MORPHOLOGY AND CULTURE OF *TOXOPLASMA**

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THE MANIFESTATIONS of toxoplasmosis depend to a great extent on the number and virulence of the organisms reaching the tissues, the reaction of these tissues to the organisms, and the ability of the cells of the affected host to overcome the proliferating organisms. Despite a considerable amount of work on the virulence of various strains of *Toxoplasma*, and their interactions with antibody and the host cells of various species,[1, 2, 3, 4, 5, 6] much needs to be done before we can completely comprehend the pathogenesis of congenital and acquired toxoplasmic infections. There has been a good deal of interest in the past few years concerning the morphology of *Toxoplasma*, its methods of propagation, and the reasons for variation in virulence of strains. A large number of puzzling observations have been made with regard to variation in virulence, for example, Lainson[4] recovered low-virulence strains from rabbits, which barely produced infections in mice on repeated passage for over a year. He found that these same strains produced rapidly fatal infections in multimammate rats and canaries, and that when they were reinoculated into mice from the rats and canaries they had become quite virulent. Another observation by Erichsen and Harboe[7] and Harboe and Erichsen[8] of an epidemic of toxoplasmosis in a flock of chickens in Norway is of unusual interest. Organisms were recovered from the affected birds. At a later date attempts to reproduce the disease in birds of the same flock with this

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same strain were unsuccessful. Jacobs and Melton[1] studied a strain of *Toxoplasma* which was isolated from a pigeon. This strain was propagated in two ways: One line was passaged in fertile eggs, and the other in mice. The mouse-maintained strain became quite virulent, whereas the egg-maintained strain retained its original low virulence.

All these studies emphasize the importance of understanding the morphology of *Toxoplasma*, its metabolism, its methods of attacking cells, and the effect of antibody on its morphology and function. This report is the first of a series and concerns the morphology of the organism, and its behavior in some types of tissue culture.

MORPHOLOGY

Toxoplasma is a crescentic organism when examined in the *fresh* unstained state. Quite often it is crescentic when examined in *fixed* tissue specimens. Fixation, however, usually results in some shrinkage, therefore the organisms seen in tissue sections are often rounder. All the strains have a similar appearance and cannot be distinguished on a morphologic basis. Harboe and Erichsen[9] measured four strains of *Toxoplasma* which had different degrees of virulence. The minor differences in length and breadth they found were not sufficient to be of value in distinguishing between strains. A summation of the findings of a number of observers[9, 10, 11, 12] with regard to measurements shows that the length varies between 3.5 and 7 μ and the width between 1.2 and 4.5 μ. One end of the organism is more pointed and the other somewhat rounded. A definite *wall* cannot be made out in fresh or stained specimens, but the organism is surrounded by a conspicuous limiting membrane which is slightly thickened at the poles.

The cytoplasm of fresh specimens is lighter in the center and contains a considerable amount of granular material. Pulvertaft, Valentine, Lane[13] described a prominent vacuole usually near the blunter end and an occasional vacuole at the sharper end. Stained specimens show chromatic granules in the cytoplasm, and with the PAS stain some of these granules are red. Jacobs[5] has suggested they may be glycogen. The nucleus originally was thought by Sabin[14] not to have a membrane, but subsequent observations by light and electron microscopy show a definite membrane.[9, 15] With the Giemsa stain the nucleus shows clumps of chromatin embedded in a loose meshwork.

Frenkel and Friedlander[16] characterize those organisms which are seen during acute infections as *proliferative* forms. During this stage the organisms proliferate within cells, are liberated rapidly, and invade

new cells. The organisms in this stage show little or no glycogen, and when seen within cells do not show a tendency to form cysts. These authors indicated that in chronic and latent infections the organisms parasitize cells and form cysts, which have a wall (Figure 1). Formerly it was believed by some that this wall was derived from that of the parasitized cell, but others such as Lainson[17] and Frenkel[18, 19] believe that there is a true cyst wall which is derived from the organisms. Within the cyst the organisms multiply slowly for a limited period, then remain quiescent.[17] The cyst wall stains lightly with PAS, and is

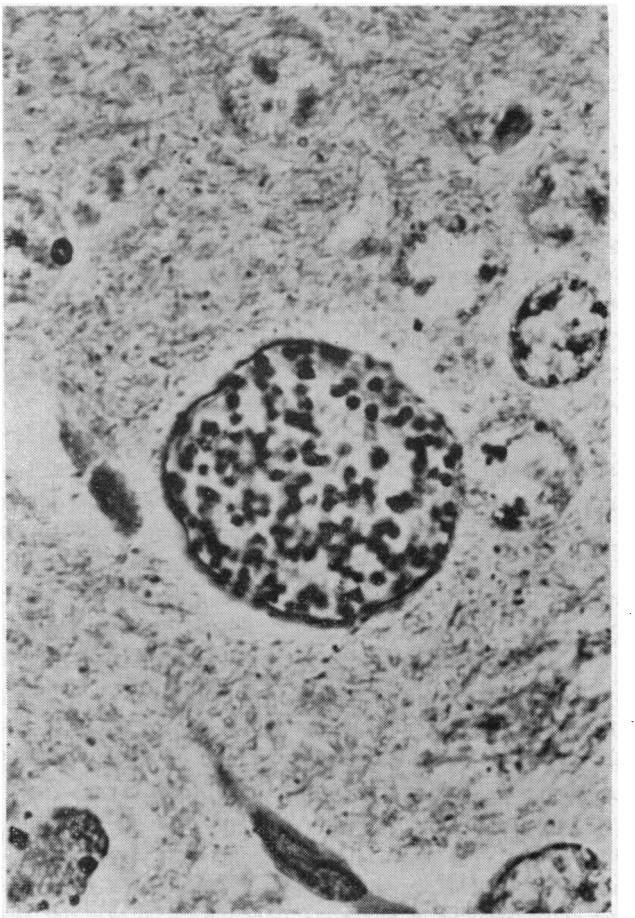

FIGURE 1. *Toxoplasma* CYST IN GUINEA PIG BRAIN, WILDER RETICULIN STAIN (× 800)

argyrophilic.[16] A considerable amount of discussion has arisen as to the nature and method of cyst-formation. By strict definition a cyst in the microbiologic sense is an investing, protective, or resistant structure formed wholly or partially from substances secreted from the body of the organisms. With respect to *Toxoplasma*, Nicolle and Conor[20] in 1913 first used the term pseudocyst to indicate the intracellular accumulation of proliferating toxoplasma organisms. It was not until 1928 that Levaditi *et al.*[21] made the important discovery that *Toxoplasma* may persist for many months in cyst-like aggregates within the tissues of healthy animals. Subsequent studies[22, 23, 16] showed this observation to be correct, and that the cysts could be liberated from host tissues by shaking infected brain with glass beads in saline, also, that the wall of the cyst was a conspicuous limiting membrane which was rather tough. This wall, however, is not resistant to hydrochloric acid and pepsin in the concentration found in gastric juice. It is now known that cyst formation occurs in almost all tissues of various species and may well account for latent or chronic infections. Also, it is possible that acute infections may be due to cyst rupture. The exact mechanism which produces such a rupture is not known.

Cysts vary in size from 5 to 10 μ to 50 to 60 μ, and they contain from 50 to 3,000 organisms.[17, 18] The organisms within young cysts stain like the proliferative forms for up to 14 days, after which the cytoplasm gradually loses its affinity for dyes. After 26 days, the cytoplasm loses its affinity for dyes, but the nucleus continues to stain.[17]

Many observers believe that *Toxoplasma* divides by longitudinal binary fission, first by the usual mitotic nuclear changes, then by cell division at either end which gradually spreads toward the center. In the later stages of division a butterfly appearance is produced, the daughter cells being attached centrally, Siamese-fashion.[13] Studies with colchicine show that nuclear division is of the mitotic type.[24] Braunsteiner *et al.*,[25] however, have shown by electron microscopy that nuclear division is amitotic, preceded by formation of two nucleoli and a horseshoe-shaped nucleus. A membrane extending from the ends produces final separation of the cytoplasm.

Electron microscopic studies have been carried out by a number of observers, employing various methods. Bringmann and Holz[26] in 1953 extracted osmium-fixed or unfixed *Toxoplasma* in distilled water at 60°C. This caused clearing of the cell and differentiated the nucleus and granules. An important finding was a system of ectoplasmic criss-cross fibers extending from the more pointed end toward the nucleus. They observed a pole structure at this end from which the fibrils

seemed to take their origin. vanThiel[27] in 1956, utilizing similar methods, demonstrated the same type of surface membrane at the smaller end, extending toward the nucleus. He also found a darker area at the pole, surrounded by a circular line. Gustafson *et al.*[15] in 1954 studied *Toxoplasma* after fixation with osmium, dehydration, and embedding in methacrylate. Thin sections were cut and examined, and both extracellular as well as intracellular organisms were described. The organisms were found to be highly organized with mitochondria, endoplasmic reticulum, granules of various types, and some well-developed juxtanuclear round granule clusters, possibly a Golgi apparatus or a centriole in the cytoplasm. A cell membrane was observed and the cell wall in certain sections showed a longitudinal fibrillar component, similar to that described by Bringmann and Holz,[26] and vanThiel.[27] In some preparations a second layer appeared below the surface membrane. No flagellae or cilia were seen. A most interesting finding was a polar organization at the blunt and round ends of many organisms. At the acute end a distinct organelle (the conoid) was seen, having the form of a truncated cone, 0.15 to 0.25 μ in diameter and 0.2 to 0.3 μ in depth. The base of this cone was open to the cytoplasm, but it was not certain if there was an opening through the cell membrane. In occasional cells this conoid was seen to protrude. Fourteen to eighteen circular or cylindrical osmophilic deep staining rod-like structures extended from the base of the conoid into the cytoplasm in much the same fashion as the streamers from a maypole. These were called toxonemes. They extended a variable distance toward the blunt end of the cell, but most often seemed to terminate near the nucleus.

The nucleus was round to oval, occasionally lobed and elongated. It occupied the middle one-third of the cell, and measured 1 to 1½ μ in diameter. There was a definite nuclear membrane, measuring 10 mμ in diameter.

It was suggested the conoid might be a mouth structure, a penetration device, or a vestigial protozoal structure such as a cytopharynx. The toxonemes were thought to be a secretory mechanism for nutrition or penetration, or a modified locomotor apparatus, like a kinetoplast.

Holz and Bringmann[28] in 1954 studied the effects of aureomycin on *Toxoplasma* by electron microscopy. Mice were infected with a virulent strain of *Toxoplasma* and treated with 10 mg./Kg. of aureomycin. The treated mice lived 14 to 21 days longer than the controls. Organisms were removed from the peritoneal cavity at intervals during the illness. The organisms eventually showed loss of cytoplasmic RNA, as evi-

denced by a less dense cytoplasmic structure, and a slowing of cell division (stages of mitosis were seen). There also was a tendency for autolysis.

Meyer and Mendonca[29] in 1957 studied the growth of *Toxoplasma* in tissue cultures, employing explants of chick embryo muscle and subcutaneous tissue in hanging drop slides. After a period of growth the explants were fixed, embedded, thin-sectioned in plastic, and studied with the electron microscope. The sections showed the growth of *Toxoplasma* to produce rosettes in the cells. The *Toxoplasma* possessed a cell membrane, nuclear membrane, nucleolus, and a ring-like structure at one end of the body, to which was attached a number of rod-like solid structures, similar to the toxonemes described by Gustafson *et al.*[15] At the opposite end there was another ring-like structure having an opening in many sections. The surface membrane often was doubled.

PERSONAL OBSERVATIONS

METHODS

1. The RH strain of *Toxoplasma* was studied. Four days after inoculation into the peritoneal cavity of mice the fluid was removed and injected directly into osmic acid fixative, after the method of Gustafson *et al.*[15] The fixed cells and organisms were centrifuged to a pellet, transferred through various alcohols, and finally embedded in methacrylate. Thin sections, cut at 1/40 μ were studied with the electron microscope.

2. The RH strain of *Toxoplasma* was inoculated into cell tissue cultures. Fifty, 500, 5,000, and 50,000 organisms were inoculated into monolayer cell cultures of retinoblastoma cells, fibroblasts, and HeLa cells. The coverslips were removed after 24, 48, 72, and 96 hours, and one, two, three, and six weeks, fixed in Zenker's, stained with Giemsa, and studied. Other cultures in retinoblastoma cells were allowed to grow for three weeks, following which the cells were fixed with osmic acid, removed from the slides, embedded in methacrylate, and sectioned for viewing with the electron microscope.

RESULTS

1. Thin sections of *Toxoplasma* embedded in methacrylate were studied with the RCA EMU3E electron microscope. The organisms were found free in peritoneal fluid, and within cells. A certain number of organisms showed separations of the cytoplasm from the cell mem-

brane, with shrinkage of the membranes, and probably were dead, but most organisms were well fixed and appeared normal. The cytology of the peritoneal fluid exudate was primarily mononuclear, but a number of eosinophils and an occasional polynuclear leukocyte were observed. Macrophages were found to contain six to 20 organisms. Most organisms were well preserved, and lay within membrane-bound vacuoles in the cytoplasm, but we believe these to be an artifact, due to shrinkage from Zenker's fixation. Some were surrounded on one or two sides by mitochondria of the parasitized cell, a finding also observed by Gustafson *et al.*,[15] and Braunsteiner *et al.*[25] Occasional cells were found to have just ruptured, and numbers of free organisms were seen in close juxtaposition to the cell. No cyst-like structures were seen in the fluid. The free organisms were cut in many planes, and a varying picture was seen, depending on the type of section. Within cells the organisms often were seen to be cut along a similar plane, probably because of their recent divisions and similar positioning in the cell.

The extracellular organisms most often have a double cell membrane, the outer one being wavy and continuous, the inner one straighter and appearing discontinuous, somewhat like ribs. The "joists" described by Braunsteiner *et al.*[25] between the inner and outer membranes were not seen. These membranes are prominent at the poles. The distance between the inner and outer cell membrane of the extracellular organisms is less than 200 Å. Many intracellular organisms have triple or quadruple membranes, all of which appeared to be of equal thickness (Figure 2). The surface fibrillar pattern which was seen by Bringmann and Holz,[26] and vanThiel,[27] and Gustafson *et al.*[15] was not seen in any of these sections, no matter how tangential, and we are investigating this concept at present by a replication technique.

The polar organization described by Gustafson *et al.*[15] also was observed by us. Sections tangent to the sharper end of the organisms showed the conoid most often to protrude from the cell at its pointed end (Figure 3). In this case it is circular, and is attached to the cell membrane at a circular base (Figure 4). An opening through the cell membrane of the conoid was not seen, although the cytoplasm within the conoid was continuous with that of the organism. The rounder end of the organism showed an occasional indentation, and a concentration of cytoplasmic structures near the cell membrane.

The toxonemes described by Gustafson *et al.*[15] were observed in nearly all cells examined. They arise at the base of the conoid, are strongly osmophilic, uniform, circular, and have no apparent internal

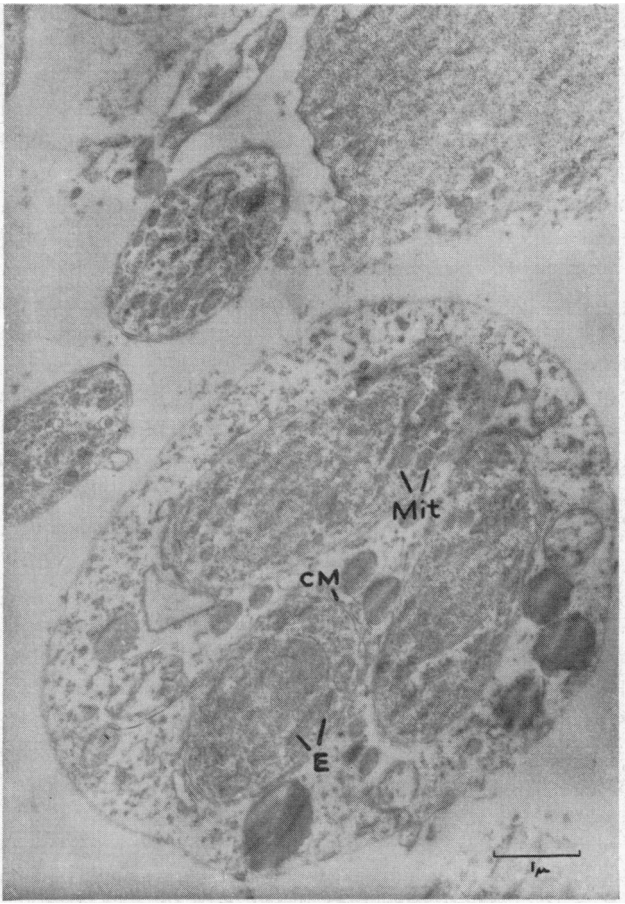

FIGURE 2. EXTRACELLULAR AND INTRACELLULAR TOXOPLASMAS IN MOUSE PERITONEAL FLUID. THREE TOXOPLASMAS WITHIN AN EOSINOPHIL (× 17,400)

CM, Double membrane around periphery of organism. The apparent third membrane belongs to the cytoplasm of the cell; E, Endoplasmic reticulum; Mit, Mitochondria.

structure or surface membrane except that the outer surface seems slightly more osmophilic. They extend from the conoid to the central portion of the organism, or even to the blunter end (Figure 4). At the conoid they are 20 to 40 mμ in diameter, at their distal end 0.10 to 0.18 μ in diameter.

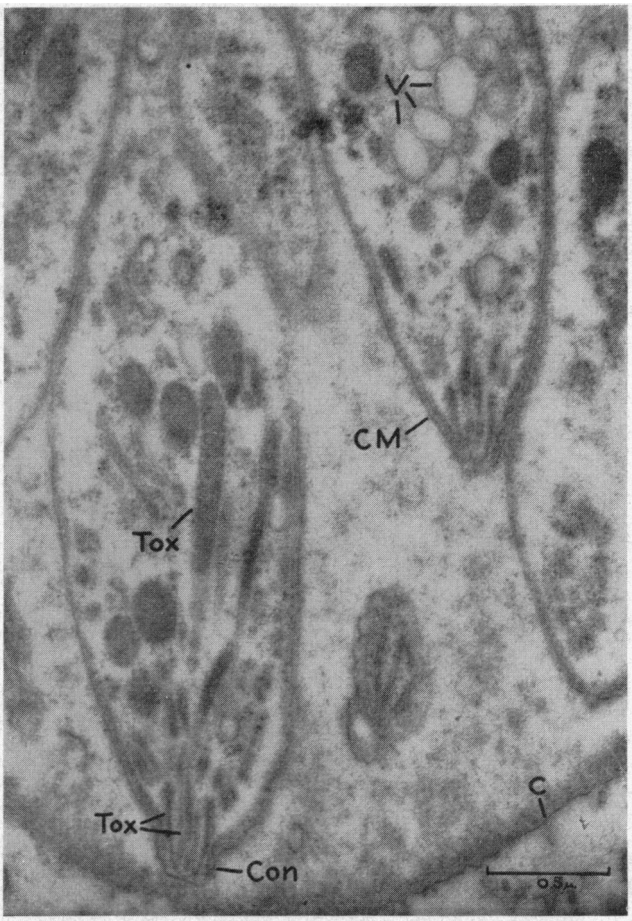

FIGURE 3. INTRACELLULAR CYST CONTAINING TOXOPLASMAS (× 49,000)

C, Cyst wall: note dense, thin, outer osmophilic membrane and broader less dense, ill-defined inner membrane; CM, Cell membrane; Con, Conoid; Tox, Toxonemes; V, Vacuoles.

The cytoplasm of the cells is finely granular, and contains highly organized structures. Numerous large, branched mitochondria 0.1 to 0.2 μ wide and 1 to 2 μ long containing cristae which do not seem to extend completely across the mitochondria are scattered through the cytoplasm. Endoplasmic reticulum is present in most cells. It usually

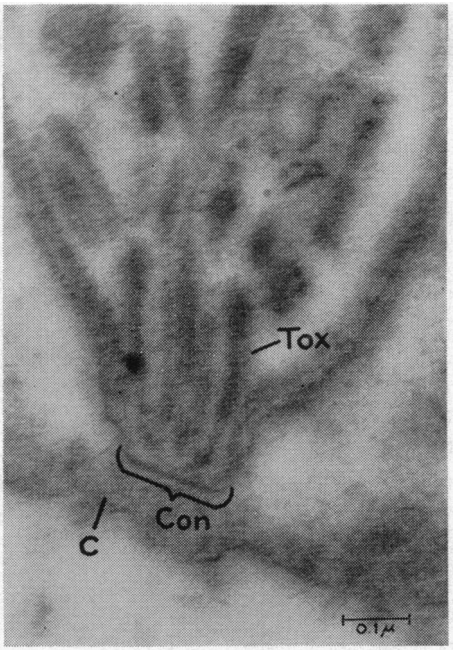

FIGURE 4. *Toxoplasma* SHOWING THE CONOID AND TOXONEMES ADJACENT TO A CYST WALL
(× 126,900)
C, Cyst wall; Con, Conoid; Tox, Toxonemes.

is located parallel to nuclear membrane. A number of large vacuoles surrounded by membranes were seen in many cells. Some of these are empty and some contain amorphous material in the center or attached to the membrane surrounding the vacuole. The vacuoles measure 0.1 to 0.3 μ. They are more prominent in the proliferating organisms within cells. The juxtanuclear round granule clusters seen by Gustafson *et al.*[15] were not seen.

An interesting finding, not previously reported, is a rather large organelle. This structure lies between the nucleus and the blunter end. It is surrounded by a light membrane and contains a large number of circular varying-sized, uniform, osmophilic structures, and a few less dense granular structures surrounded by membranes. The nucleus lies near the middle portion of the cell, and is round to oval. The nuclear membrane appears to be double, with rather large openings in its

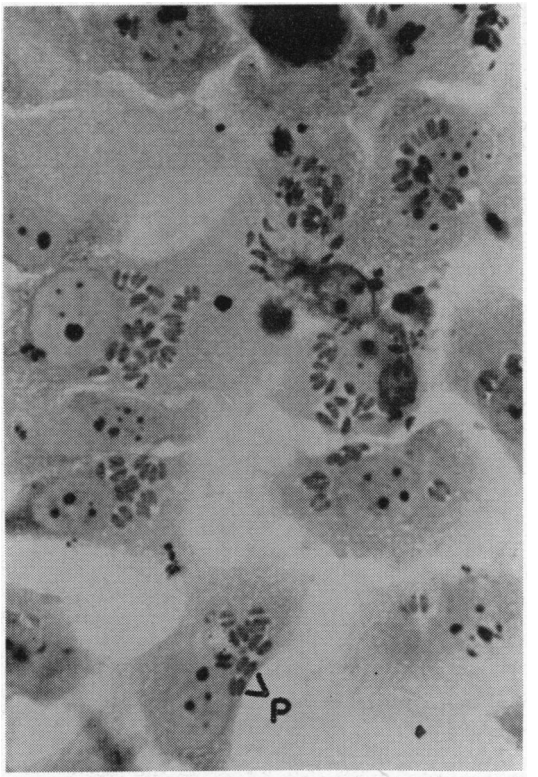

FIGURE 5. *Toxoplasma* TISSUE CULTURE IN HELA CELLS, TWENTY-FOUR HOUR CULTURE (× 480)
P, Pairs of *Toxoplasma*. Note the paired intracellular organisms and a tendency for rosette formation.

surface. The margins of these openings are rounded. There is a well defined nucleolus. The nuclear chromatin is evenly dispersed through the nucleus, and a fine granular material occupies most of the nuclear substance.

2. *Toxoplasma* grows well in all types of cell culture.[30, 31, 32, 33, 34, 35, 36] Our studies show that within 24 hours a large percentage of cells contain organisms (Figure 5). The organisms are most often seen in pairs at this time, probably as a result of division by longitudinal binary fission. Soon the cells contain one or more rosette-like structures, also probably due to the method of division (Figure 6). The rapidly

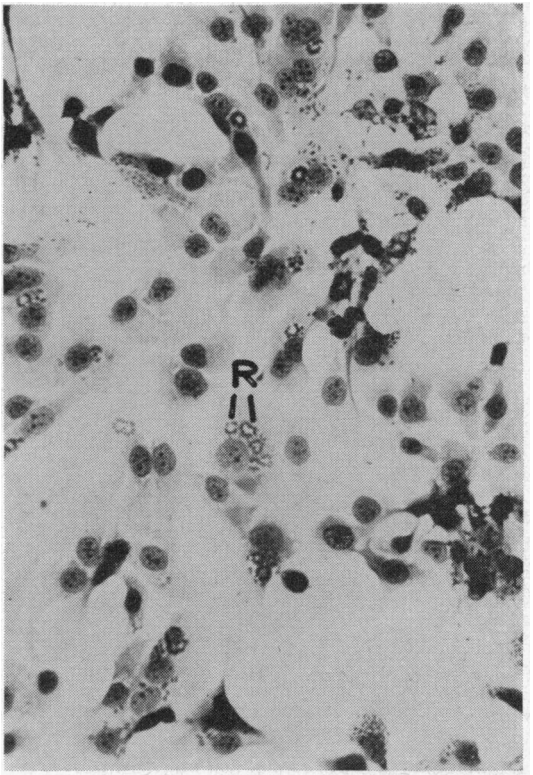

FIGURE 6. *Toxoplasma* TISSUE CULTURE IN HELA CELLS, SEVENTY-TWO HOUR CULTURE (× 192)
R, Rosettes. Note the numerous rosettes.

growing HeLa and fibroblast cultures showed larger numbers of organisms and cytopathogenic effects were noted early. By the seventh to tenth day a large percentage of cells were destroyed. The retinoblastoma cells, which grow more slowly, contain large numbers of organisms, often multiple rosettes, and cytopathogenic effects are less severe and occur at a later period. These cultures could be held for six weeks at which time many cells were destroyed, but about 10 percent had survived. A number of these contained proliferating organisms, and ball-like clusters of organisms (Figure 7). Some of these clusters had definite membranes and exhibited the changes of cyst formation, with decrease in size and staining of the contained organisms.

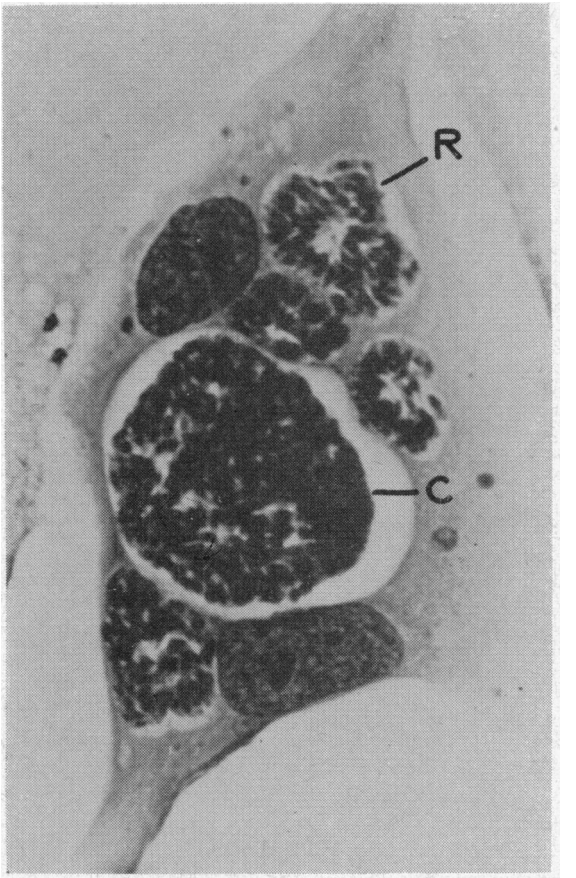

FIGURE 7. *Toxoplasma* IN TISSUE CULTURE OF RETINOBLASTOMA CELLS, THREE WEEKS' CULTURE (× 768)
C, Cyst; R, Rosette.

Electron microscopic studies of three-week cultures of the retinoblastoma cells containing the ball-like and cyst structures showed the organisms to be contained within a definite cyst wall 0.1 to 0.2 μ wide which has a thin solid outside osmophilic layer resembling a membrane, and immediately beneath it a thicker, less osmophilic, granular layer having no definite boundary at its inner aspect with the contents of the cyst. This layer contains numerous tiny vesicles (Figures 8, 9, 10). The wall of this cyst was definitely distinct from the wall of the cell, which could be seen in the same section. Within the cyst is a fine

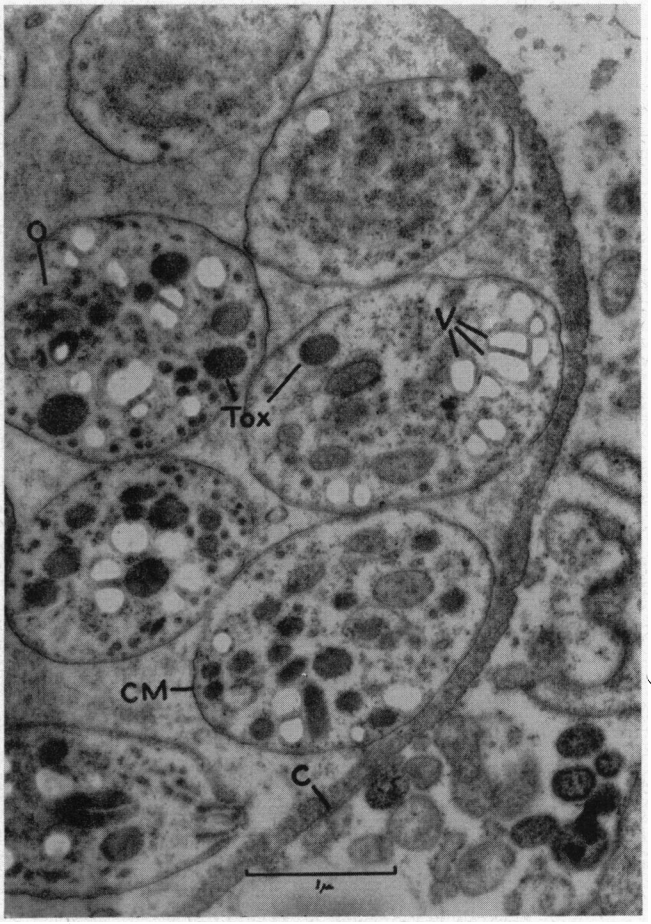

FIGURE 8. *Toxoplasma* ORGANISMS IN A CYST WHICH IS LOCATED WITHIN THE CYTOPLASM OF A RETINOBLASTOMA CELL (× 29,400)
C, Cyst wall; Cm, Cell membrane; O, Organelle; V, Vacuoles; Tox, Toxonemes.

granular material which surrounds the *Toxoplasma*. The toxoplasma organisms within the cyst definitely contain more and larger vacuoles in their cytoplasm than do proliferating organisms.

DISCUSSION

Our findings in retinoblastoma cell tissue cultures indicate that cyst formation can occur in tissue culture as early as three weeks after

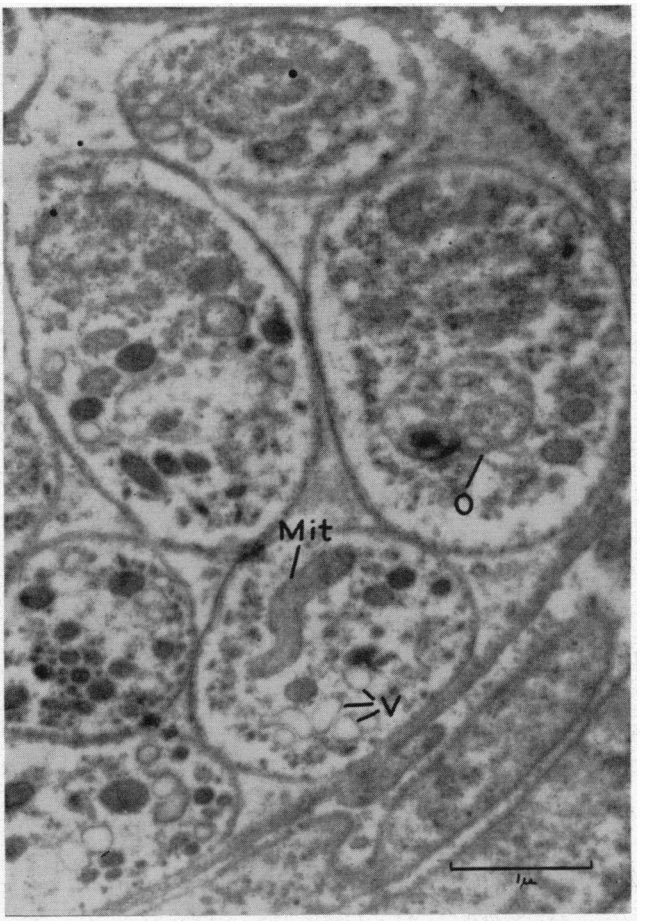

FIGURE 9. *Toxoplasma* ORGANISMS IN A CYST WHICH IS LOCATED WITHIN THE CYTOPLASM OF A RETINOBLASTOMA CELL (× 28,000)
Mit, Mitochondria; V, Vacuoles; O, Organelle.

introduction of organisms into the culture. This correlates with the observations of Lainson[17] who found cyst formation at seven to 21 days in experimental animals which were infected with relatively avirulent rabbit strains of *Toxoplasma*. He showed cyst formation in the brain at eight days with the cysts containing two to four parasites. The number of contained organisms and the size of the cysts increased up to four months. After 10 months no larger forms were found. In the

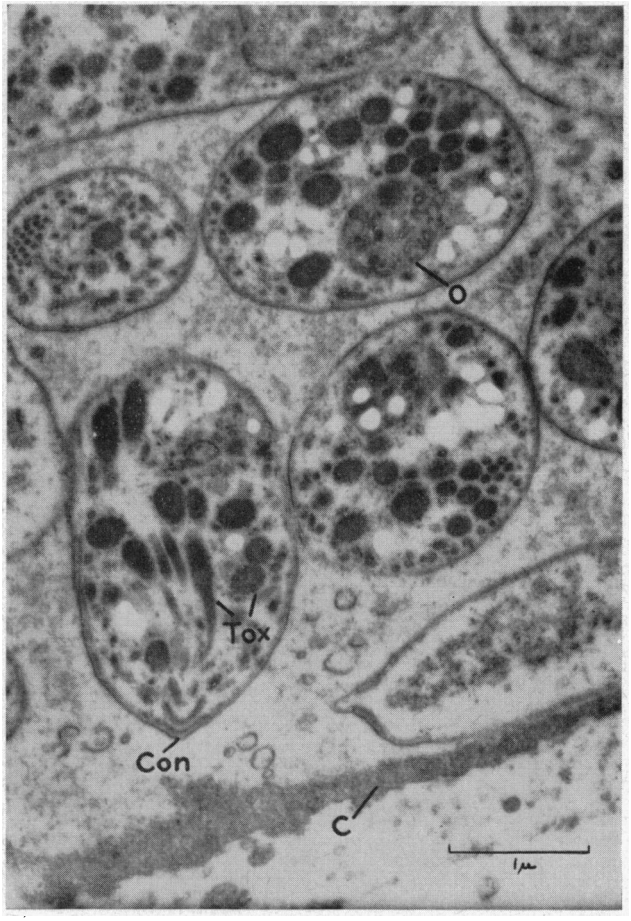

FIGURE 10. *Toxoplasma* ORGANISMS IN A CYST WHICH IS LOCATED WITHIN THE CYTOPLASM OF A RETINOBLASTOMA CELL (× 28,000)
Con, Conoid; C, Cyst wall; Tox, Toxonemes; O, Organelle.

lung, cysts were found in alveolar macrophages at seven days, and by the 21st day the cysts bulged into the alveoli and traces of the host cell were gone. Our observation of cyst formation in the retinoblastoma cultures correlates with this finding, even though the more virulent RH strain was used. These large, slow-growing cells could be shown to gradually develop larger and larger ball-like clusters of organisms. Within three weeks a thin wall was formed around the ball and the wall gradually increased in thickness up to termination of

the study at six weeks. At three weeks we often observed formation of several cysts in the cytoplasm of a single cell, while at the same time in another part of this cell one or more rosettes were present. Several pairs of proliferating organisms also might be observed in another portion of the cytoplasm of such a cell.

Our observations as to the morphology of *Toxoplasma* by electron microscopy were similar to those of Gustafson *et al.*,[15] except that we found two to four thin membranes in the outer envelope of the cells of many organisms, and interruptions in the nuclear membrane. Such interruptions in nuclear membranes have been found in other cells, but not of the size observed in this study. We also have the feeling that the normal position of the conoid is in the extended position, and that in use for attachment to cells it is retracted.

The function of the newly described organelle in the cytoplasm of *Toxoplasma* is unknown.

SUMMARY

1. Electron microscopic study of thin sections of osmium-fixed *Toxoplasma* embedded in methacrylate shows the extracellular organisms to have two and the intracellular organisms to have two to four thin membranes in their outer envelope. They also have a double nuclear membrane showing several interruptions. The interruptions are surrounded by the rounded edge of the nuclear membranes. The conoid most often is seen in the extended position, and the impression is gained that it is retracted upon fixation to the parasitized cell. A new organelle is described in the cytoplasm.

2. Growth of the RH strain of *Toxoplasma* in human retinoblastoma cells, HeLa cells, and fibroblasts shows less tendency for lysis of the cultures to occur in the slower-growing retinoblastoma cells. A series of events occurs following penetration of the cell: (a) division occurs by binary fission, resulting in accumulation of many pairs of organisms; (b) one to four rosettes form in the cytoplasm of the cells as a result of this division; (c) an increase in number of organisms within the rosettes leads to the formation of ball-like, dark-staining agglomerations of organisms; (d) at about the third week a thin cyst wall forms around these ball-like structures; this membrane increases in thickness in the ensuing weeks.

3. Electron microscopic study of the cysts which have formed in the cells at the third week shows the wall to be distinct from the cell membrane of the host cell. The outer portion of the wall is densest, and

beneath this is a more granular, and less osmophilic layer, often containing minute vacuoles.

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DISCUSSION

DR. JAMES H. ALLEN. I suppose the Program Committee asked me to discuss this paper because they knew I was interested in ocular bacteriology, but they forgot that protozoology is quite far afield from bacteriology. Therefore I accepted this assignment only because of my respect for the Chairman and the authors, knowing as little as I do about protozoology. I discussed this paper with Drs. Beaver and Yeager in our Departments of Parasitology and Tropical Medicine, and found that most of this information is new.

Toxoplasma organisms have resisted detailed study of their morphology for a long time, as a result partly of their small size, and partly of their

refractility in the unstained state. With the addition of staining and light microscopy, or phase-contrast microscopy, studies of either the whole specimen or thick sections have added little additional information. However, recently ordinary microscopy after the application of silver impregnation methods and electron microscopic study of thin sections have added a great deal of information so further interest in this field has proven rewarding. Dr. Hogan and his co-workers not only have confirmed the important observations of several other groups but also have presented us with two important new findings: first, the development of cysts in tissue culture, and second, a previously undescribed organelle within the parasite. In addition, their paper has raised several other interesting points.

In the photographs furnished to the discusser by the authors with the preliminary draft of the paper, the cysts in the tissue culture cells appear degenerated as compared to those normally seen in animal tissues. Normally the cysts form a heavier wall than those shown in the photographs by these workers. Also multiple cysts, as shown in the tissue culture cells, have not been reported in animal tissues. Is this perhaps a reflection of abnormal environment, or has this phenomenon, thus far, been overlooked in tissues?

Another question I would like to ask the authors is, whether they have been able to elucidate the question regarding multiplication. There is some doubt as to multiplication by simple binary fission. It has been suggested by Goldman and others that internal multiplication occurs before fission occurs. I wonder if the authors' observations have shed any light on this problem?

The clearly defined organelle observed by the authors has not been observed before, although Cross described two fine granules in the location described for this organelle, and a bizarre mass composed of indefinitely grouped fine granular material was described by Goldman and his co-workers. I wonder whether there is any possibility that these workers were referring to the same structure appearing differently as the result of variations in technique?

The present authors are to be commended for using the terminology of Gustafson's group who originally described several of these structures, rather than confusing the literature with new terms, as has been done by several other groups.

The authors have set themselves a huge task in evaluating pathogenecity and in trying to correlate structure and pathogenecity. Many obstacles exist to the study of pathogenecity of intracellular parasitic organisms. However, the authors have made a fine start by showing certain differences of growth and appearance of the parasites in various types of host cells. We hope this will prove to be of practical importance. Therefore we are looking forward with great interest and expectation to subsequent reports from this group.

DR. HOGAN. I wish to thank Dr. Allen for his remarks, and constructive criticism. Some of his points are very well taken, and a few of his questions

were answered in the final manuscript, which was not available to him before this meeting.

We were not very certain at first whether we were actually dealing with cyst-formation in the cells of the tissue cultures. However, when we were able to study osmium-fixed cells with the light and the electron microscope, it was possible to be certain that cysts actually existed within the cytoplasm of the cells. Zenker-fixed tissue showed considerable shrinkage of the cell cytoplasm from the cysts, so that the detail was lost.

We have now cultured infected retinoblastoma cells up to 12 weeks and at the end of this time can demonstrate persistance of organisms by inoculation into mice. Most likely the organisms are present within cysts at this time. As far as I know, cyst-formation in cells of tissue cultures has not been accomplished previously, and it is interesting that some cells contain multiple cysts. The special conditions imposed on cells of tissue cultures probably leads to this type of cyst-formation. Rosettes have been observed in the brain and other cells of humans with toxoplasmosis. Wilder showed them in the retinal cells of eyes enucleated for chorioretinitis. Most often, however, by the time the tissues are studied the cysts have formed in tissue cells, and have been liberated into the tissues themselves.

As far as the division of *Toxoplasma* is concerned, studies show that it most likely occurs by binary fission. Lainson showed in time-lapse cinephotography studies that division occurred in this manner, and Rodhain demonstrated binary fission after mitosis of the nucleus, by the use of colchicine. Some electron studies have suggested that division is by horizontal fission, rather than longitudinal fission, but most of the evidence is in favor of the latter.

During this presentation I did not discuss the organelle we have observed in these studies. This structure has not been described by others, and we are not certain of its significance. Cross described an organelle which she called a cytostyle, lying just beneath the cell membrane, but our structure is not of this type. It is quite well organized, and contains a variety of structures. A number of consultants were unable to help us with its identification.