The intestinal flagellate *Chilomastix mesnili* is not frequently encountered on routine examination of the faeces of patients admitted into the hospitals in this country. Its incidence is said to vary from 3.5 to 11.7 per cent of the individuals examined and during one year about 10 per cent of the faeces examined showed infection with *Chilomastix mesnili* (Knowles, 1928). This parasite is non-pathogenic and lives as a commensal in the large intestine, probably in the caecum.

The morphology of the parasite in its trophic and cystic forms as revealed by iron-haematoxylin stain is given below. (Plate VIII, 1 & 2)

**Trophic form.**

*C. mesnili* is the largest intestinal flagellate occurring in man. Its length varies from 10 to 15 microns and it is somewhat pear shaped but is distinctly asymmetrical, the posterior part ending in a thin pointed tail. The body appears to be slightly twisted.

The nucleus is oval and vesicular in type and situated at the anterior extremity of the body. There are a number of chromatin granules on the nuclear membrane.
and a karyosome which is usually excentric is present.

There is a group of basal granules (number said to vary from 4—6) situated close to the nucleus. Three anterior flagella arise from individual granules and pass forward and from a fourth granule the flagellum which lies in the cytostome originates. Two fibres which strengthens the margins of the cytostome originate from two other basal granules.

The cytostome is a large and slightly spiral cleft with lateral margins supported by two fibrils. The right fibril arises from a basal granule, passes down the right border of the cytostome, loops up to the left at the posterior end of the cytostome where the cell mouth is situated. The fibril along the left border is less prominent than that along the right. The cytostome extends to 1/3 to 1/3 the body from the anterior end.

The cytoplasm is denser in the posterior half and is filled with ingested bacteria and food particles. A spiral groove is often seen running from the right of the mouth obliquely across the body surface on the ventral aspect.
The cystic form.

The cysts are typically lemon shaped but more spherical forms may also be seen. These measure from 7.5—8.5 microns. The contents are slightly shrunken from the cyst wall at the anterior pole. The nucleus is generally nearer to the posterior pole or may be situated about the centre of the cyst. The chromatin is condensed to single mass applied to the inner aspect of the nuclear membrane giving a signet ring appearance. Shadow outline of the cytostome is seen as well as the looped right marginal fibre of the cytostome are seen. The cyst shows volutin granules in fresh specimens.

CYTOCHEMICAL OBSERVATIONS

The available literature does not contain any reference to cytochemical study of this parasite.

Mitochondria. (Plate VIII, 3)

Supravital staining with Janus green B showed that mitochondria were present in the flagellate. These were stained within 2 minutes and by about 10 minutes several had changed to pink colour. By about ½ hour most of the mitochondria were lavender or pink in colour and others had already turned colourless. The number of mitochondria was not very large and these were situated in the cytoplasm in areas excluding those corresponding to the nucleus, the cytostome and the thin tapering tail.
The Golgi complex were visualised as neutral red vacuoles by supravital staining. These were seen to be spheroidal in nature under the phase contrast microscope. (Plate VIII, 4)

The Feulgen reaction.

The nuclear membrane showed a few granules or small rods of Feulgen positive material and the karyosome appeared as a dot of DNA containing material. The distribution of the DNA containing material in the nucleus showed considerable variation and the shape was also variable. The blepharoplasts or basal granules were Feulgen-negative. (Plate VIII, 5 - 8)

Brachet's pyronin-methyl green stain.

The cytoplasm was stained with pyronin and the nucleus including the nuclear membrane and nucleoplasm also showed pyroninophilic material. The minute grains of methyl green stained material could be seen in the nuclear membrane and the karyosome was also stained with methyl green. (Plate VIII, 9 & 10)

Danielli's coupled tetra-azotised benzidine reaction.

Simple protein could be demonstrated in the protoplasm including the flagella of the parasite by this method. The nuclear membrane and the karyosome showed strong reaction, i.e., deeper colour; the
nucleoplasm relatively paler colour. The cytoplasm was stained orange red but the tail end was relatively pale, the right border of the cytostome appeared darker in colour. (Plate VIII, 11)

**Periodic acid-Schiff reaction.**

With the PAS reaction, the cytoplasm was usually faintly stained red indicating the presence of only slight polysaccharide content. In areas however stronger reaction was noted in a fair proportion of flagellates, e.g., near the anterior end close to the site of the basal granules. Ingested bacteria if present gave a positive reaction. (Plate VIII, 12)

**Toluidine blue stain.**

No metachromasia was found with this stain. The cytoplasm appeared blue in colour and the nucleoplasm a pale blue. Dots of darker blue were seen on the nuclear membrane and the karyosome was similarly stained. (Plate VIII, 13)

**Hale's method for hyaluronic acid type of polysaccharides.**

The chromatin granules in the nucleus including the karyosome showed positive reaction with the Hale method. A few minute grains of HAP appeared to be present in the cytoplasm.
Alkaline phosphatase.

The morphology of the flagellate was well shown up in specimens subjected to Gomori's modified technique for localisation of alkaline phosphatase. The nuclear membrane showed well marked reaction, nucleoplasm somewhat weaker reaction, and the karyosome a strong reaction in most instances. The margin of the parasite and the flagella showed distinct reaction; the cytoplasm was stained grey and the ingested bacteria were well stained. The margins of the cytostome including the supporting fibrils were stained to show the presence of the enzyme. (Plate VIII, 14 - 16)

The cysts.

The cysts showed similar cytochemical reaction as the trophic form in the different structures. The cyst wall showed well marked reaction for the enzyme and the cytostome appeared quite prominent in preparations showing the distribution of alkaline phosphatase.

Conclusions.

The mitochondria present in the parasite showed oxidation-reduction phenomena, viz., change of janus green to diethyl saffranine and ultimately to a colourless leucobase. The Golgi complex were found to be present as neutral red vacuoles.
The protoplasm of the parasite was made up of simple protein containing tyrosine, tryptophane or histidine. The reaction for protein appeared stronger in nuclear membrane and sites corresponding to the chromatin granules. The cytoplasm contained RNA and a low concentration of polysaccharide. The enzyme alkaline phosphatase was present diffusely in the cytoplasm. Hyaluronic acid type of polysaccharide was present in a few granules in the cytoplasm.

The nucleus of C. mesnili is made up mainly of RNA-protein which was present in the nuclear membrane and the nucleoplasm. DNA-protein was present in the chromatin grains present in the nuclear membrane and in the karyosome; HAP was also present in these structures. Alkaline phosphatase was present in the karyosome and the nuclear membrane and the nucleoplasm. Occasionally the site corresponding to chromatin could be made out as showing stronger reaction.

The basal granules did not contain any DNA but alkaline phosphatase was present. At times stronger reaction for polysaccharide could be made out in the region of the basal granules. It is possible that this store of energy giving polysaccharide was utilised during flagellar movement. The margins of the cytostome
showed alkaline phosphatase which may possibly mediate movement of these margins. Alkaline phosphatase at the cell margin was apparently concerned with transfer of food material across it.
PLATE VIII

Chilomastix mesnili

Figs. 1 & 2 Iron haematoxylin stain, showing the morphology of the trophic and cystic forms of C. mesnili.

Fig. 3 Supravital staining with Janus green-B showing the mitochondria in the trophic form.

Fig. 4 The Golgi complex visualised by supravital staining with neutral red.

Figs. 5-8 The Feulgen reaction showing the distribution of DNA in the trophic form (Figs. 5-8) and the cyst (Fig. 8).

Figs. 9-10 Pyronin-methyl green stain showing the distribution of RNA and DNA in the flagellate.

Fig. 11 Danielli's coupled tetra-azonium reaction showing localisation of simple protein.

Fig. 12 Periodic acid-Schiff reaction showing the distribution of polysaccharides.

Fig. 13 Toluidine blue stain showing the nuclear pattern and ingested bacteria in the cytoplasm.

Figs. 14-16 Reaction for alkaline phosphatase in the cyst (Fig. 14) and the trophic form of the flagellate.

Magnification: Figs. 11 & 16, ca, X1500; Others, ca, X1500.