For the production of an extract containing cysts only, a method would have to be devised to remove the small particles present. It is unlikely that sieving or filtering will be able to eliminate these particles and other methods, such as differential centrifugation in a sucrose gradient, would have to be used. It is, however, unlikely that a more purified extract than the one obtained by the above method would ever be required.

If it were decided to mass produce the cysts for an attempted large scale infection of field populations, the method of extraction would have to be considerably streamlined. Using the present method it was found that one worker could process two handfuls of faeces (approx. 85 gms.)

Cages, food jars and sand containers could all be sterilized by dipping them into boiling water for a short time. This could only be

/ done

in a morning. This meant that one day's production of faeces from the Locust Unit required two mornings' work. Methods would therefore have to be devised for the bulk handling of faeces should large quantities of cysts be required.

The figures obtained for the amount of cysts in the extract can only be taken as approximations. The reasons for this are, firstly, the extraction process is not 100% efficient and numbers of cysts are lost at each stage of the process and, secondly, the method of counting the cysts is also an approximation. Even although the figures obtained are approximate they give a very good idea of the astronomical numbers of cysts present in the total daily weight of faeces from infected locusts and underline the necessity for the maintenance of clean laboratory conditions.

D. THE CONTROL OF N. LOCUSTA IN LABORATORY POPULATIONS OF LOCUSTS.

Of all the methods tested, very few proved efficient in killing the cysts. Those that gave the best results were heat, glacial acetic acid and phenol (carbolic acid).

The results of the temperature experiment (Table 10) show that a minimum temperature of 65°C . acting on the cysts for 10 minutes is able to kill 49% (approximately 50%) of the cysts, i.e. the LD 50 is 65°C . for 10 minutes. At temperatures higher than 65°C . the percentage mortality rises rapidly to 100% and at a temperature between 75°C . and 80°C . the cysts are killed by an exposure of 1 minute.

This method of killing the cysts has been exploited in the laboratory. All sand into which the locusts lay their eggs is sterilized by heat. Heat could be more extensively used in the combating of the amoeba. Cages, food jars and sand containers could all be sterilized by dipping them into boiling water for a short time. This could only be

done to the cages when they did not contain locusts. The food containers could be removed from the cages and sterilized more often. The heat treatment would also be useful in killing harmful fungi and bacteria.

Chemical methods of control have the advantage of being able to be applied while the cage contains a population of locusts. They are limited, however, in the time it takes for the liquid to evaporate and the harmful effects that they or their residue might have on the locusts.

Acetic acid (glacial) is capable of killing 56% of the cysts at a concentration of 10% acting for 1 minute. Higher concentrations acting for longer times give a correspondingly higher percentage of mortality. (Table 12).

Phenol gives a 56% mortality of the cysts at a concentration of 50% saturated solution (1 gm./13 ml. water) acting for 15 minutes. To be able to kill at least 50% of the cysts in one minute, the phenol must have a concentration of 70% saturated solution.

The treatments using ethyl alcohol, cresol, chlorocresol and formalin did not show any promising signs of killing the cysts at the concentrations and time intervals used in the experiments. (Tables 13, 14, 15). Of the three, ethyl alcohol seemed to give the highest percentage mortality.

The results of the action of the ultra violet light show that the treated cysts have a consistently higher mortality than that of the control. This can be interpreted by assuming that the ultra violet does kill the cysts but is only able to act on the top layer of the sample and is unable to penetrate to the underlying cysts.

In a preliminary test, 50% glacial acetic acid and 70% phenol remained wet for at least 5 minutes after being brushed on to the bottom

SUMMARY - 49 -

1. of the locust cages (at the temperature of the laboratory $\pm 30^{\circ}\text{C.}$), and this would give them sufficient time to kill a large percentage of the cysts. Adult locusts in the cage did not seem to be affected
2. A morphological study was undertaken using light and electron microscopy to investigate the size and morphology of the parasite to be carefully assessed before the large scale introduction of these chemicals as a means of disinfecting the cages.

(a) gain more information about the life history of the parasite;

(b) The substances tested represent only a very few of the numerous substances which could be tried in an attempt to kill the cysts, but even so they show that this avenue of attack could prove successful. The

(c) find a way to eliminate the infection from the laboratory colony method of testing the various chemicals has proved satisfactory and of locusts.

further work would be a matter of the routine screening of new substances.

The methods used in these investigations are described.

3. The size of the amoeba was found to differ from that of previous workers and the reason for this difference is discussed.

The light microscope ***** the morphological findings of the previous workers, while the use of the electron microscope added new information as to the structure of the parasite and the morphological changes during encystment.

Investigation into the life history revealed possible lines of study to determine the factors responsible for eclosion and also helped to clarify the general sequence of events in the life history.

A reasonably efficient method for obtaining large numbers of cysts of Malawha locustae was devised.

Certain chemical and physical treatments proved capable of killing the cysts of the parasite.

S U M M A R Y .

ACKNOWLEDGEMENTS.

1. An outline of previous studies on the parasite Malameba locustae is given and the direction of the present investigation proposed.

I wish to thank Dr. D.J. Nolte for his advice and encouragement

2. A morphological study was undertaken using light and electron microscopy to investigate the size and morphology of the amoeba.

Thanks are also due to Mr. A. Lee, Chief Locust Officer, for

Investigations were also undertaken in order to -

(a) gain more information about the life history of the parasite;

(b) find a method for obtaining large quantities of the infective stage; and

(c) find a way to eliminate the infection from the laboratory colony of locusts.

The methods used in these investigations are described.

3. The size of the amoeba was found to differ from that of previous workers and the reason for this difference is discussed.

The light microscope observations confirmed most of the morphological findings of the previous workers, while the use of the electron microscope added new information as to the structure of the parasite and the morphological changes during encystment.

Investigation into the life history revealed possible lines of study to determine the factors responsible for eclosion and also helped to clarify the general sequence of events in the life history.

A reasonably efficient method for obtaining large numbers of cysts of Malameba locustae was devised.

Certain chemical and physical treatments proved capable of killing the cysts of the parasite.

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Besis, I wish to thank Dr. D.J. Nolte for his advice and encouragement during the investigation.

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Thanks are also due to Mr. A. Lea, Chief Locust Officer, for making literature available.

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I am also indebted to Mrs. K. Borthwick for all her help and to Mr. and Mrs. F. Warminger for typing and duplicating the manuscript.

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Scale for Plate 1.

Each division represents 0.01 cm. (10 microns).

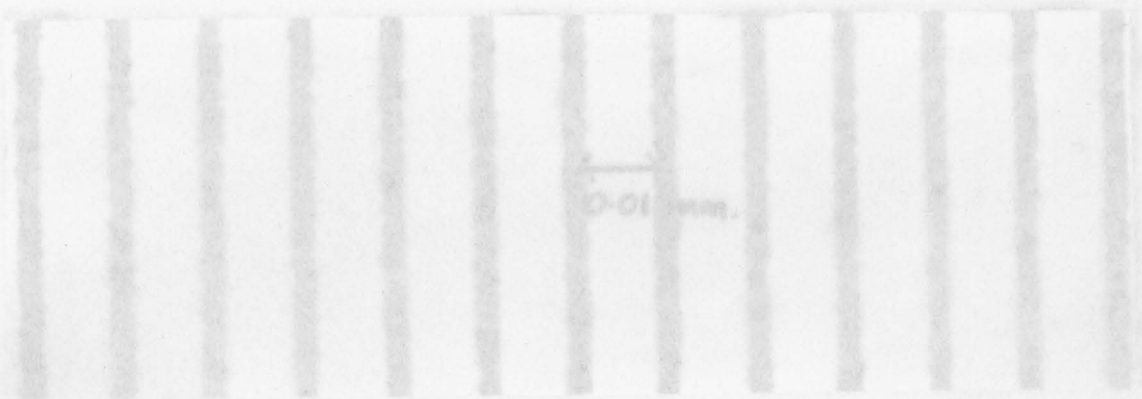
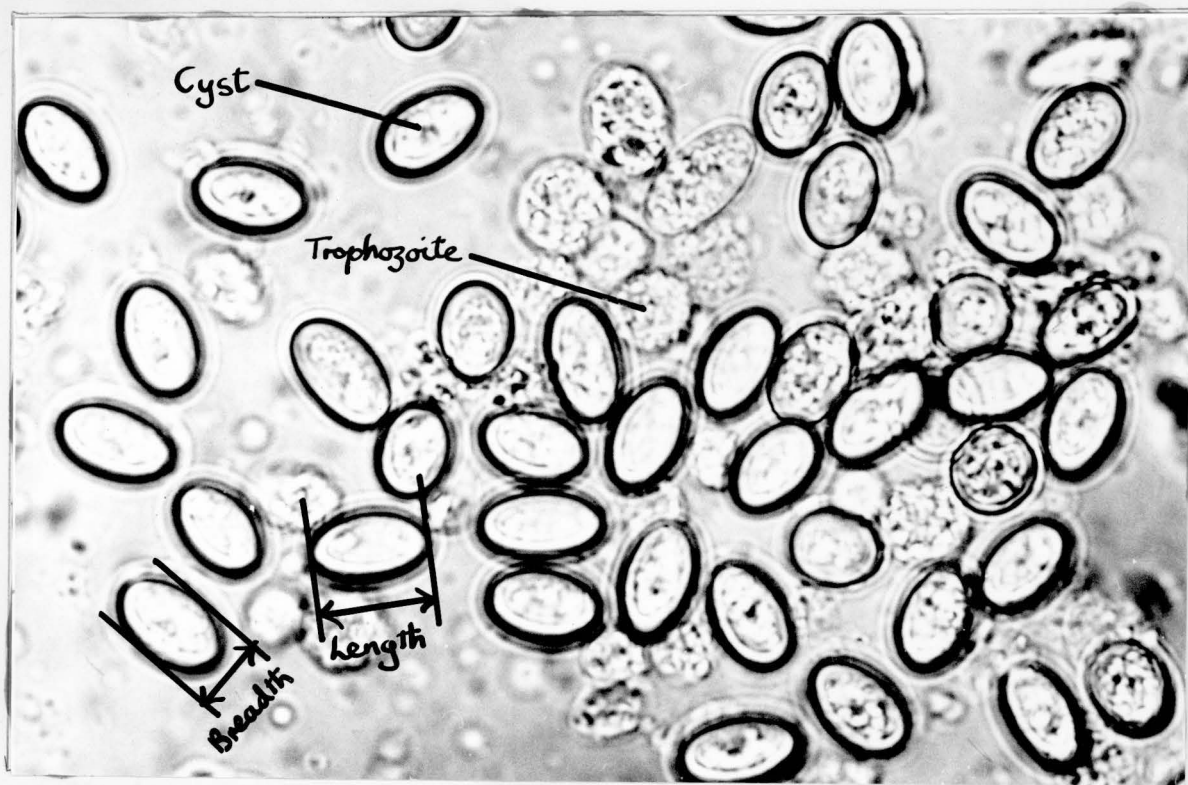


PLATE 1.



Scale for Plate 1.

Each division represents 0.01 mm. (10 microns).



PLATE 2.

Concentration of the cysts of *Malameba locustae* from locust faeces.



Faeces + Water.

Blend.



Sieve.



PLATE 3.



Filter.



Centrifuge.



Dry and Store.

PLATE 4.

Transverse Sections of malpighian tubes containing Malameba locustae.

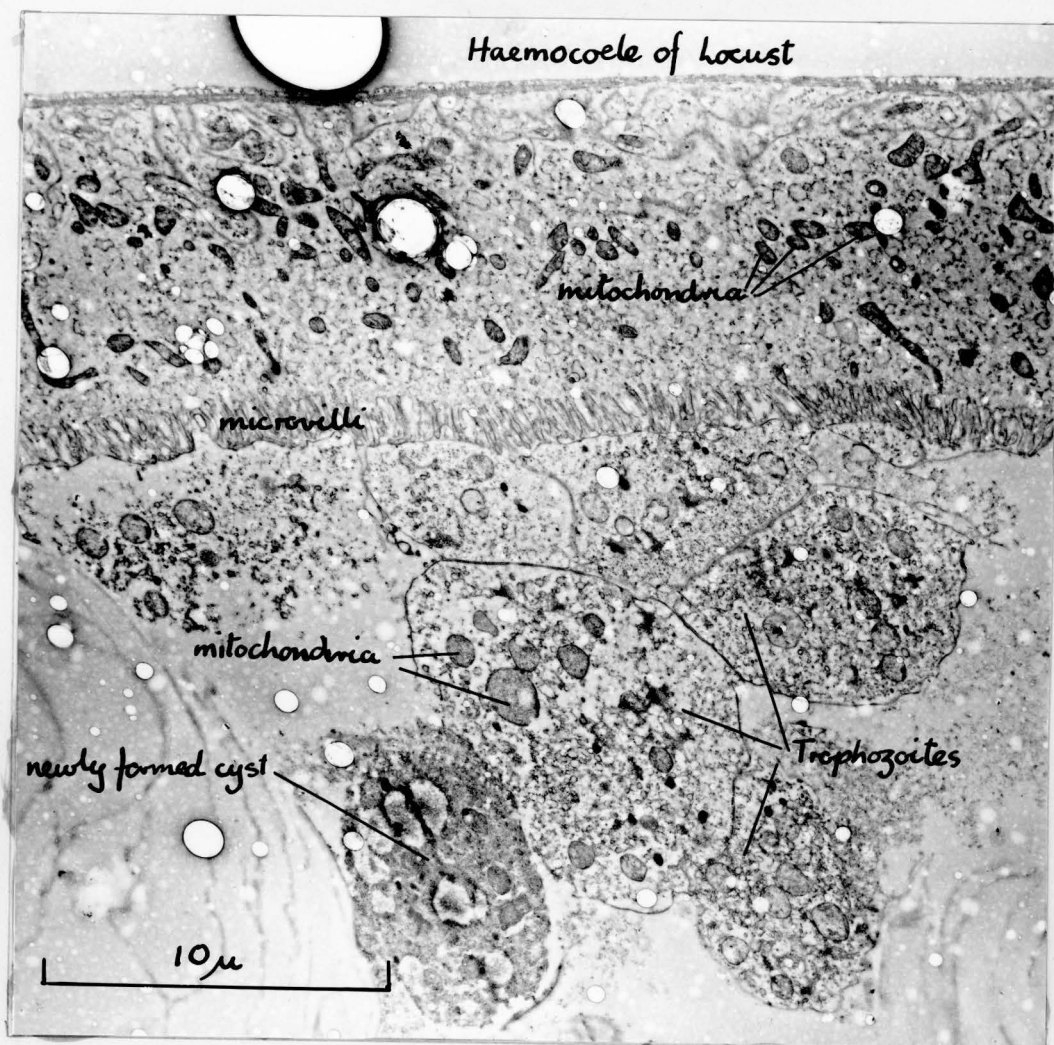
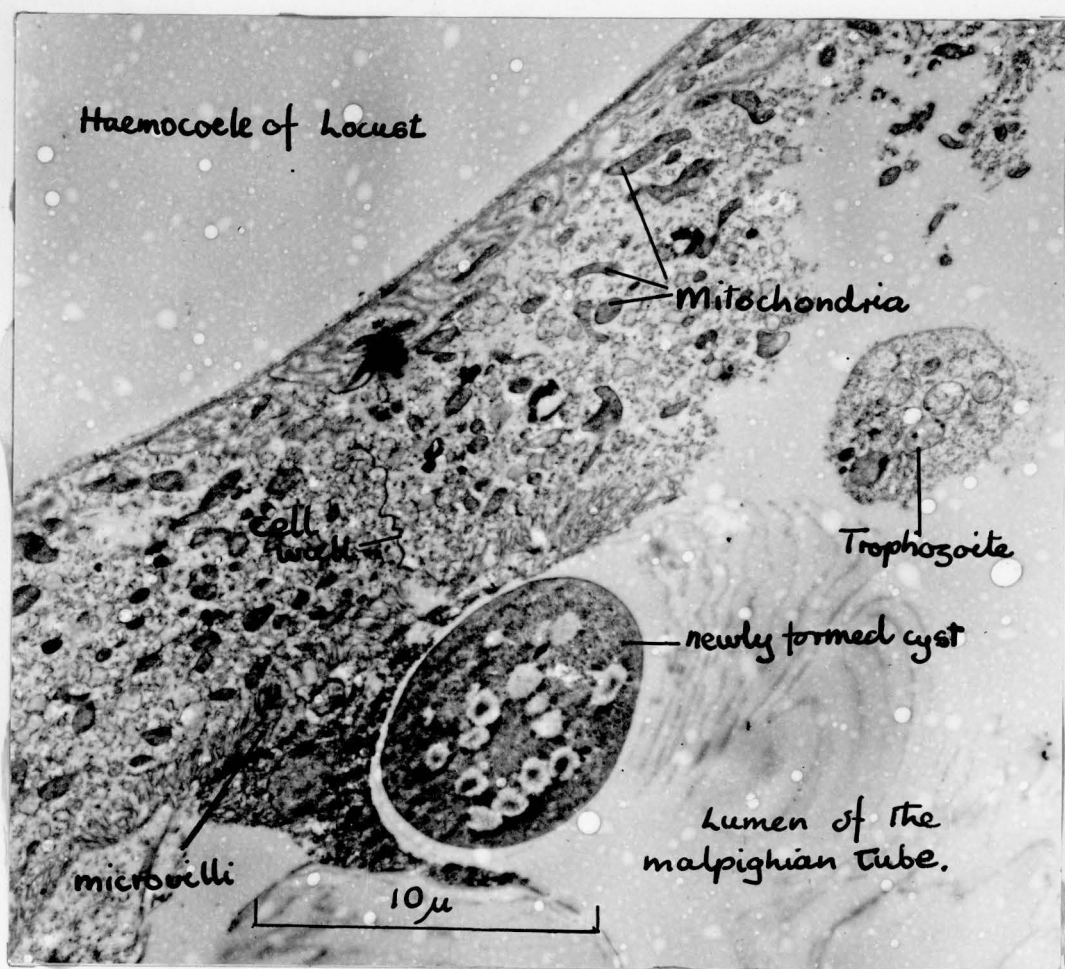
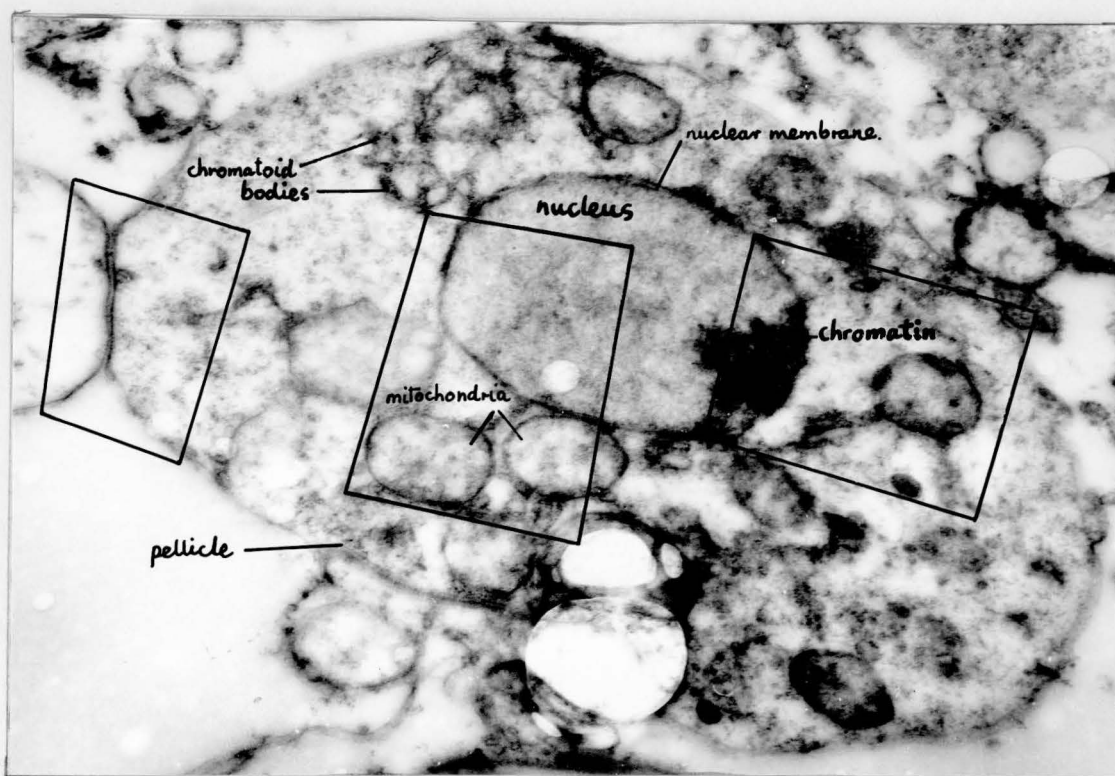


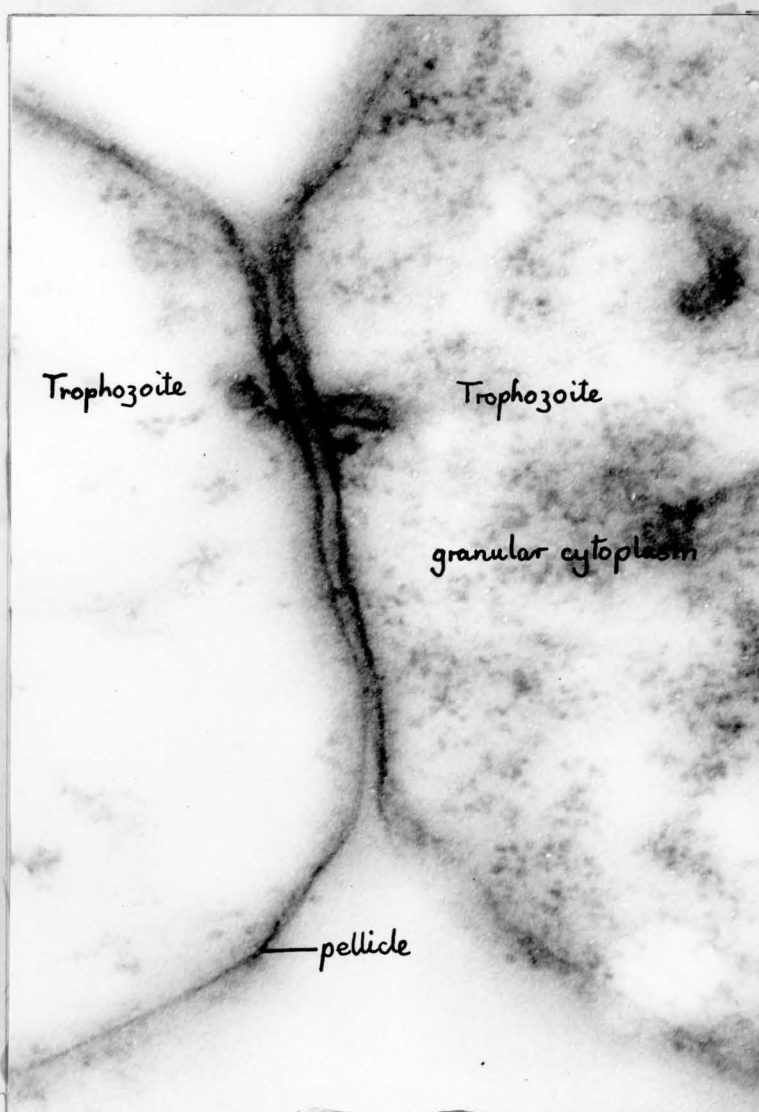
PLATE 5.

Trophozoite.



a.

X24,000

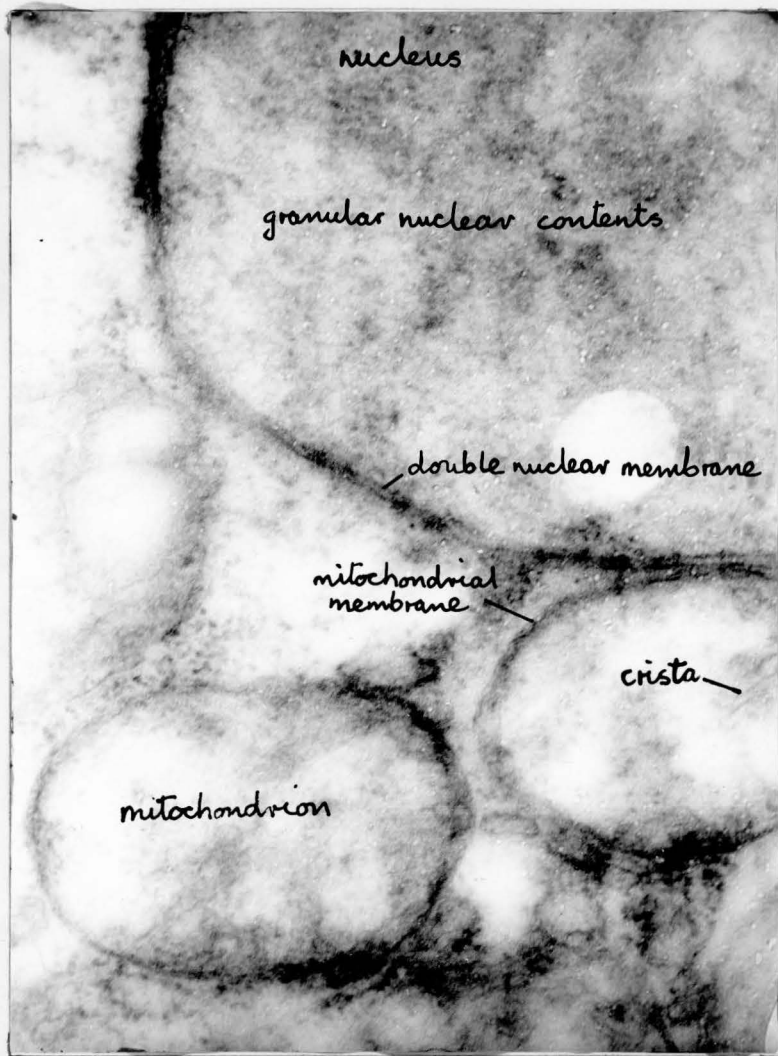


b.

X100,000

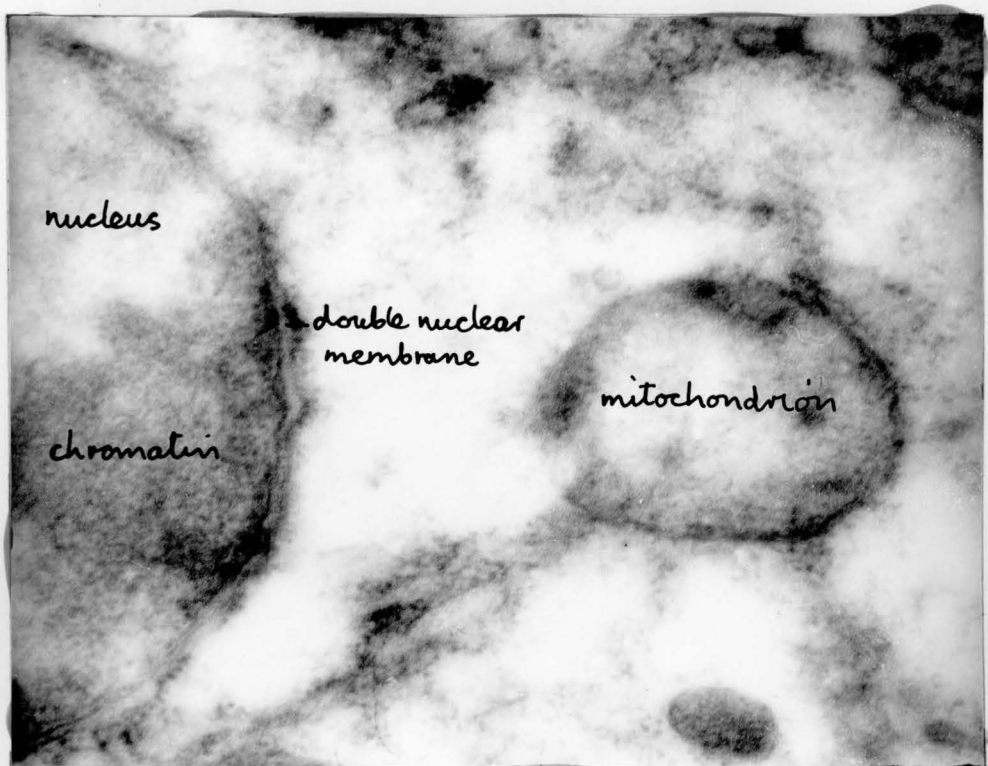
PLATE 6.

Trophozoite.



a.

X80,000

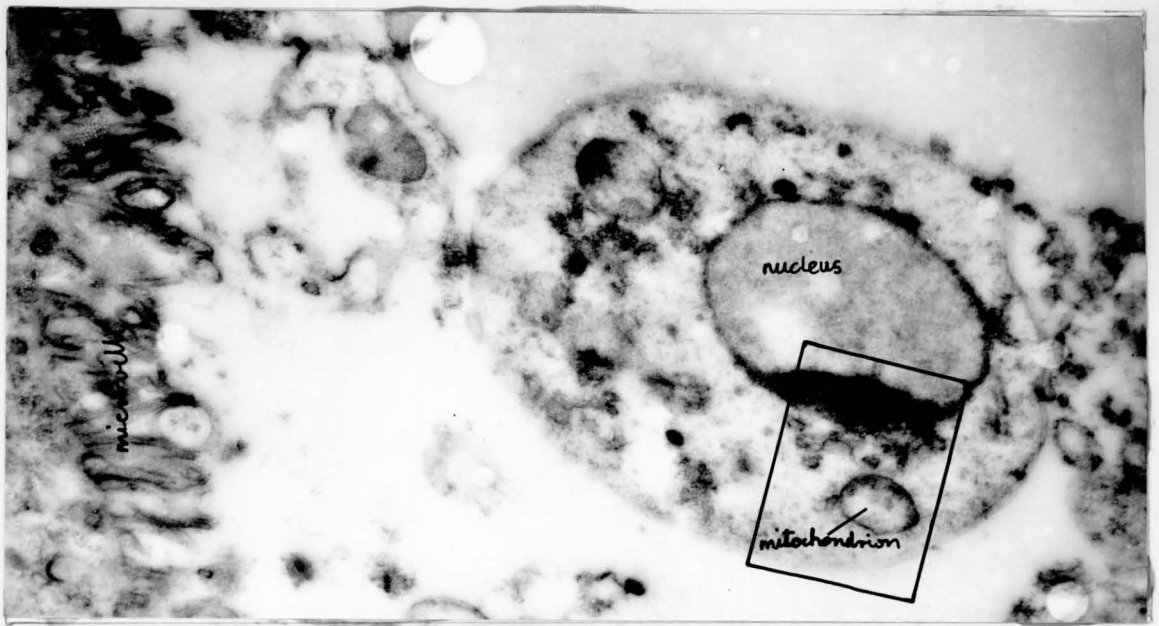


b.

X80,000

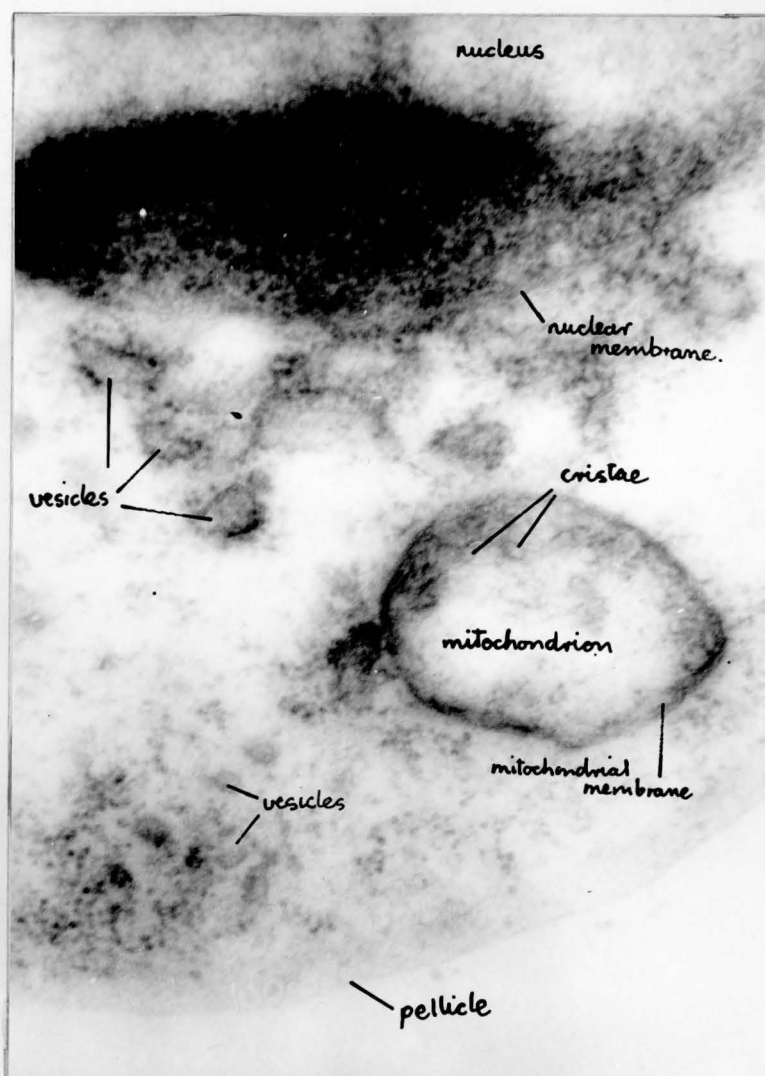
PLATE 7.

Trophozoite.



a.

X20,000

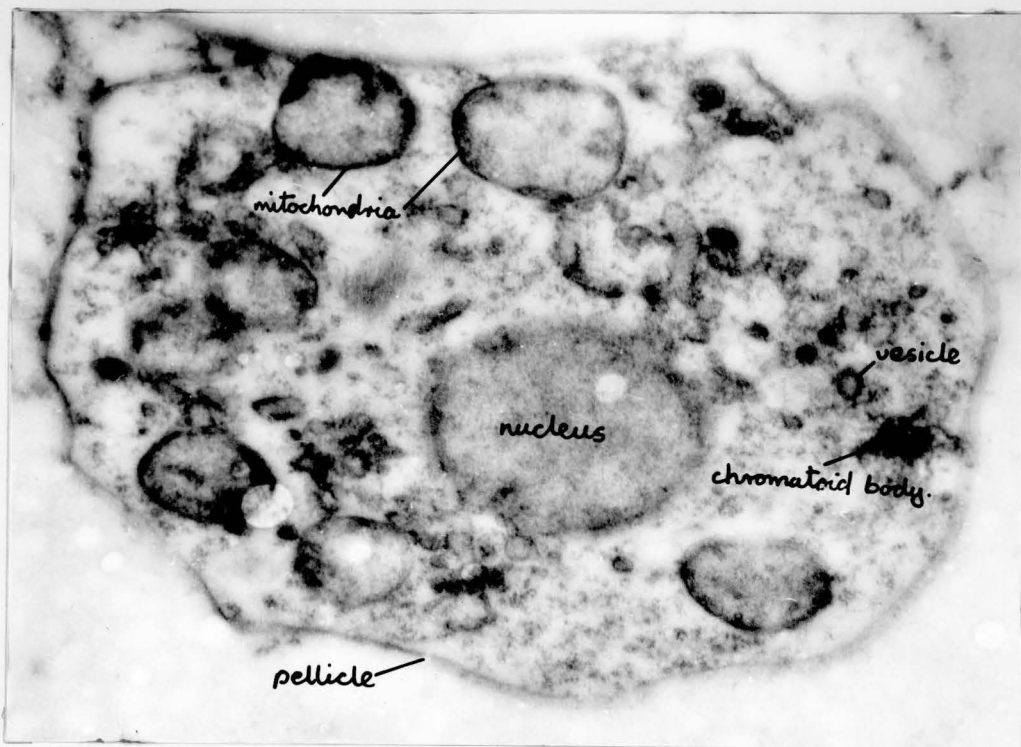


b.

X80,000

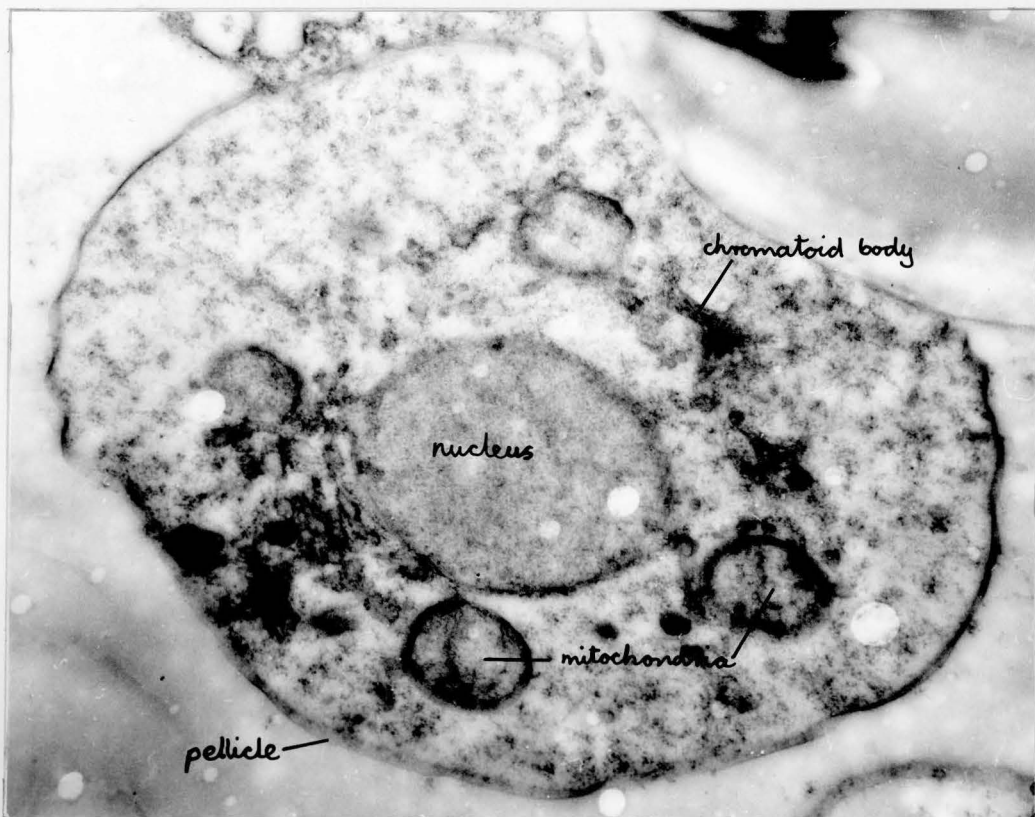
PLATE 8.

Trophozoites.



a.

X24,000

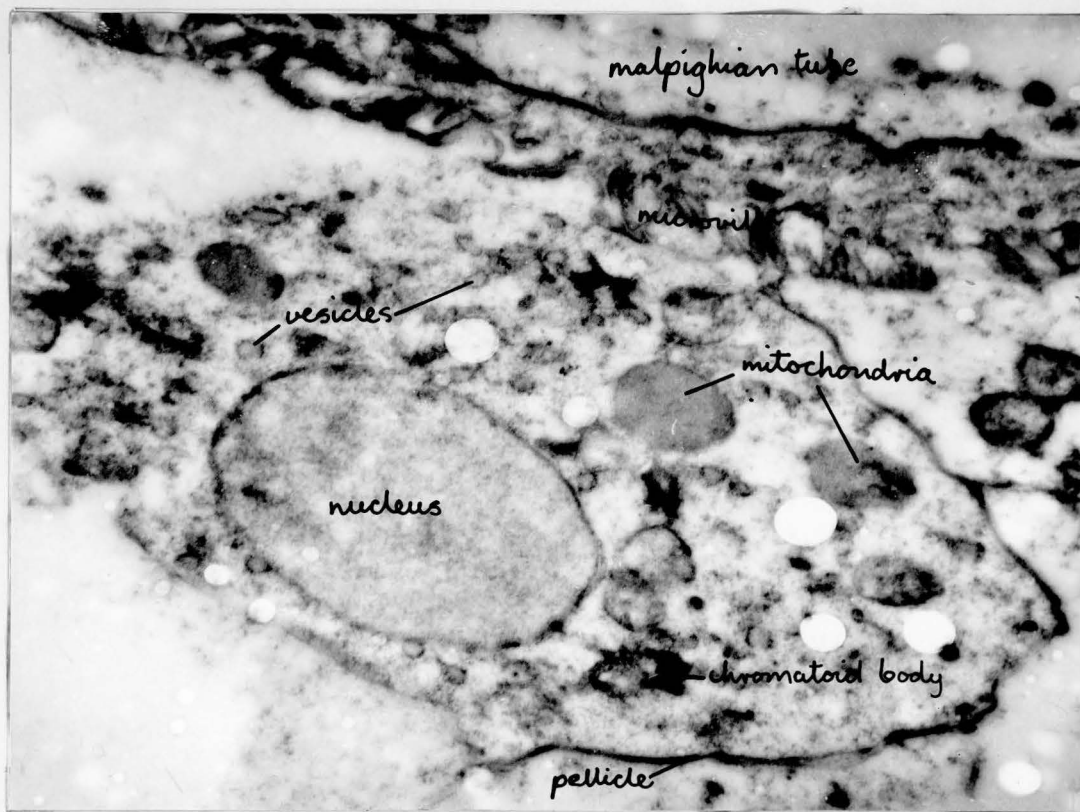


b.

X24,000

PLATE 9.

Trophozoite.

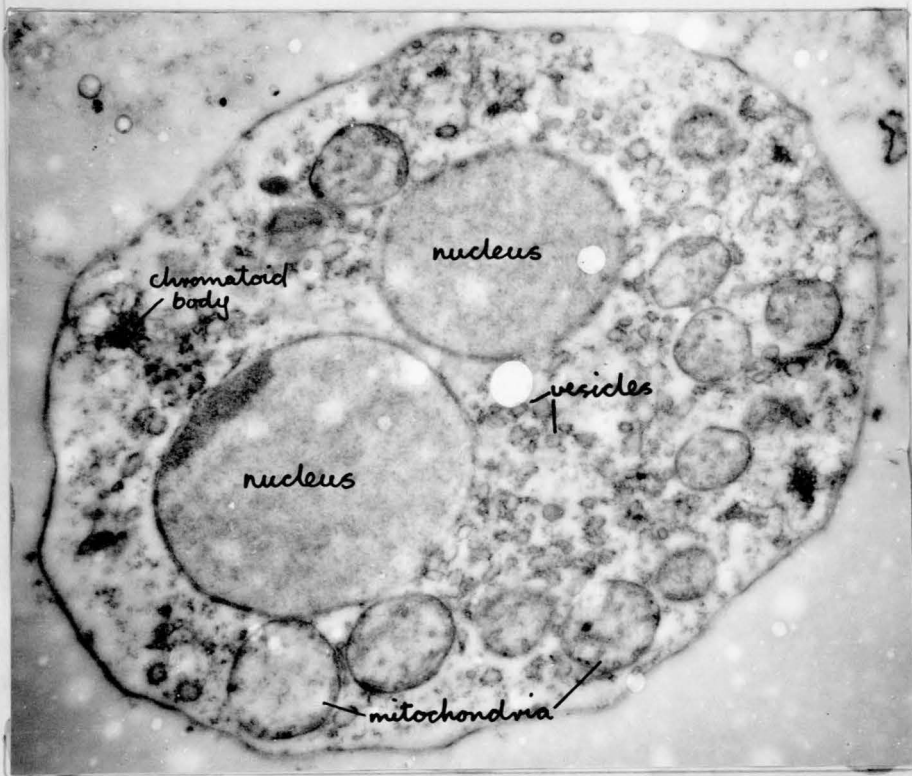


X20,000

Trophozoites.

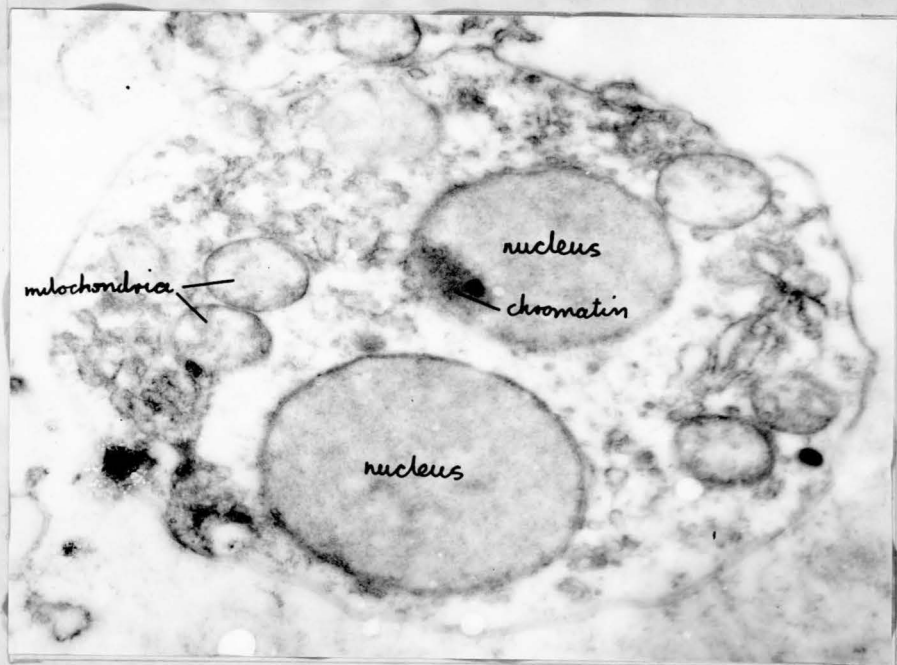
X20,000

a.



X16,000

b.



X16,000

c.

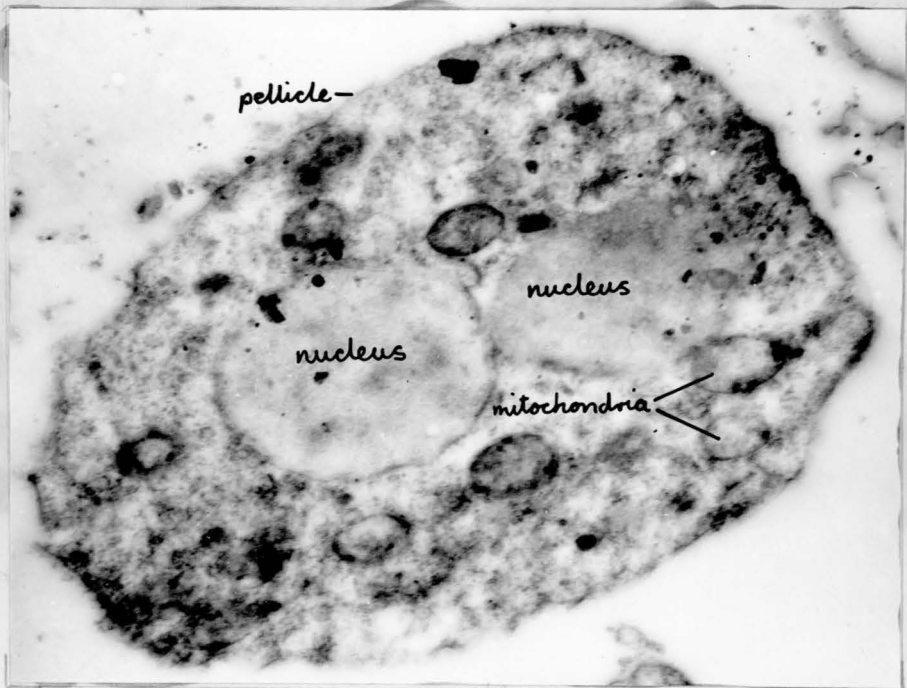
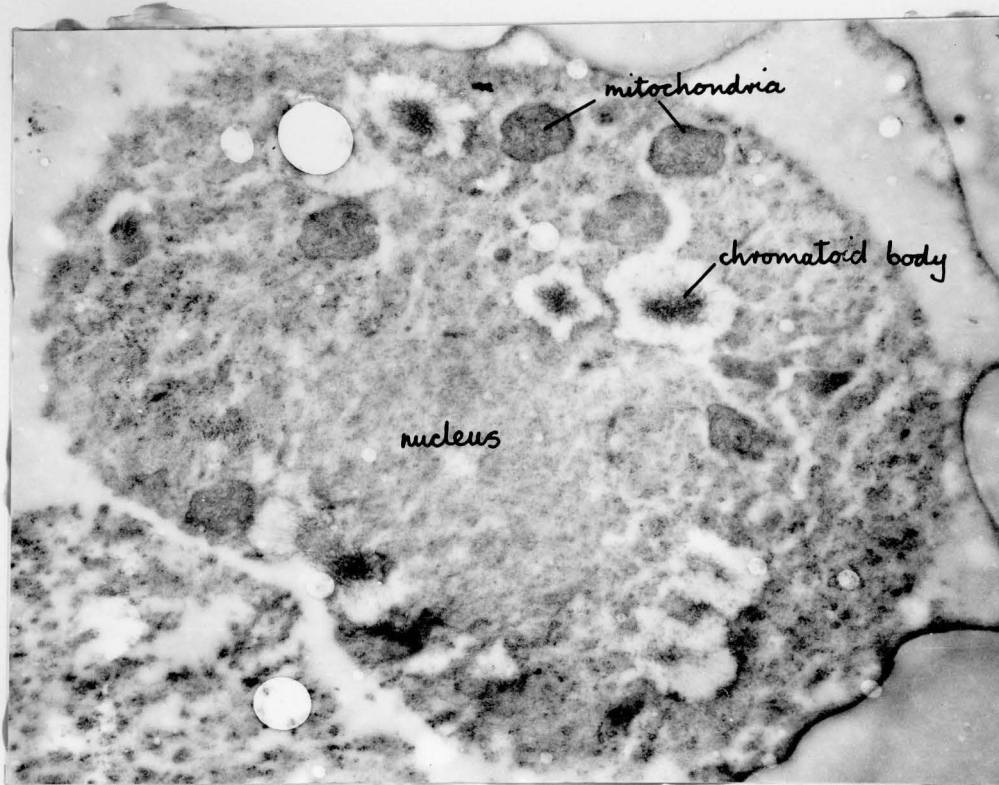


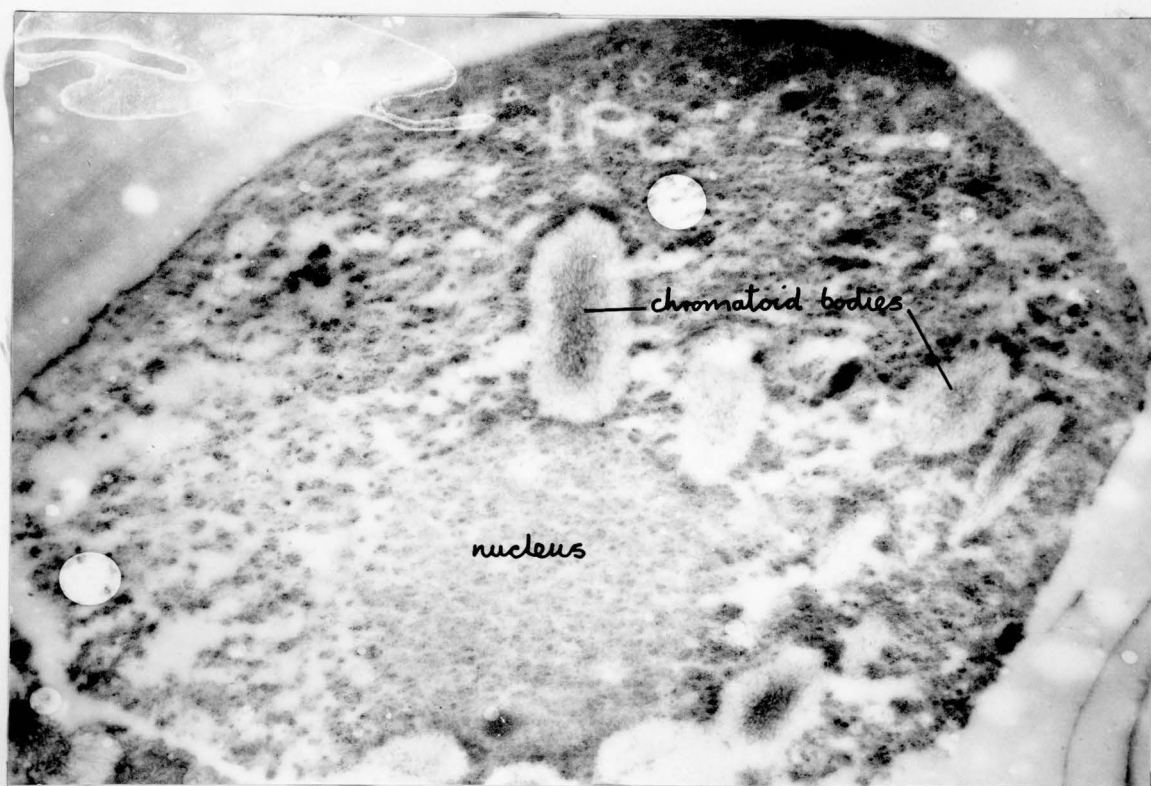
PLATE 11.

Trophozoites beginning to encyst.



a.

X20,000

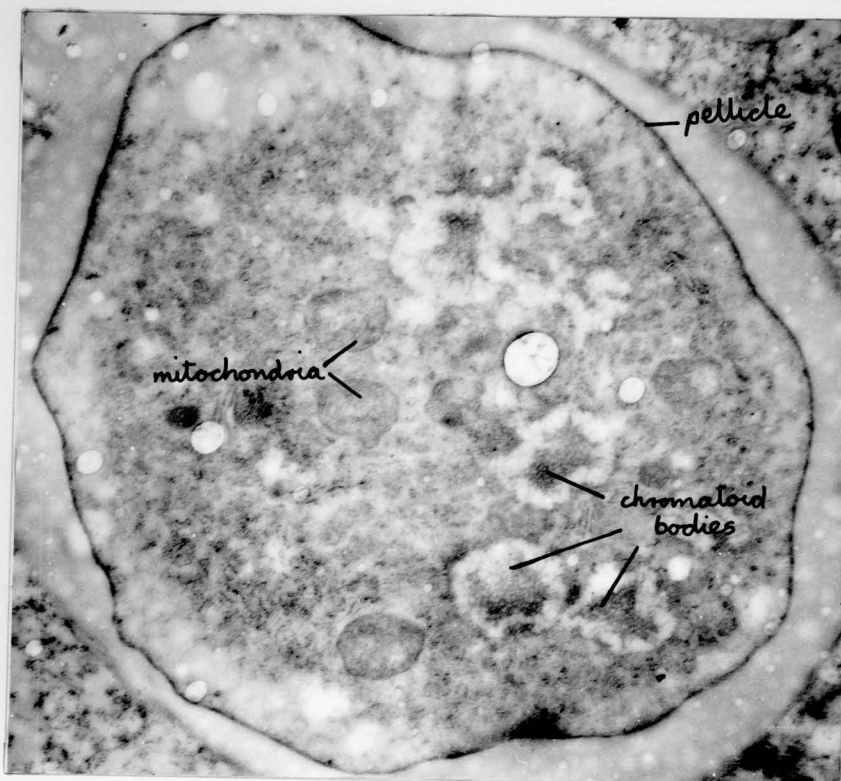


b.

X20,000

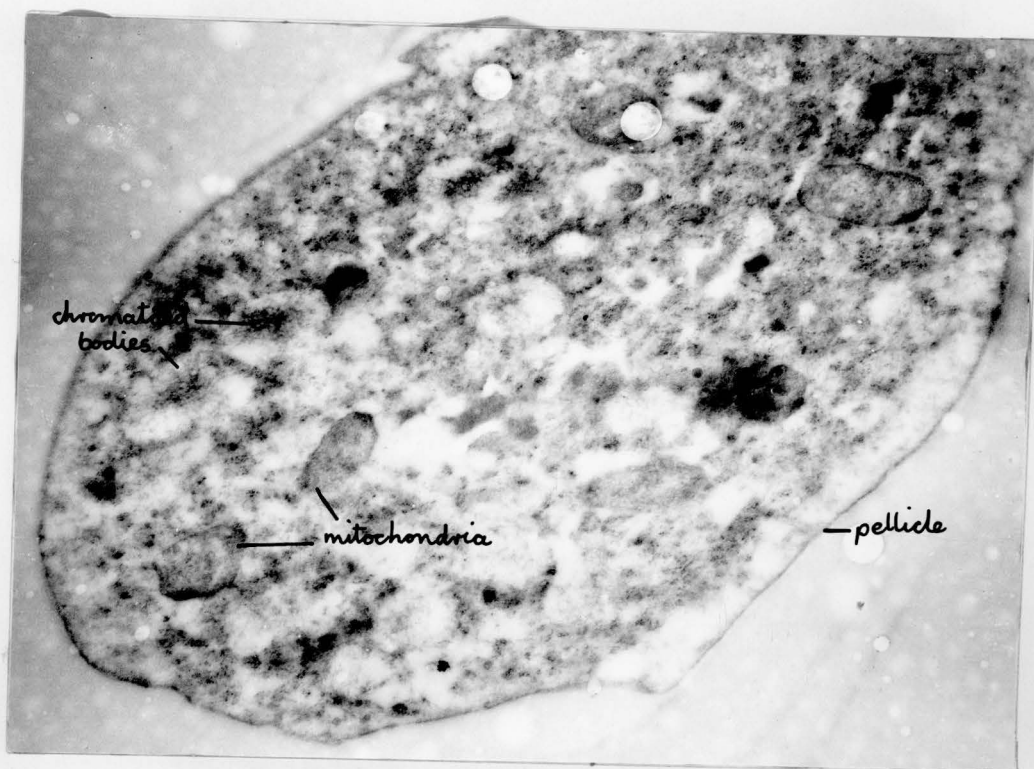
PLATE 12.

Trophozoites beginning to encyst.



a.

X20,000

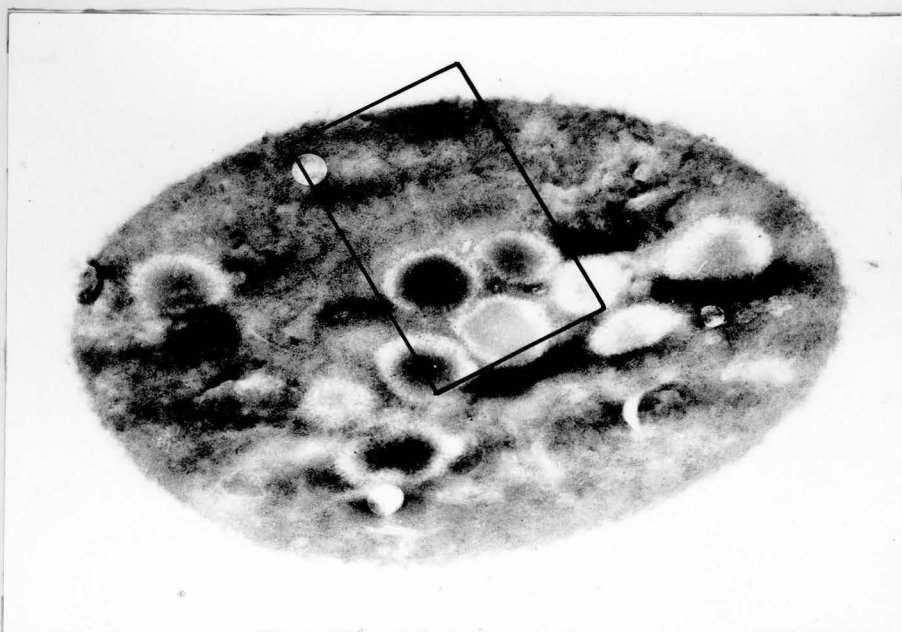


b.

X20,000

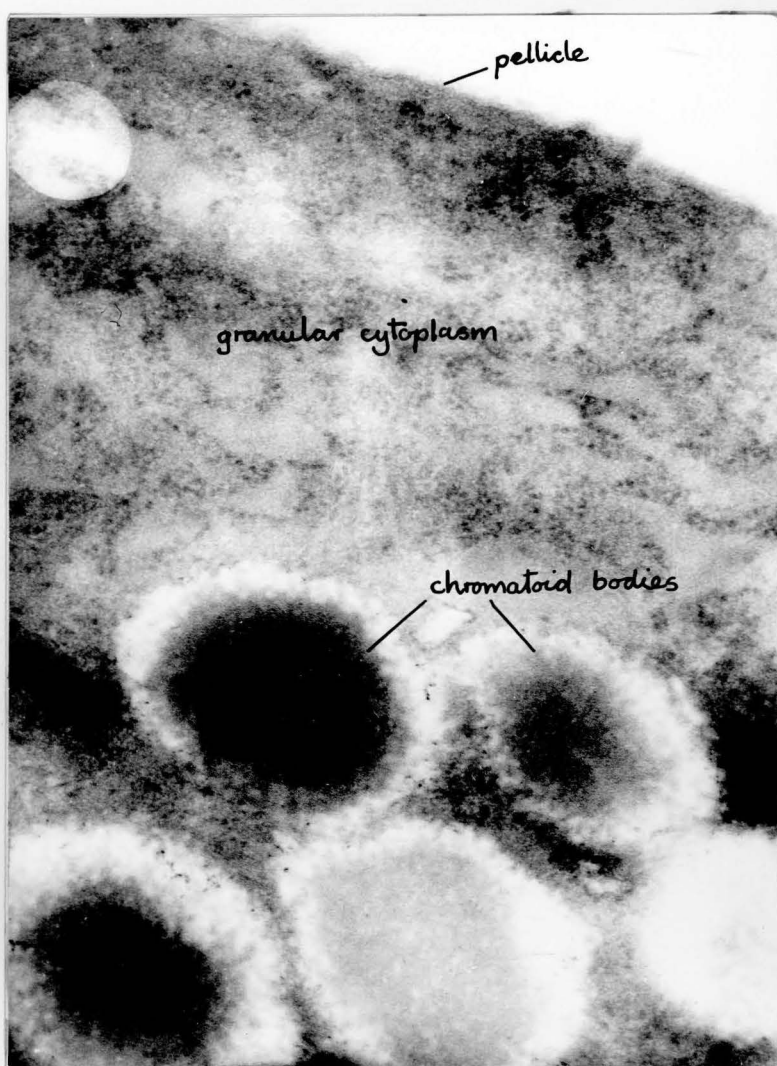
PLATE 13.

Newly formed cyst.



a.

X24,000

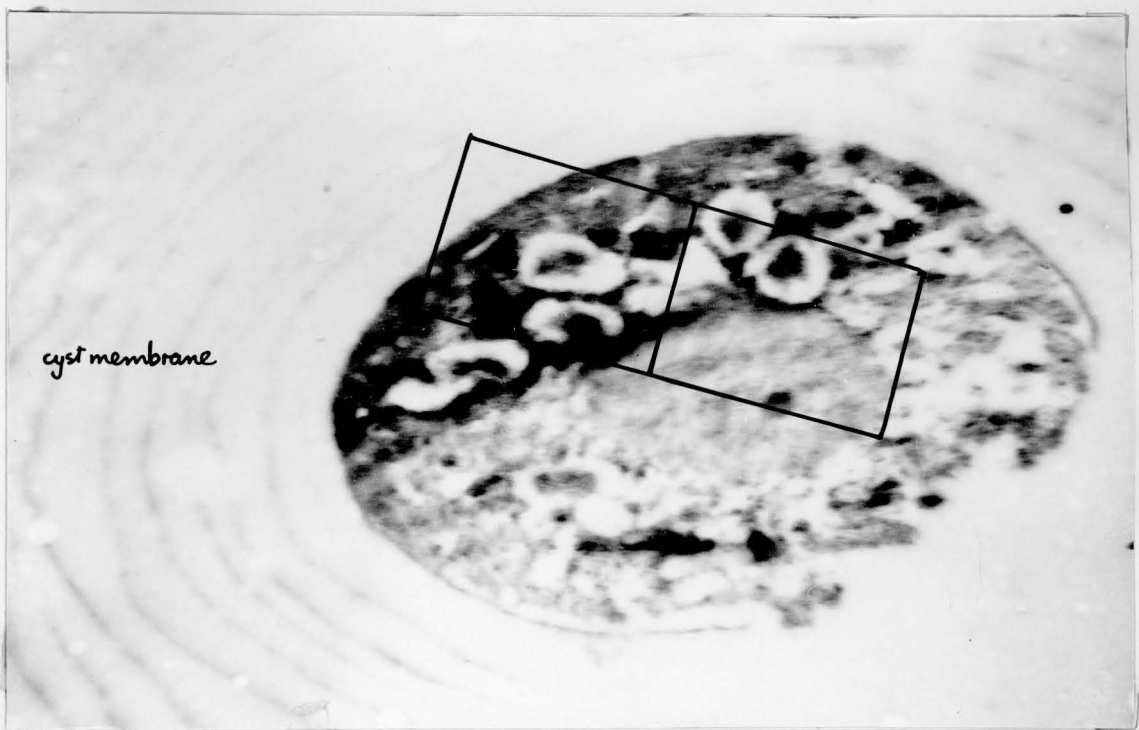


b.

X80,000

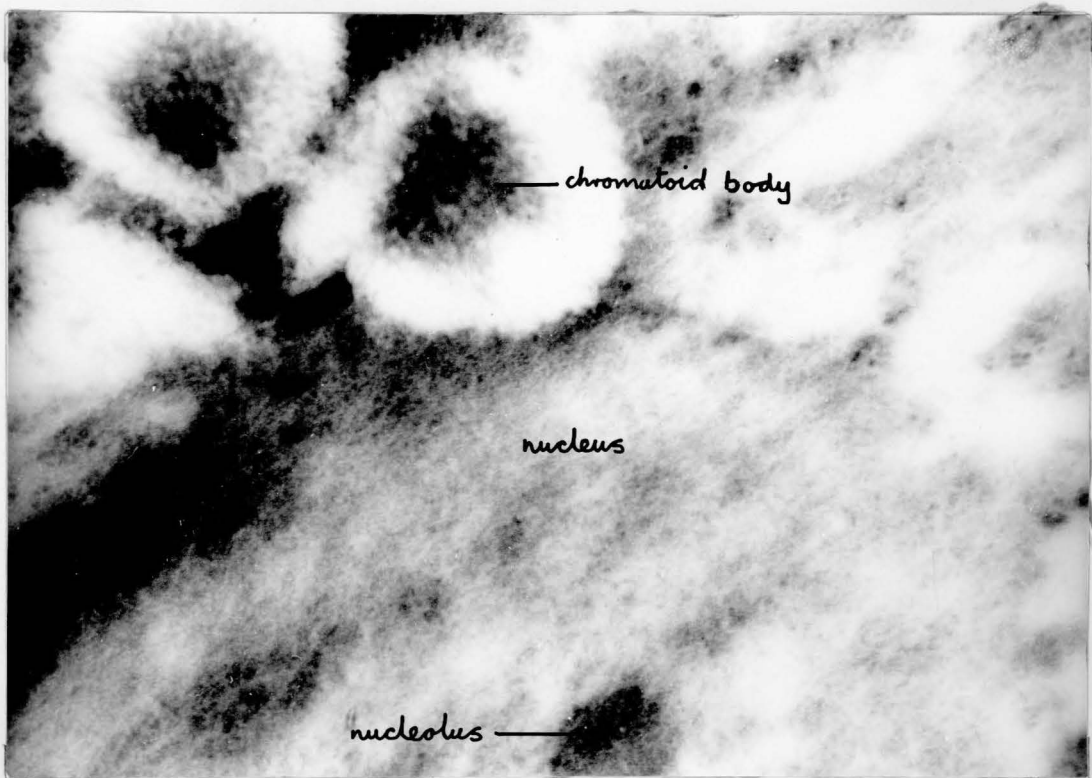
PLATE 14.

Newly formed cyst.



a.

X24,000

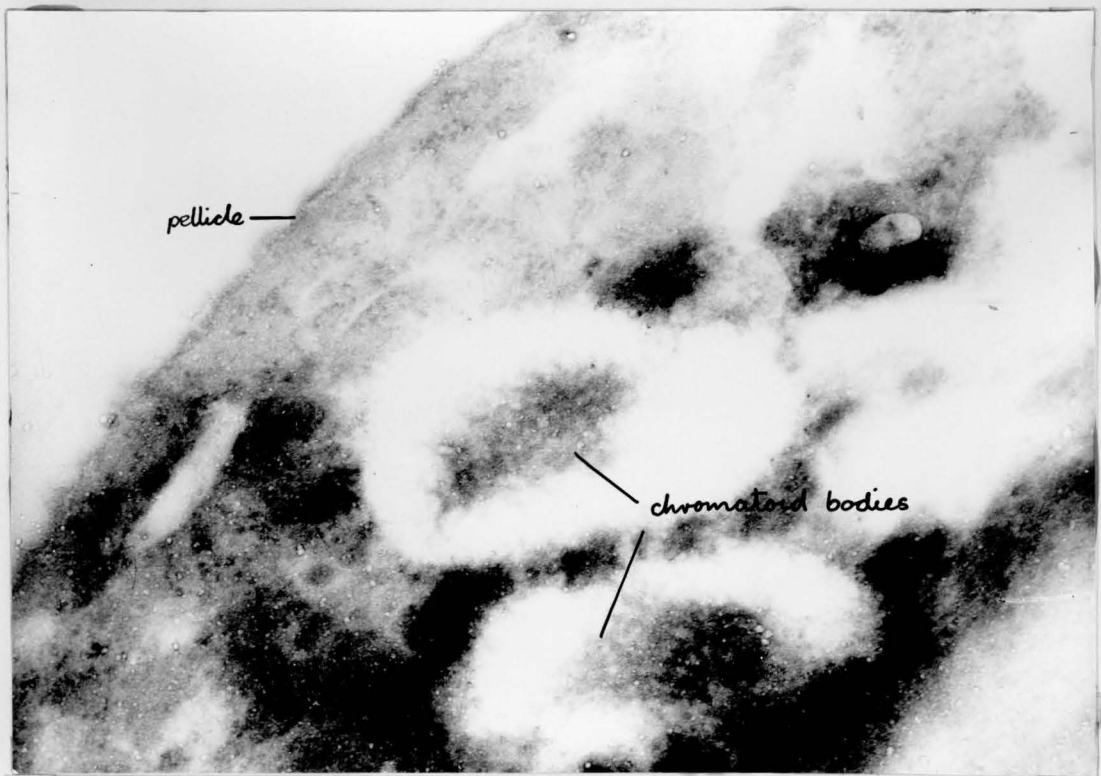


b.

X80,000

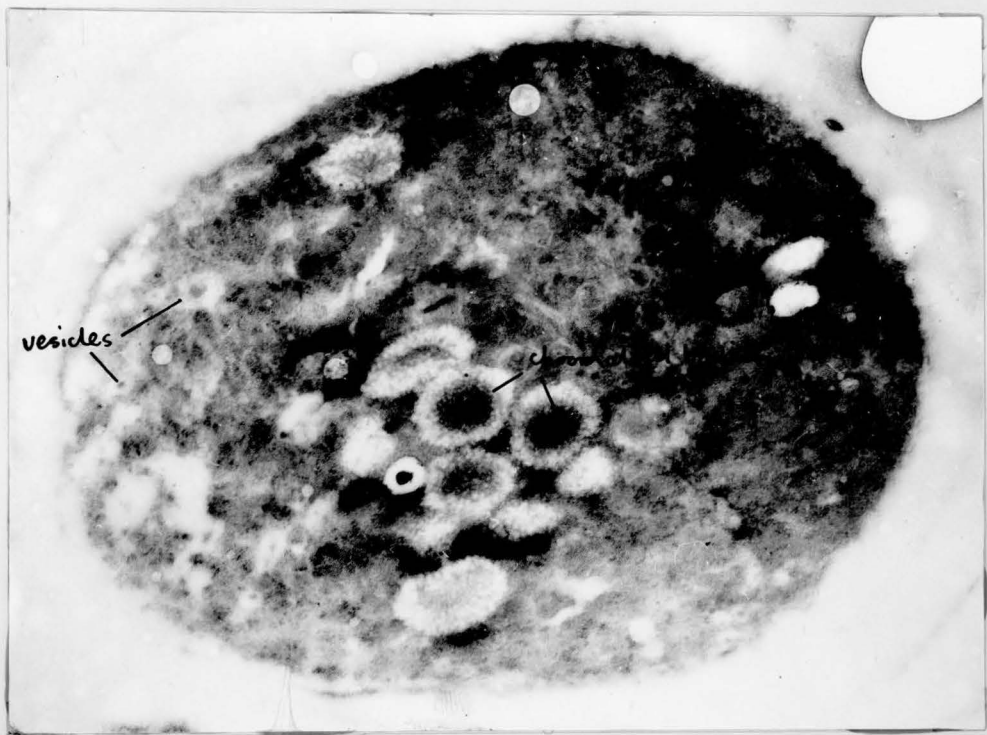
PLATE 15.

Newly formed cyst.



a.

X80,000



b.

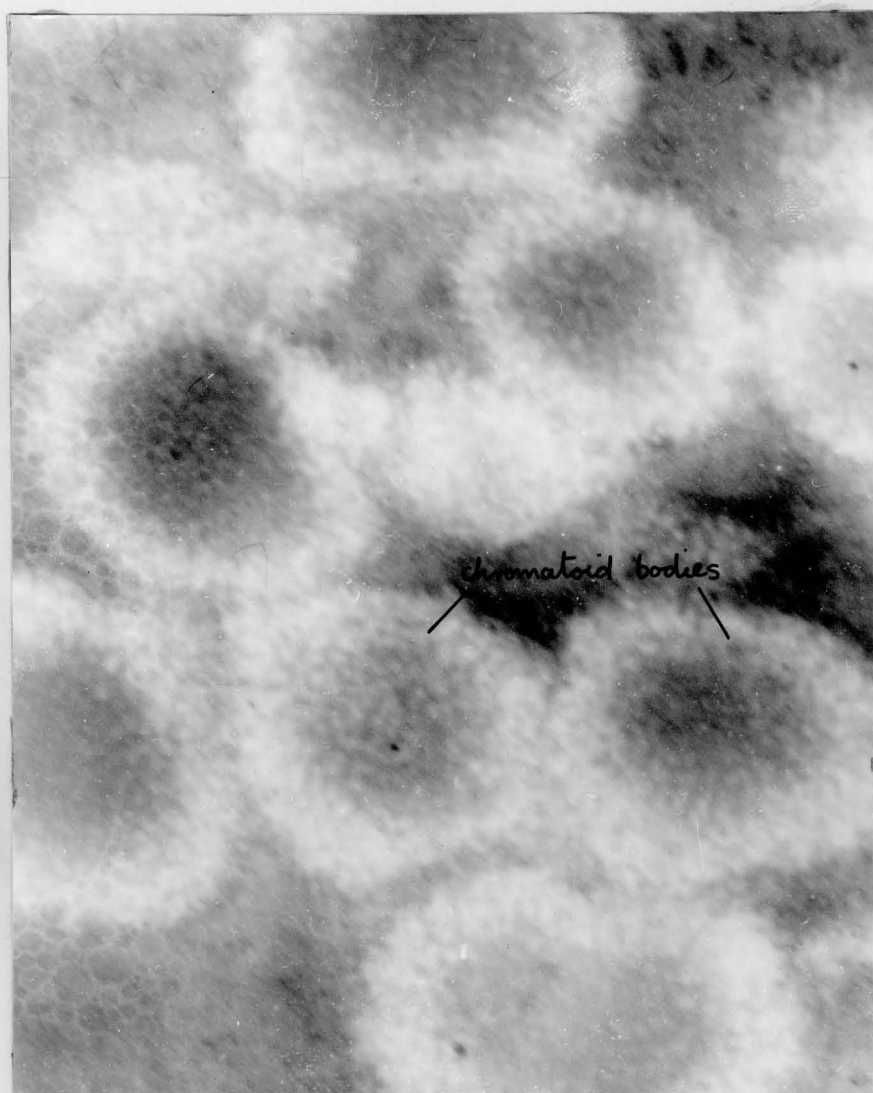
X24,000

PLATE 16.

Newly formed cyst.



X24,000



X80,000

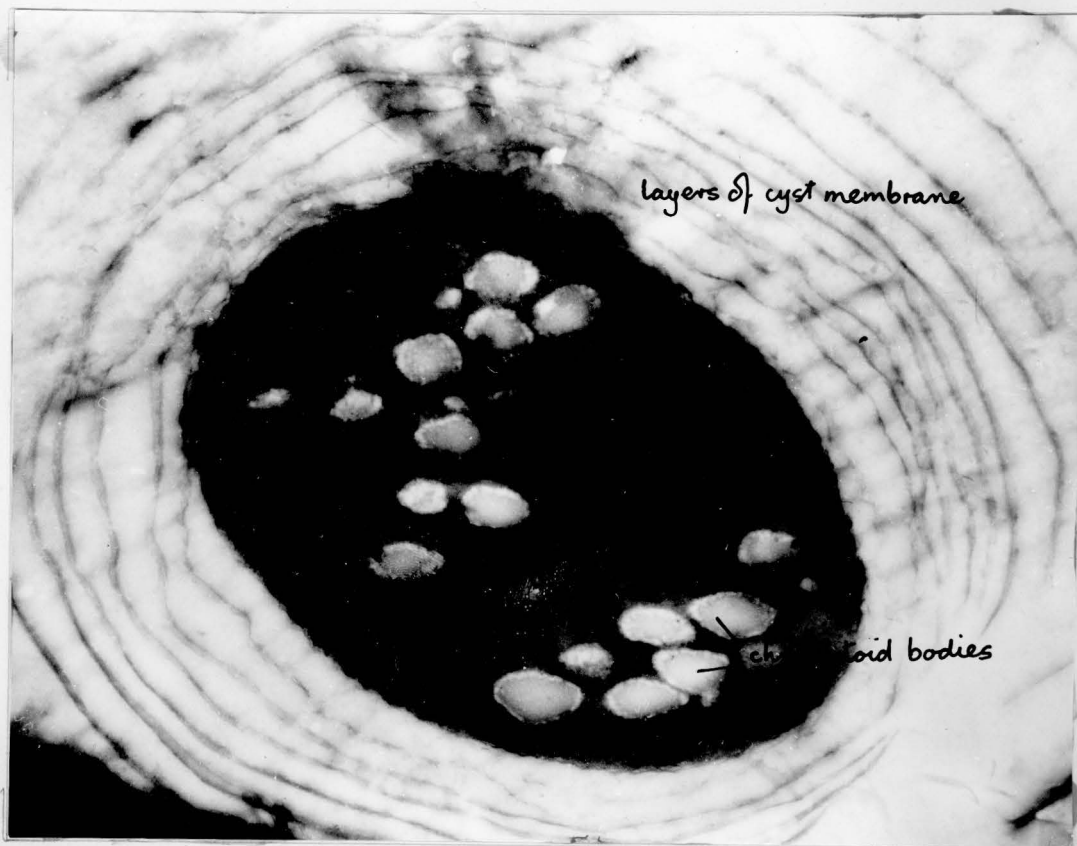
PLATE 17.

Newly formed cysts.



a.

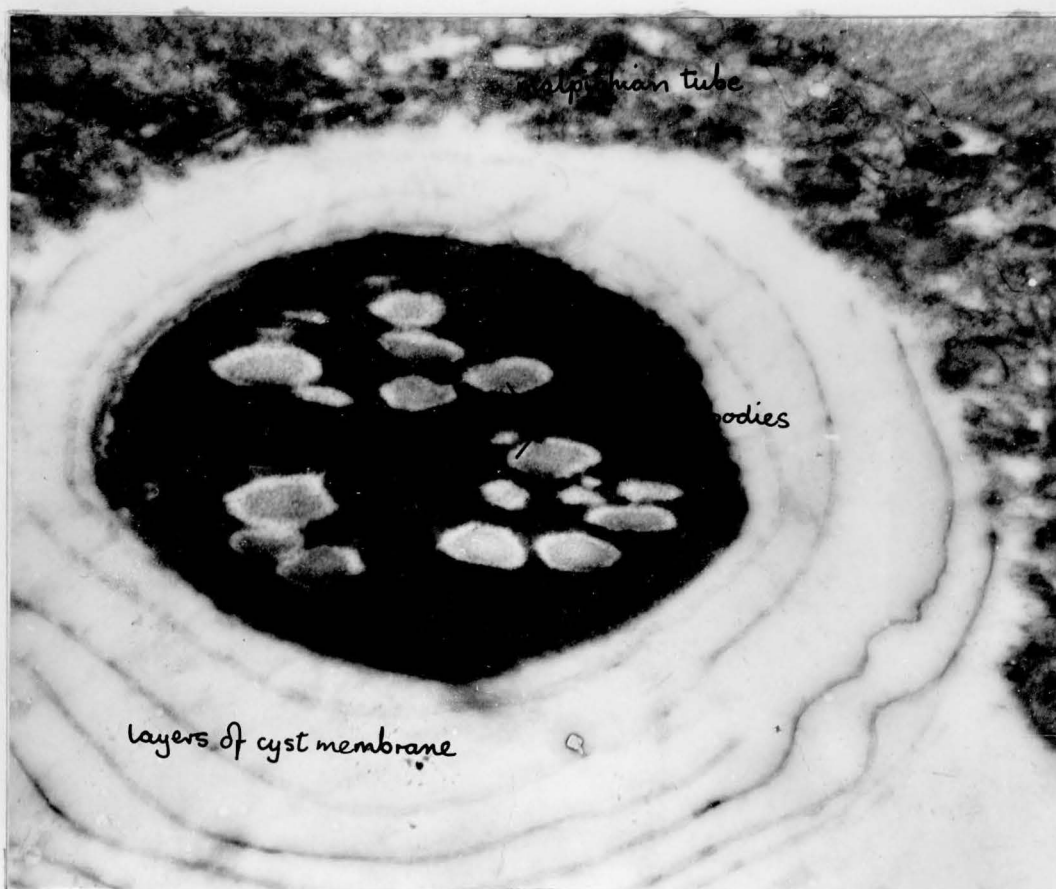
X20,000



b.

X32,000

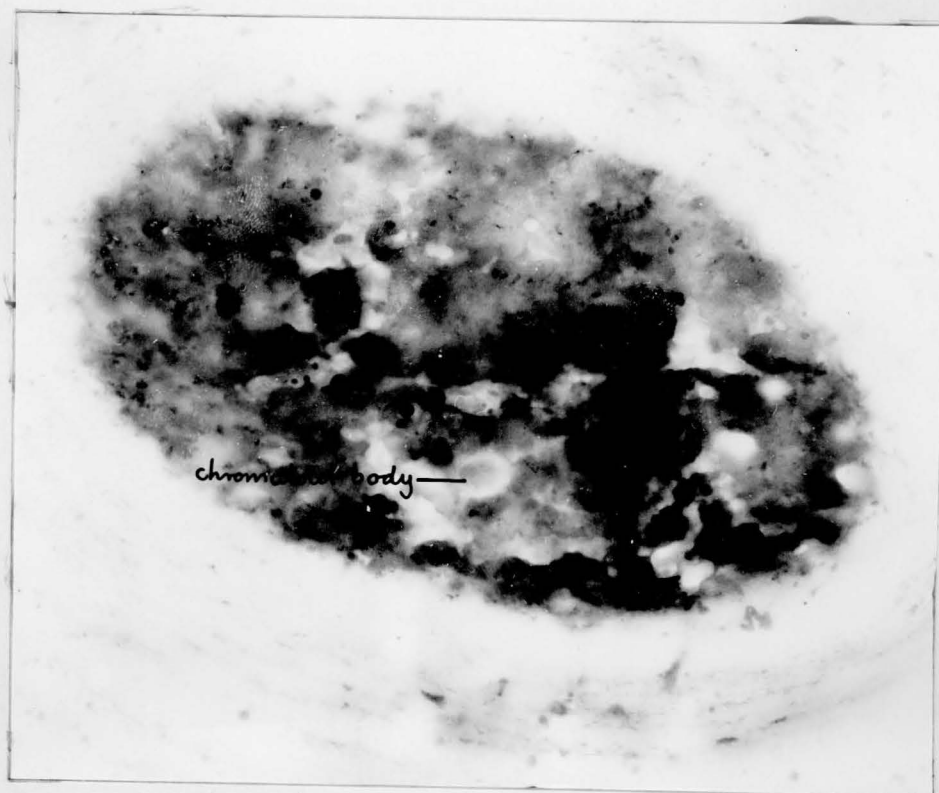
PLATE 18.



a.

X16,000

Newly formed cyst.



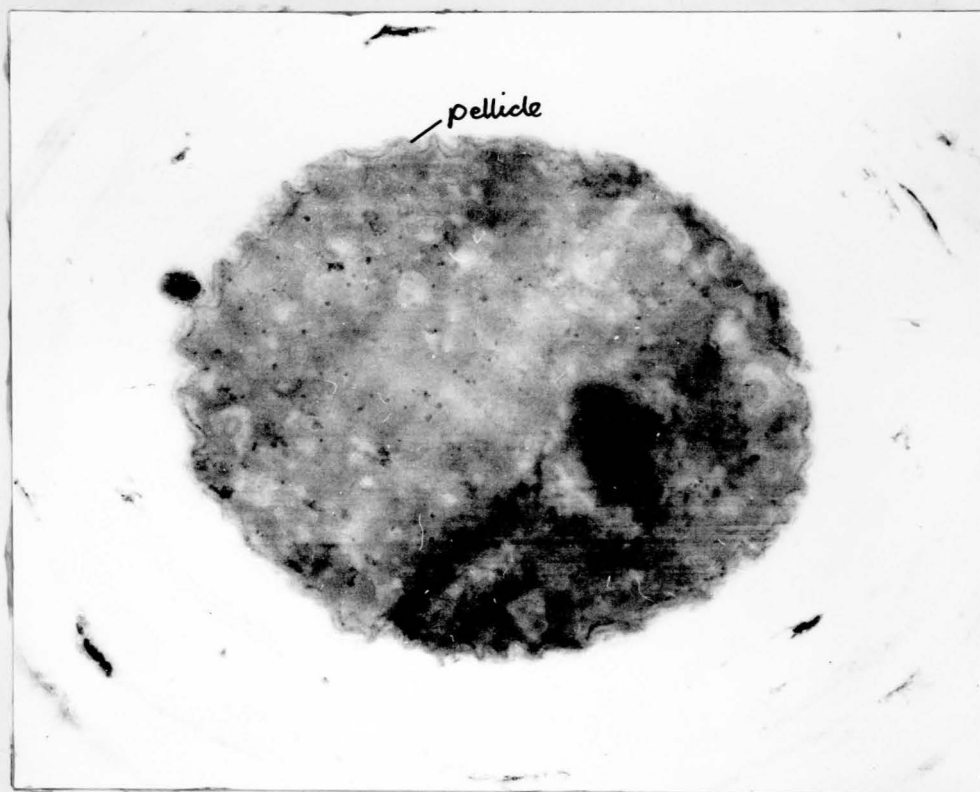
b.

X24,000

Old cyst.

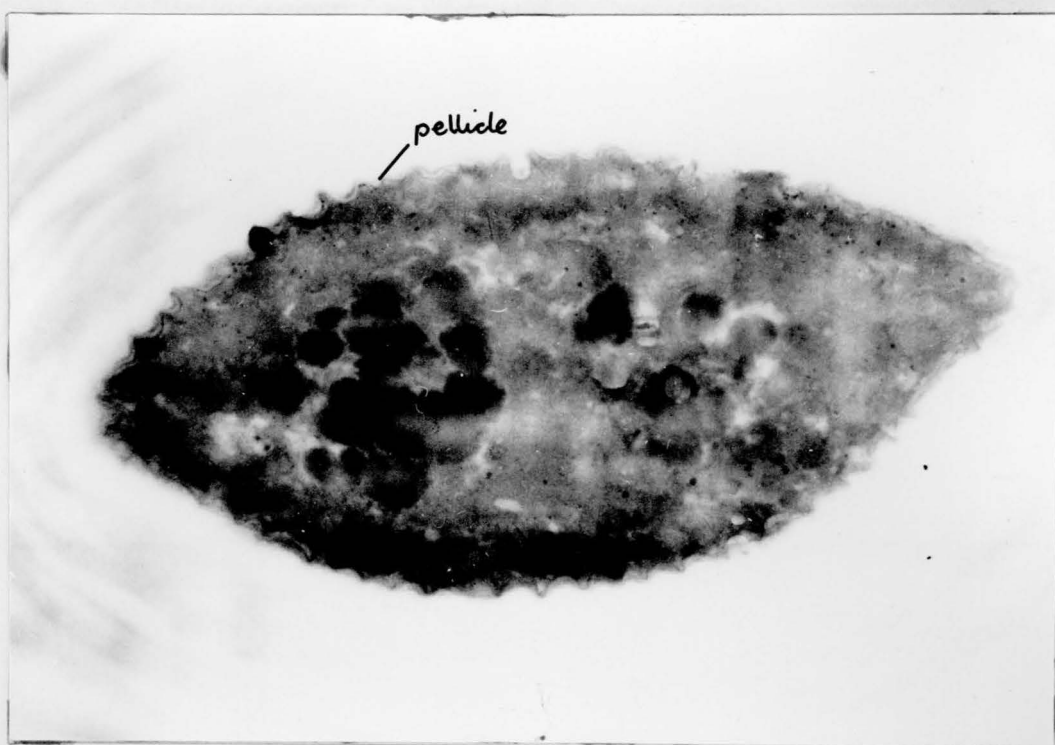
PLATE 19.

Old cysts.



a.

X24,000

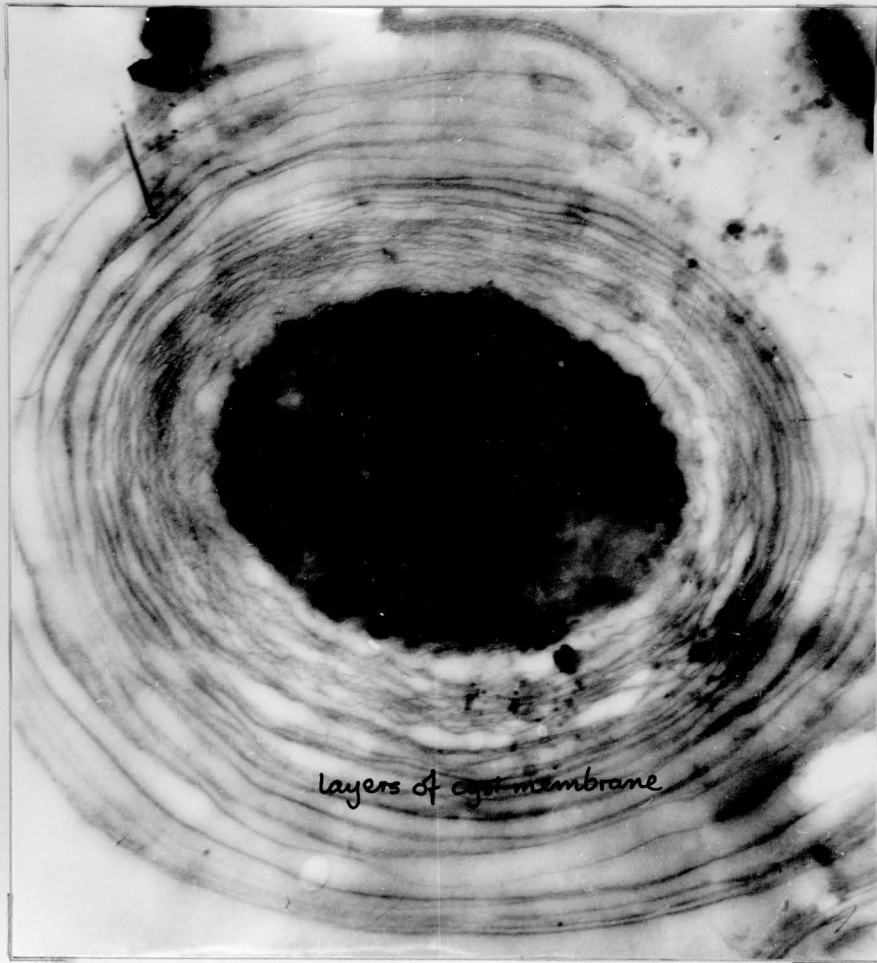


b.

X20,000

PLATE 20.

Old cysts.



a.

X16,000



b.

X16,000

PLATE 21.

Cysts before and after extraction from the faeces.

