FINE STRUCTURE OF CYSTIC FORM OF TOXOPLASMA GONDII

BY

P. C. C. GARNHAM, M.D., D.Sc.

J. R. BAKER, Ph.D.

AND

R. G. BIRD, M.B., B.S.

London School of Hygiene and Tropical Medicine

[WITH SPECIAL PLATE]

The importance of toxoplasmosis as an infection of man is being increasingly realized to-day. Over 2,000 papers have been published in recent years dealing with the subject, and yet the mode of transmission of the infection remains undiscovered, with the single exception of the congenital form. Great Britain shares with much of the rest of the world a high incidence of infection, and Robertson (1960, 1961) has disclosed an excessive perinatal mortality, probably toxoplasmic in origin, in towns in or near the Humber, where in 1961 transmission seems to have been exceptionally active.

The full life-history of the organism has not been worked out, but the cystic stage undoubtedly represents an essential part of the process. The morphology of the cyst has until now been studied only under the relatively low magnification given by the light microscope; however, the fine structure of the proliferative and "pseudocystic" stages has frequently been studied by electron microscopy. We thought that the examination of cysts by the latter method might reveal details of structure which would throw light on the life-cycle itself and on the—still obscure—taxonomic status of the organism.

Cysts of *Toxoplasma gondii* are most easily found in the brain, and this organ is the favoured site of its development in man, particularly in congenital cases, where extensive damage often results—for example, in the case described from Woolwich by Morris *et al.* (1955). Cysts for electron-microscopic examination are, however, more easily obtained from mice or rats suffering from chronic infections, and our material came from such a source.

Material and Methods

Mice which had been chronically infected with *T. gondii* (strains S 90 and RB 35 originally isolated from a sheep and a rabbit respectively) for 10–11 months were killed and smears were prepared from their cerebral hemispheres by crushing a small piece of tissue between two glass slides and then drawing the latter apart with a longitudinal sliding motion. These smears were immediately placed into ice-cold 1% osmium tetroxide solution containing 4.5% (w/v) sucrose and buffered to *p*H 7.4 (Caulfield, 1957) for 15–30 minutes. They were then rapidly dehydrated with a graded series of ethanol solutions. When in 70% ethanol the smears were temporarily covered with a coverslip and examined microscopically, face downwards; suitable cysts (which could be identified easily at a magnification of ×120) were marked with a circle on the back of the slide with a diamond object-marker. The dehydration of the smears was then completed, and they were treated with a 1% solution of tungstophosphoric acid in ethanol for 30 minutes.

Embedding was in the epoxy resin "araldite," slides being first immersed overnight in a mixture of equal parts of pure ethanol and araldite at room temperature, then, next day, given two changes of undiluted araldite at 37° C. for 3–4 hours. Finally each slide was balanced on top of three or four No. 00 gelatin capsules filled to overflowing with araldite.

The capsules were arranged directly beneath the cysts selected for sectioning, a small cardboard-box lid with punched-out holes being used as a rack for supporting them. Polymerization was carried out by heating to 60° C. for two to three days in a hot-air oven. The hardened blocks with cysts embedded in their surfaces were easily separated from the glass slides when chilled in the refrigerator (Bird, in preparation).

For comparative purposes, proliferative ("pseudocystic") forms of *T. gondii* (strain RH) were prepared by squirting infected mouse peritoneal fluid into the fixative and then centrifuging to form a pellet, which was embedded in the usual way (Garnham *et al.*, 1961).

Sections were cut with a glass knife at 60–90 mμ on a Huxley pattern Cambridge Instrument Co. ultramicrotome and examined in an AEI.EM6 electron microscope, using 75 kV and a 0.025-mm. objective aperture. Micrographs were recorded on Ilford "lantern" contrasty plates.

Structure of the Cyst of T. gondii and its Contents

The true cyst of *T. gondii* grows to at least 100 μ in diameter in the brain of warm-blooded vertebrate animals, and in such forms many thousands of individual *Toxoplasma* are present, enclosed by the fine, but tough, cyst wall (Fig. 5*).

The organism within the cyst exhibits the usual features, which have been described in detail for the proliferative forms by Ludvík (1956) and others, though the general structure appears to be more "tattered" than usual. This may be because fixation is less adequate (the fixative having failed to penetrate the cyst wall and get adequately into contact with the *Toxoplasma*) or because the organisms are older in the cyst, a structure which is known to persist for months (Beverley and Fry, 1957).

The organism is enclosed in a two-layered pellicle (Figs. 8, 12) about 6 mμ in width. A well-defined micropyle 100 mμ in depth (Fig. 8) breaks the continuity of the surface membrane at some point towards the centre of the organism. Immediately inside this pellicle run the peripheral fibrils or rib cords, terminating at the polar ring (Figs. 6, 10). In transverse sections there can be seen lateral connexions between the fibrils, of which at least 22 may be counted (Fig. 11).

The anterior tip is provided with a strong conoid or truncated cone (about 20 mμ thick and 250 mμ deep), a device used for the penetration of a host cell (Figs. 6, 12). Inside the conoid are to be found the anterior parts of the paired organelle (Figs. 7, 12). Fig. 7 indicates clearly the apparent secretory nature of this structure, which extends to almost the whole length of the *Toxoplasma*, and in its posterior part becomes swollen and sac-like. Other structures possibly of a secretory nature also proceed to the polar ring (Fig. 7); these have been designated under various names—toxonemes, osmio-

*Figs. 1–15 appear on the Special Plate.

phile fibrils, lysosomes, sarconemes, convoluted tubes—we prefer the last name as being least committal; they are present throughout the cytoplasm.

The nucleus shows nothing of special interest; it possesses a surface membrane and one or more nucleoli or bodies of high electron density (Figs. 4, 13). At least one large mitochondrion was found in the cystic form (Figs. 14, 15).

The cyst itself has a peculiar serrated profile and appears to be made up of the following layers (Figs. 4, 4a, 13): an outer electron-dense layer 5–10 mμ thick and an indefinite granular inner layer 200–300 mμ thick. The cyst lies in the brain tissue without provoking any reaction around it.

Discussion

Figs. 1, 2, and 3 are taken from electronmicrographs of sections of the proliferative or pseudocystic stage of *T. gondii*, and are presented here in order to effect a comparison between the two stages of the parasite. The main features are present in both forms—the conoid, paired organelles, convoluted tubes, nucleus with well-defined nucleoli, and micropyle. In the proliferated form the peripheral fibrils are less conspicuous. We have found evidence of internal budding or endodyogeny as described by Goldman *et al.* (1958) and Ludvík (1962) in this stage of the organism.

The origin of the cyst wall has been a matter of speculation for a long time. We suggest that the parasite continues to divide by internal budding, with the persistence—and enormous hypertrophy—of the pellicle of the original organism, inside which the further multiplication proceeds. The cyst wall would thus be part of the parasite itself, not derived from a host cell and therefore not "pseudocystic." The "cogs" may be analogous to the much more developed "villi" as described by Ludvík (1960) in the cyst wall of *Sarcocystis* spp.

The presence of a micropyle in both stages of *T. gondii* is of great significance. We (Garnham *et al.*, 1961, 1962) described this structure originally in the sporozoites of malaria parasites and later in *Lankesterella*; Ludvík (1962) subsequently reported its occurrence in *Sarcocystis* and the "M-organism." A micropyle has never been found in the flagellates, amoebae, or ciliates, and we think it may be justifiable to surmise that if a protozoon possesses such an organelle then it belongs to the Sporozoa. In view of the uncertain systematic status of *Toxoplasma*, this conclusion is of interest, because it suggests the return of *Toxoplasma* into the class where it had reposed for decades.

It was disappointing to discover no major difference between the two stages: no evidence of sexuality was found, but the life-cycle of *T. gondii* is still incompletely known, and it may yet be shown to undergo such a phase.

Summary

1. The cystic and pseudocystic (proliferative) stages of *Toxoplasma gondii* were compared under the electron microscope.

2. Both stages show the conoid, paired organelle, convoluted tubes (toxonemes), double-layered pellicle, nucleus with nucleoli, and mitochondria.

3. A single micropyle is present in both stages. Its presence suggests that *Toxoplasma* belongs after all to the class Sporozoa.

4. Peripheral fibrils are more conspicuous in the cystic stage.

5. The cyst wall is composed of a thick layer with jagged "cogs" bounded by a thin outer layer.

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CARDIAC INFARCTION AND THE GLUCOSE-TOLERANCE TEST*

BY

EDGAR SOWTON, M.A., M.B., M.R.C.P.
Medical Registrar, King's College Hospital, London

The association of cardiac infarction with diabetes mellitus is well known, as is the increased insulin requirement of patients with diabetes after infarction, but the presence of latent diabetes in patients presenting with cardiac infarction is often not recognized, perhaps because it is rarely looked for.

Glycosuria during or immediately after the acute state of cardiac infarction may occur and occasionally insulin is needed for control (Cruickshank, 1931). The belief that this glycosuria is of temporary significance only was challenged by Goldberger *et al.* (1945) when they investigated 14 patients and found six with definite diabetes and four with abnormal glucose-tolerance tests. The abnormal curves sometimes took several months to develop, and most curves tended to become more diabetic as time went on, although a few returned towards normality.

In the present series the incidence of abnormal glucose-tolerance in 40 patients presenting with cardiac infarction has been investigated, together with the changes in the glucose-tolerance curves of those followed for periods of up to five years.

Method

Glucose-tolerance tests were carried out on all patients admitted under one consultant to a general hospital during one year with a diagnosis of cardiac infarction. Care was taken that these were not done, so far as could be determined, after a period of low carbohydrate intake, and it was found that the procedure did not upset ill patients. All patients were receiving the usual treatment for cardiac infarction, including anticoagulants, and in most cases the glucose-tolerance test was carried out on the morning after admission.

The diagnosis of cardiac infarction was made on history and clinical state, E.C.G. changes, and serum glutamic oxaloacetic transaminase levels, at least two of the criteria being positive. The criteria adopted were

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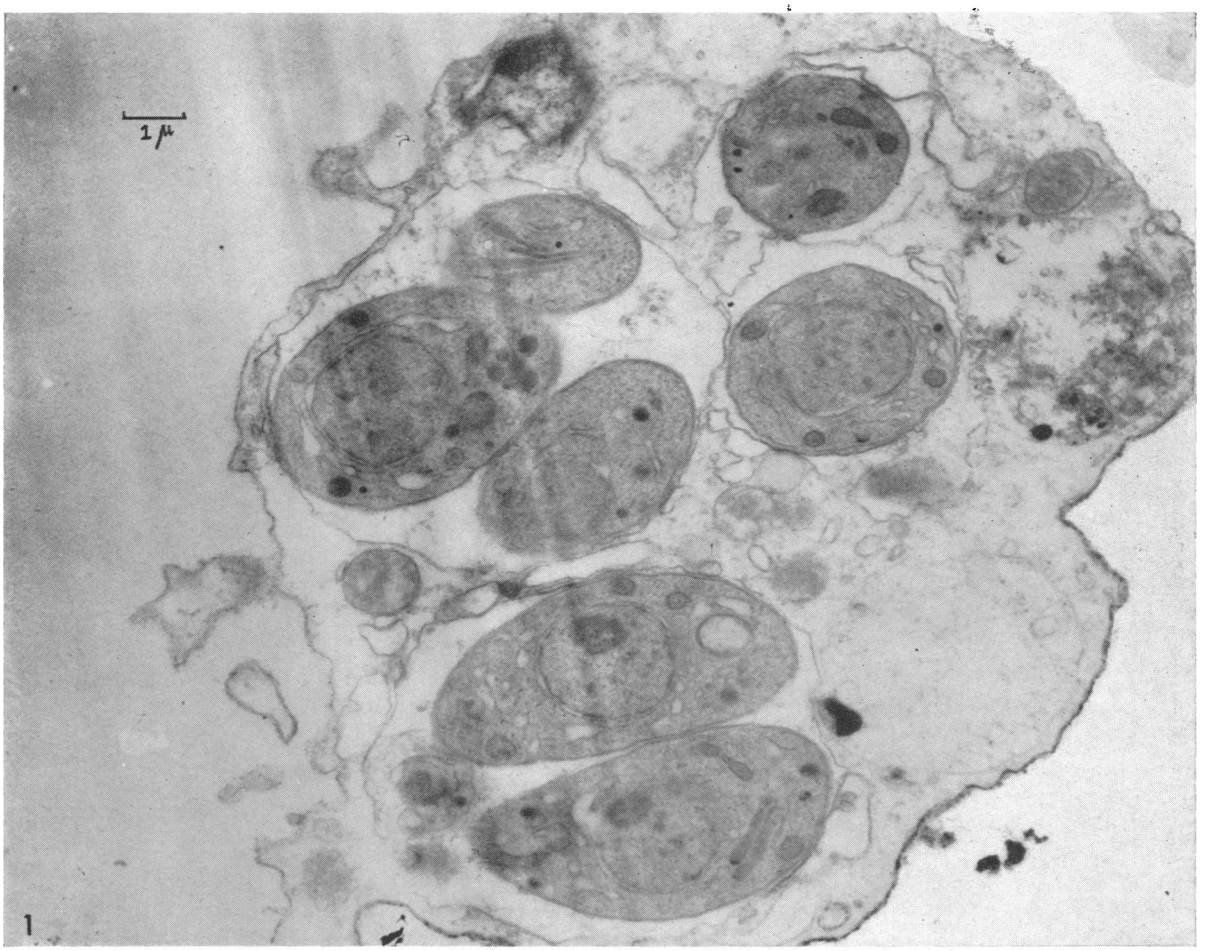
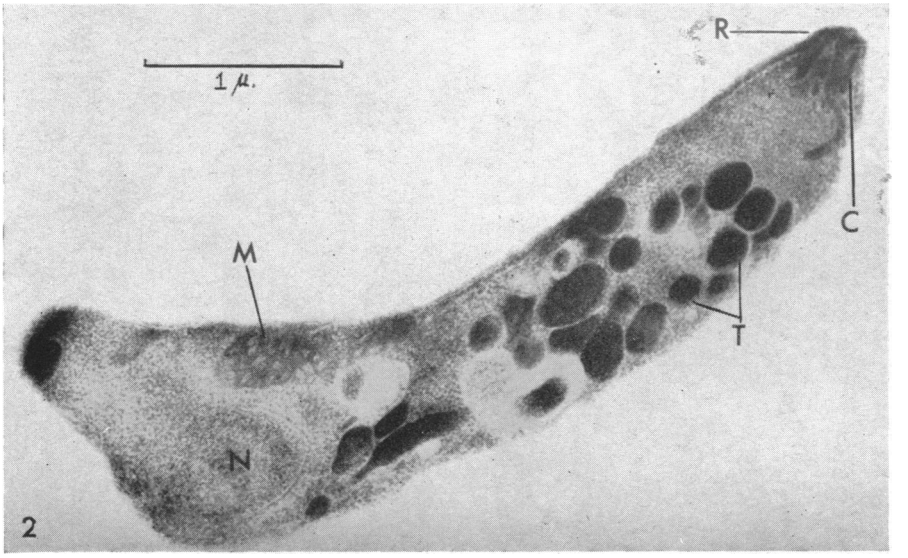
All figures except Fig. 5 are electronmicrographs of thin sections of *Toxoplasma gondii*; Figs. 1-3, proliferative (pseudocystic) stage from mouse peritoneal fluid (strain RH); and Figs. 4-15, cystic stage from mouse brain (strains S 90 and RB 35).





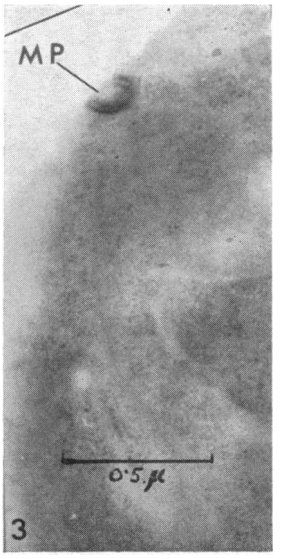
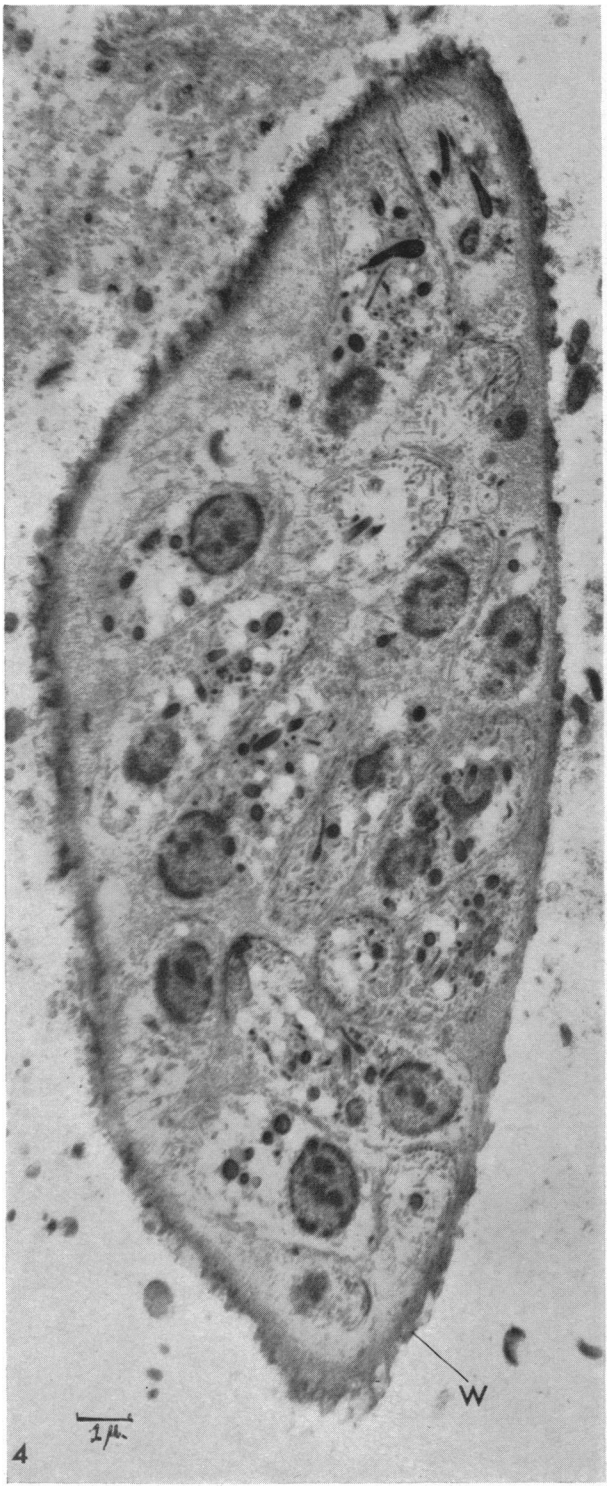


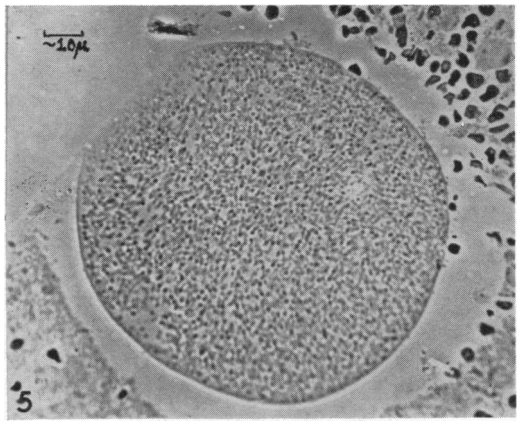
FIG. 1.—Low-power view of group of organisms within a cell (pseudocyst).

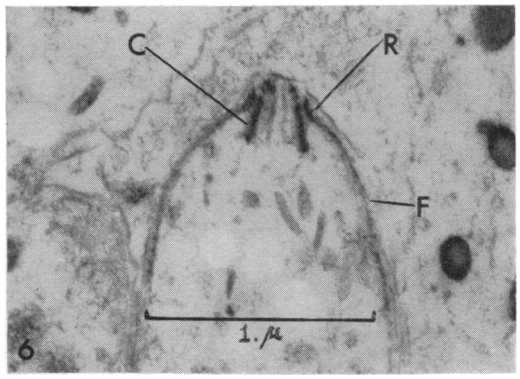
FIG. 2.—Longitudinal section of a proliferative form (prepared from material kindly supplied by Dr. J. D. Fulton).

FIG. 3.—Oblique section of micropyle of proliferative form.

C=conoid; M=mitochondria; MP=micropyle; N=nucleus; R=polar ring; T=convoluted tubes.







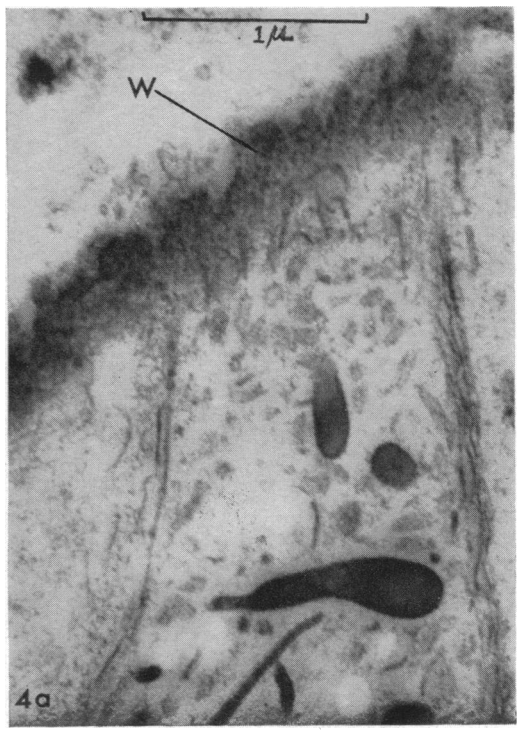
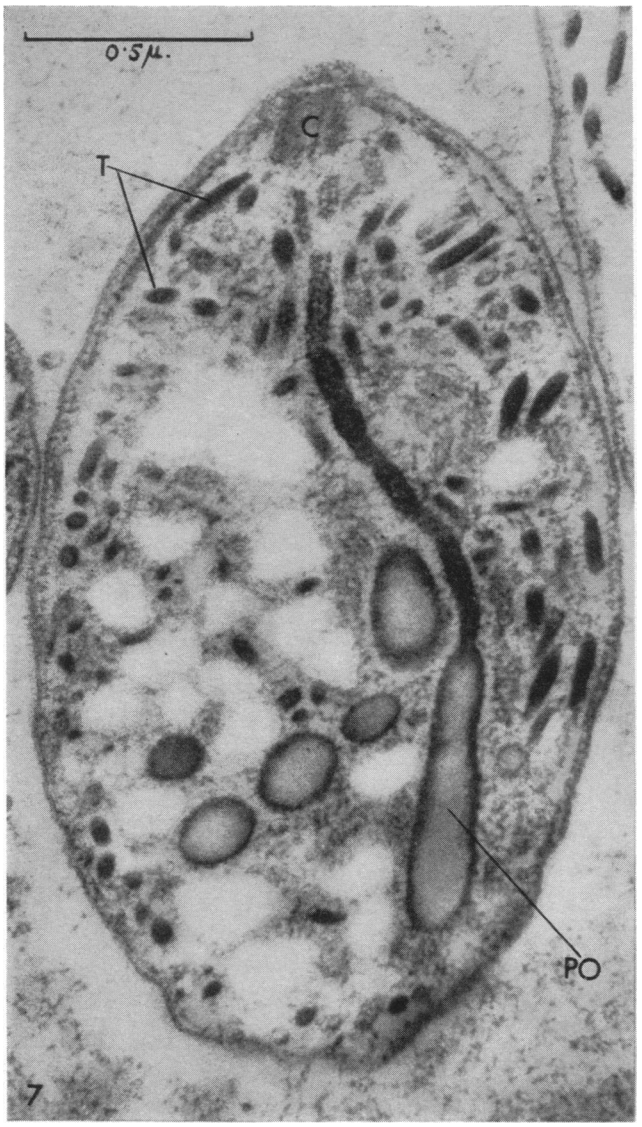
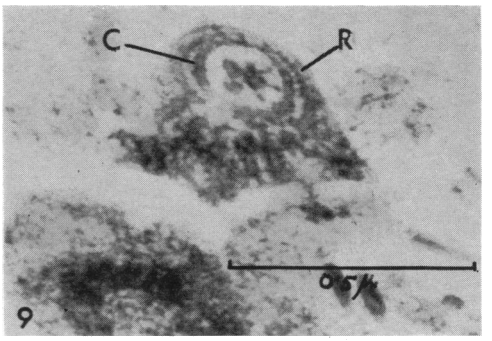
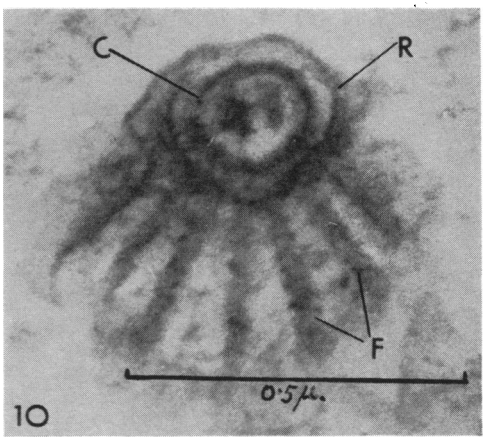


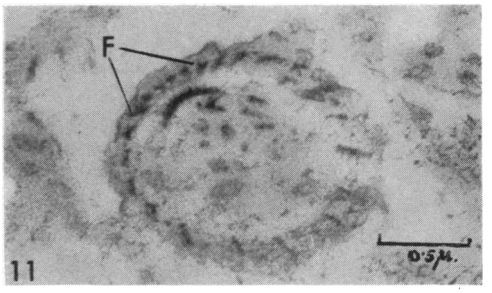
FIG. 4.—Low-power view of a section through a cyst near its periphery.
FIG. 4*a*.—Enlargement of part of the cyst wall from Fig. 4.
FIG. 5.—Light-microscope picture (phase contrast) of a section through a cyst.
FIG. 6.—Longitudinal section through the anterior end of an organism in a cyst.

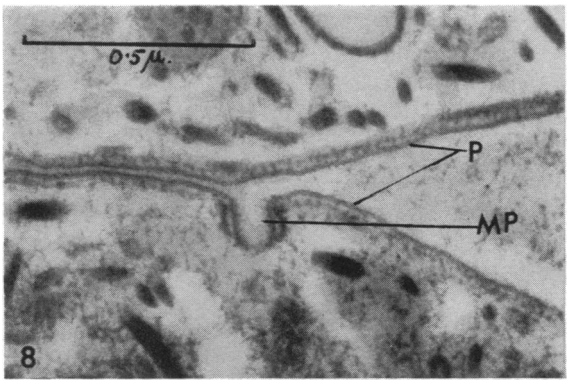
C=conoid; F=peripheral fibrils; R=polar ring; W=cyst wall.











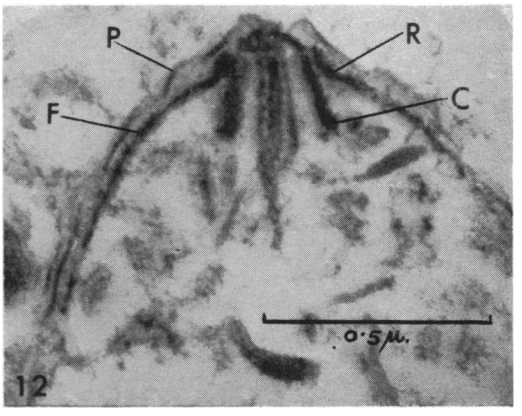
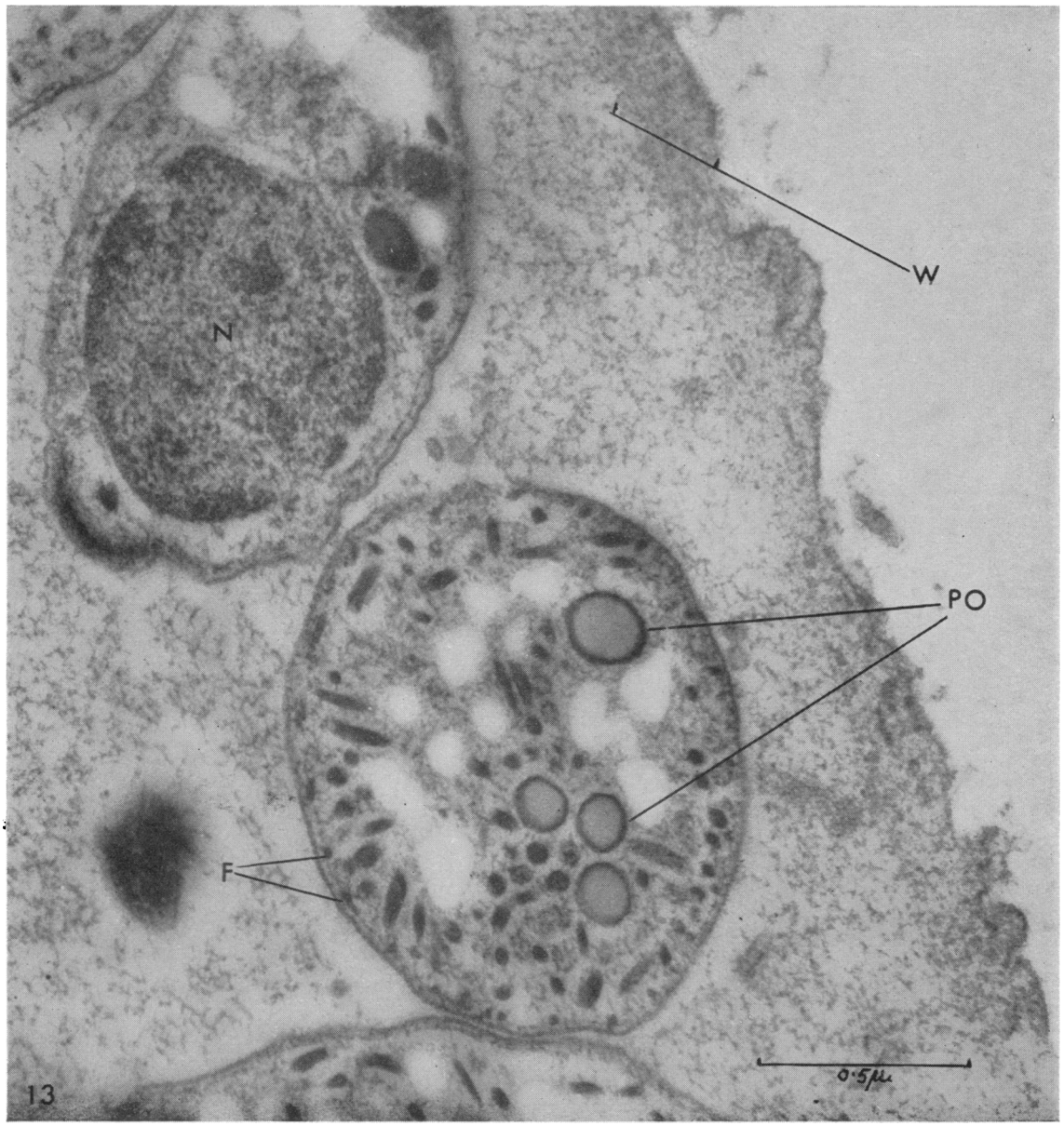
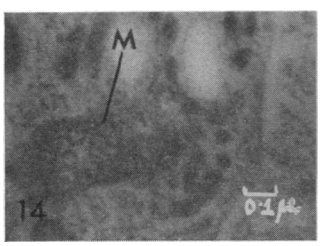


FIG. 7.—Longitudinal section through one organism, showing one limb of the paired organelle.
FIG. 8.—Section through a micropyle.
FIGS. 9 and 10.—Surface views of the anterior tip of two organisms.
FIG. 11.—Oblique section near the anterior tip.
FIG. 12.—Longitudinal section of the anterior end of an organism.

C=conoid; F=peripheral fibrils; MP=micropyle; P=pellicle; PO=paired organelle; R=polar ring; T=convoluted tubes.





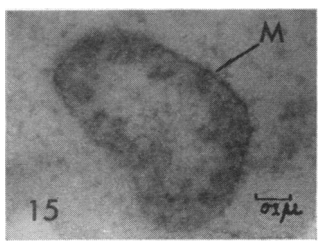


FIG. 13.—Transverse section through two organisms lying near the cyst wall.
FIGS. 14 and 15.—Sections through mitochondria from two cystic individuals.

F=peripheral fibrils; M=mitochondria; N=nucleus; PO=paired organelle; W=cyst wall.