However, amyl alcohol(13) retards growth in peptone media while being an excellent carbon source in simple media.

*P. caeca* differs from *P. agilis*(5) and *C. paramecium* (4) in utilizing amyl alcohol when grown in simple media. Both species of *Polytomella* utilize propionate, in contrast to *C. paramecium*. Caproic and caprylic acids, not utilizable by *P. caeca*, support growth of *C. paramecium*.

## REFERENCES


1. Bebbington, A. E., Bourne, E. J., Stacey, M. & Wilkinson, I. A. (1952). The Q enzyme of *Polytomella caeca*. *J. Chem. Soc.* (*London*), **1952**, 240-245.
2. Bebbington, A. E., Bourne, E. J. & Wilkinson, I. A. (1952). The conversion of amylose into amylopectin by the Q enzyme of *Polytomella caeca*. *J. Chem. Soc.* (*London*), **1952**, 246-253.
3. Bourne, E. J., Stacey, M. & Wilkinson, I. A. (1950). The composition of the polysaccharide synthesized by *Polytomella caeca*. *J. Chem. Soc.* (*London*), **1950**, 2649.
4. Cosgrove, W. B. & Swanson, B. K. (1952). Growth of *Chilomonas paramecium* in simple organic media. *Physiol. Zoöl.*, **25**, 287-292.
5. Little, P. A., Oleson, J. J. & Williams, J. H. (1951). Growth studies on *Polytomella agilis*. *Proc. Soc. Exptl. Biol. Med.*, **78** (2), 510-513.
6. Lwoff, A. (1935). L'oxytrophie et les organismes oxytrophes. *Compt. rend. soc. biol.*, **119**, 87.
7. ——— (1935). La nutrition azotée et carbonée de *Polytomella agilis*. *Compt. rend. soc. biol.*, **119**, 974.
8. ——— (1941). Limites de concentration en ions H et OH compatibles avec le developpement *in vitro* du flagellé *Polytomella caeca*. *Ann. Inst. Pasteur*, **66**, 407-416.
9. Lwoff, A. & Dusi, H. (1937). La pyrimidine et le thiazol, facteurs de croissance pour le flagellé *Polytomella caeca*. *Compt. rend.*, **205**, 756.
10. Lwoff, A., Ionesco, H. & Gutmann, A. (1949). Métabolisme de l'amidon chez un flagellé sans chlorophylle incapable d'utiliser le glucose. *Compt. rend.*, **228**, 324-344.
11. Pringsheim, E. G. (1937). Zur Kenntnis saprotropher Algen und Flagellaten. II. Mitteilung. Ueber eine neue, farblose Polyblephoridine, *Polytomella caeca*. *Arch. Protistenk.*, **88**, 150-160.
12. ——— (1937). Beiträge zur Physiologie saprophytischen Algen und Flagellaten. II. *Polytoma* und *Polytomella*. *Planta*, **26**, 665-691.
13. Provasoli, L. (1938). Studi sulla nutrizione dei Protozoi. *Boll. zool. agrar. e, bachicolt. Univ. studi Milano*, **9**, 59-64.
14. Schoenborn, H. W. (1946). Studies on nutrition of colorless Euglenoid flagellates. II. Growth of *Astasia* in an inorganic medium. *Physiol. Zoöl.*, **19**, 430-442.


J. PROTOZOOL., **2**, 158-167 (1955).

# The Development of Megaloschizonts of *Leucocytozoon simondi* Mathis and Leger.*

ARCHIBALD B. COWAN†
*University of Michigan, Ann Arbor, Michigan*‡

SUMMARY. Susceptible ducklings were exposed to natural infection during a limited time, and infections of supposedly known duration were obtained. Slides of tissues from these ducklings were prepared by common histological techniques. Comparative materials were provided by cooperators. On the basis of study of these tissues what seems a logical description of development of megaloschizonts of *Leucocytozoon simondi* was formulated.

The earliest stage found was the trophozoite. There is no evidence of schizogony in this form; the only internal structure is the nucleus. Prior to any schizogony there is a marked increase in the amount of chromatin in the nucleus, especially on the periphery.

It is hypothesized that as the chromatin increases, buds are evaginated from the margin of what may then be called the central body and are set off to form spherical primary cytomeres. The chromatin of primary cytomeres first diffuses and then proliferates to form peripheral clusters. These clusters of chromatin separate to form secondary cytomeres which continue to multiply in the same manner.

The production of cytomeres by the central body and by division of primary and secondary cytomeres eventually fills the cytoplasmic area of the megaloschizont. Apparently pressure within the schizont then shapes the course of further development. The central body ceases to function as a primordium and becomes compressed. The multiplying cytomeres become smaller and more granular in appearance, and their chromatin becomes more concentrated. Merozoite-like bodies are eventually produced. These reproduce until the central body is greatly compressed and the membrane enclosing the megaloschizont is ruptured and releases the merozoites.

*Leucocytozoon simondi*, Mathis and Leger (=*anatis*) has been shown by O'Roke(6) to be a serious pathogen of ducklings. In this genus only sexual stages, the gametocytes, occur in blood cells. Asexual multiplication takes place in the vertebrate host and sexual reproduction occurs in certain species of blackflies, *Simulium* spp., the only known vectors.

* A portion of a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan (1954). This study was supported in part by a predoctoral fellowship granted by the Michigan Memorial-Phoenix Project.

† The author wishes to express appreciation to Prof. E. C. O'Roke, Chairman, and the other members of his committee, all of whom gave valuable assistance and advice. He also thanks Drs. C. M. Herman, C. G. Huff, R. J. Porter, and Eli Chernin for their comments during the preparation of this paper.

‡ Present address: Patuxent Research Refuge, Laurel, Md.

In determining the life history of *L. simondi*, O'Roke described and figured some of the asexual stages but made no definitive study of their development. Huff (3) made the major contribution to existing knowledge of the mode of development of asexual forms. Fallis, Davies, and Vickers(2) provided valuable information on time intervals required for development of tissue stages.

The interpretation of a dynamic process through study of fixed materials can, at best, produce only a hypothesis. Different observers frequently reach widely divergent conclusions which may be equally valid until better techniques make possible a final and reproducible proof. With this in mind, the author presents herein a description of the development of megaloschizonts as interpreted by histological study of organs from a series of ducklings naturally infected during a limited period of exposure.

It should be stated here that the author considers megaloschizonts to be the final prepatent generation of schizonts and not representative of the complete schizogonic cycle or the earlier schizonts. It is also believed that megaloschizonts are the sole contributors to the succeeding parasitemia of gametocytes.

## MATERIALS AND METHODS

Four 8-week-old white Pekin ducklings were exposed to natural infection from August 19 to 23, 1952, in southeastern Kalkaska County, Michigan. On the morning of August 23rd, they were removed from the endemic area to Ann Arbor, Michigan. Daily blood smears were taken, and on August 30 all ducklings exhibited a light parasitemia of very young gametocytes. At 8:30 a. m. of that day one exhibited a general malaise and by 1:30 p. m. was unable to walk. This bird was killed immediately. The remaining three were killed at succeeding 24-hour intervals so that progressive development of the tissue stages of the parasite might be followed.

Portions of spleen, bursa of Fabricius, heart, small intestine, adrenal glands, brain, kidney, lung, liver, bone marrow, thyroid, ovary, testes, and pancreas were fixed in Bouin's solution, and embedded in paraffin.

Sections were cut to thicknesses of 6 and 3 $\mu$. All were mounted serially, in relatively long series. Several standard stains were used. Giemsa stain, used as described by Shortt and Cooper(7), provided a desirable differentiation of blood cells in the tissues. The Maximow technique did not prove as satisfactory, probably due to fixation.

In addition to materials thus obtained, Professor E. C. O'Roke made his entire collection of *Leucocytozoon* slides available; and Dr. A. M. Fallis, Director, Department of Parasitology, Ontario Research Foundation, graciously provided slides of tissues from ducklings with infections of known duration.

## OBSERVATIONS

*General Findings.* Spleen, heart, bursa of Fabricius, small intestine, adrenals, brain, kidney and lung taken on the first 2 days of parasitemia were positive for megaloschizonts. Of the tissues obtained on the 3rd day of parasitemia, only heart and brain were positive. By the 4th day of parasitemia the brain alone had demonstrable parasites. Surprisingly, none of the livers were infected, either with hepatic schizonts or megaloschizonts.[1] However, the slides contributed by Prof. O'Roke provided an opportunity to examine hepatic schizonts at many stages of development.

Initial observations on megaloschizonts in various tissues disclosed that in any given tissue they were all at relatively the same stage of development. This, plus the fact that tissue stages all but disappeared within a 24-hour period, led to the conclusion that the ducklings had been simultaneously infected during only a relatively short period of the entire exposure time. Megaloschizonts in tissues from an infected duckling which had been exposed for only 30 minutes and autopsied 10½ days later were at identical stages of development as those above, thus presenting further evidence that all in the initial series were infected at about the same time. It was therefore felt that they were single exposure infections and progressive development of a single generation of megaloschizonts could be followed.

*Growth and Development.* It is necessary to distinguish growth from development, because in later stages of schizogony one does not necesarily imply the other. Growth, or increase in size, of megaloschizonts appears to cease before they mature. But chromatin-like parasite substance continues to increase within the delimited schizonts during maturation. Considerable overlap in sizes at different stages of development has been observed in schizonts within the same organ, and conversely there is a wide range in size of schizonts at the same stage of development.

Despite variation that may exist within any given infection, there appears to be a very marked inverse relationship between intensity of infection and size of megaloschizonts. This is demonstrated by a comparison of sizes in three different intensities of infection. The splenic tissues used in the study were obtained 10 to 11 days after initial exposure, and the schizonts measured were all within similar ranges of develop-

[1] To preserve continuity, the nomenclature presented by Huff(3) will be used. Hepatic schizonts are those found in liver cells and do not exceed the normal limits of the host cell. Megaloschizonts are many times larger than hepatic schizonts. At times the terms megaloschizont, schizont, and parasite are used interchangeably.

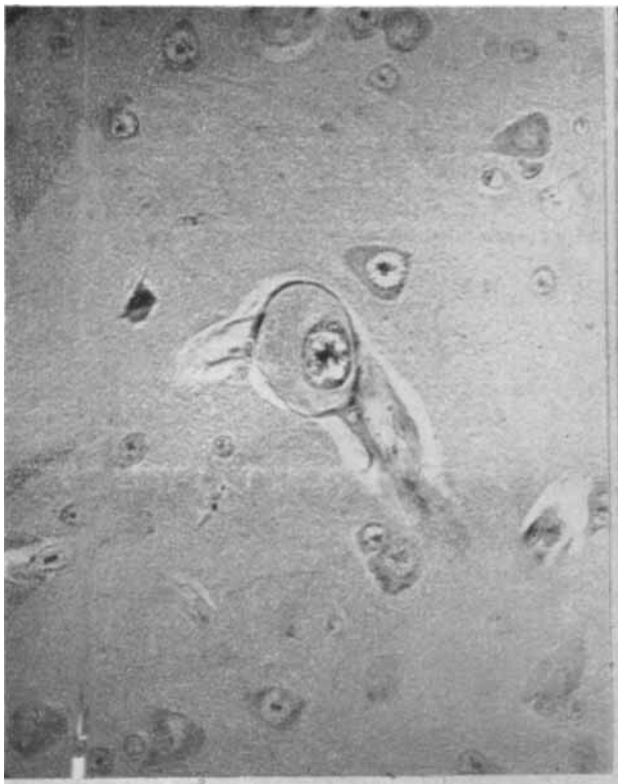

Figure 1

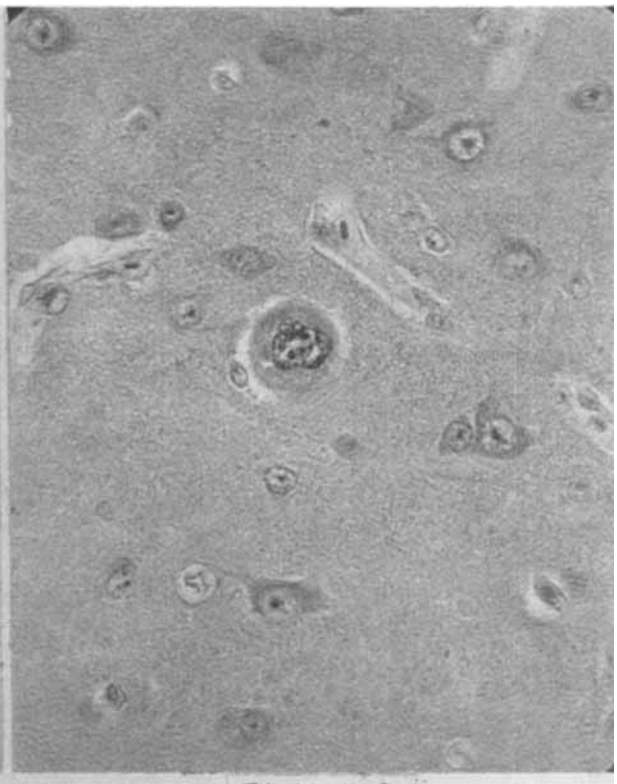

Figure 2

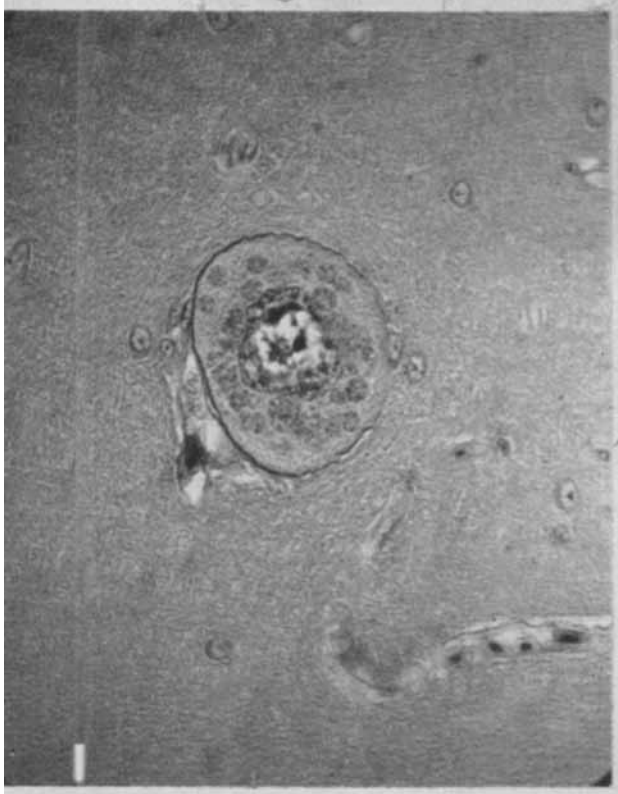

Figure 3

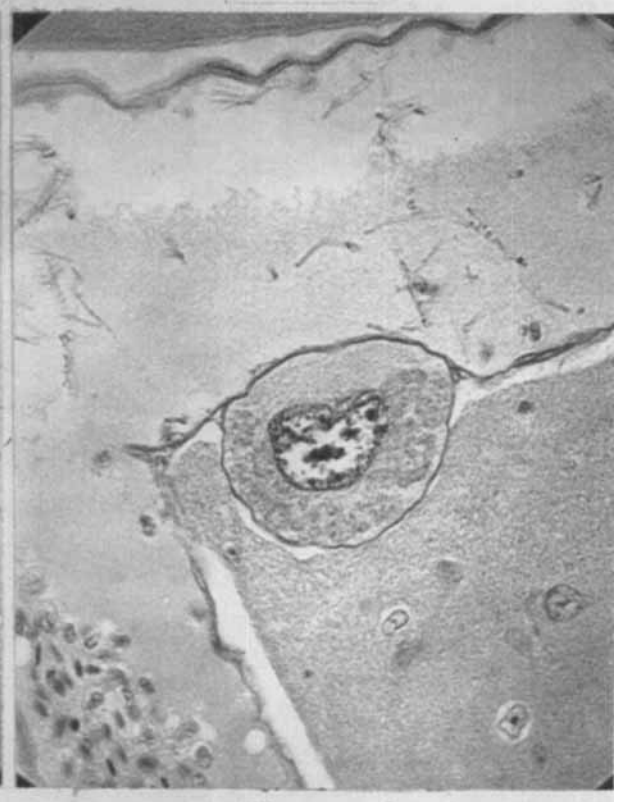

Figure 4

ment. The average diameter of the 10 largest schizonts measured in each infection was used for the comparison. Intensities of infection could be classed comparatively as moderate, heavy, and very heavy. Average diameters of megaloschizonts in these infections were 164, 145, and 133 $\mu$ respectively.

Optimum growth and development as well as greatest numbers of these schizonts appeared to occur in those organs or systems which contain much, or have many foci of, lymphoid tissue and are well vascularized. Many megaloschizonts in heart tissue seemed closely associated with small, intermuscular strands of lymphoid tissue. None were seen that appeared to have developed within heart muscle cells. Those in adrenal glands were in cortical areas. Megaloschizonts in the brain were small and retarded in development and no mature ones were found, suggesting that the brain is an unfavorable site for the parasite. However, stages found there were of great value to this study because, in their retarded state of development, they seemed to present an integrated review of what had occurred in other tissues. All megaloschizonts in the brain were closely associated with vascular endothelium. (Figs. 1 and 4).

The smallest tissue stages of the parasite were found in brain tissues taken on the first day of parasitemia (Fig. 1). They ranged in size from 27 to 31 $\mu$ average diameter as contrasted to schizonts in the spleen of the same duckling which were as large as 206 $\mu$ average diameter. At the earliest stage of development seen they looked like a large cell enclosed by a thin connective tissue membrane, the only definite internal structure being the nucleus. Careful study of these early forms through serial sections disclosed no evidence of a parasitic body.

Other parasites within the same size range contained nuclei in which there was markedly more chromatin. Along the margin, but still an integral part of these nuclei, were small bubbles or buds of chromatin evaginated into the cytoplasm (Fig. 2). At this stage of development, the nuclei began to assume the appearance of the central body of Huff(3), though there was still no evidence of a parasite in the cytoplasm of the cell.

The next-larger group of stages observed in brain tissues was approximately double the size of the smallest. Their nuclei were definitely the same as Huff's central body. The peripheral chromatin in some cases was divided up into bud-like evaginations, each of which was organized much in the same pattern as the central body. There was a peripheral arrangement of chromatin granules encompassing a centrally placed cluster of similar granules, all interconnected by delicate fibers. Unattached cytomeres occurred throughout the cytoplasmic portion of the schizonts (Fig. 3). Although the chromatin in the cytomeres was more diffused, it was distributed very similarly to that in the buds on the central body.

In other instances (Fig. 4), the margins of the central body were precise and well defined, and the cytomeres had diffused to the extent that they formed only shadowy, lightly chromatin-stained masses. However, their original identities could still be faintly discerned.

The continuity of growth and development beyond that found in brain tissues was exhibited by megaloschizonts in other organs, primarily the spleen, where the parasites were much larger and more nearly mature. In these larger megaloschizonts the central body had grown in proportion to the parasitic body. In the least mature stages, bud-like evaginations were still present, occasionally in large clusters, on the periphery. Separate clusters of cytomeres occurred throughout the cytoplasm, but still did not fill the cytoplasmic portion of the schizont. The similarity between the clusters of buds on the central body and the clusters of cytomeres was very striking (Fig. 5).

With increasing maturity of the schizonts, the buds on the central body decreased and finally disappeared (Figs. 6-9). However, the cytomeres continued to increase through regular multiplication until the cytoplasmic area was filled and the separate clusters had coalesced into a continuous mass (Fig. 6).

Multiplication of the cytomeres appeared to occur through an increase of chromatin which concentrated into peripheral clusters. These then separated to form new cytomeres (Fig. 6). Although the identity of the original cytomeres was lost, there was evidence that delicate membranes continued to hold the new genera-

Fig. 1. A trophozoite, the earliest stage of development observed, in a capillary of the brain tissue taken on the first day of parasitemia. The majority of the parasites found in this tissue were similar in morphology and location. Note the small amount of chromatin in the nucleus and the absence of any other internal structure. (6 $\mu$ thick, iron-hematoxylin and fast green stain, $\times$505. This and all subsequent tissues fixed in Bouin's.) Fig. 2. The smallest megaloschizont found was also in brain tissue taken on the first day of parasitemia. This is slightly advanced in development over Fig. 1; the chromatin in the central body has increased, and buds have begun to form on the margin. (6 $\mu$ thick, iron-hematoxylin and fast green stain, $\times$507.) Fig. 3. The production of primary cytomeres characterized schizogony in the brain tissues taken on the second day of parasitemia. Note the many buds on the margin of the central body and the manner in which the chromatin-staining material diffuses after being set off. (6 $\mu$ thick, Flemming's triple stain, $\times$504.) Fig. 4. Eccentric production of primary cytomeres. Though this schizont occurred in brain tissue taken on the first day of parasitemia, the diffusion of the chromatin of the primary cytomeres is advanced beyond that in Fig. 3. The close association of the parasite with the vascular endothelium in the brain is well demonstrated. (6 $\mu$ thick, iron-hematoxylin, and fast green stain, $\times$512.)

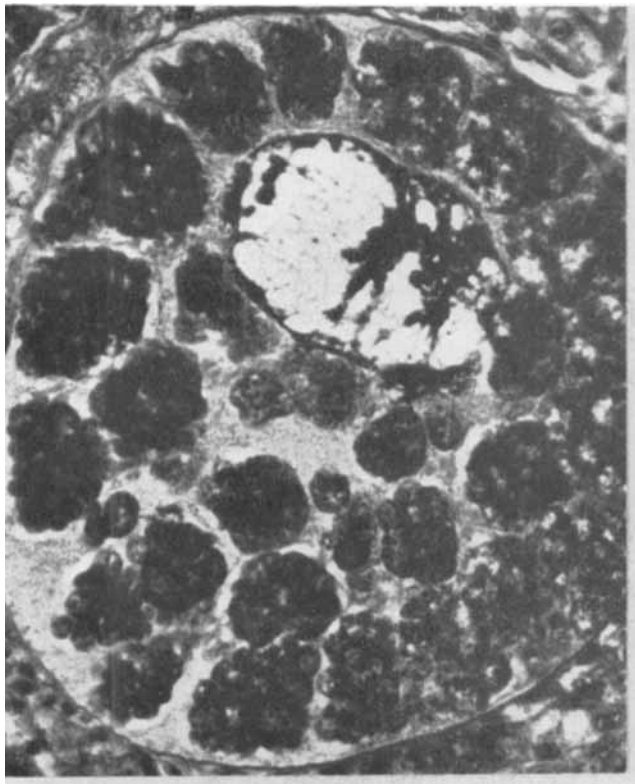

Figure 5

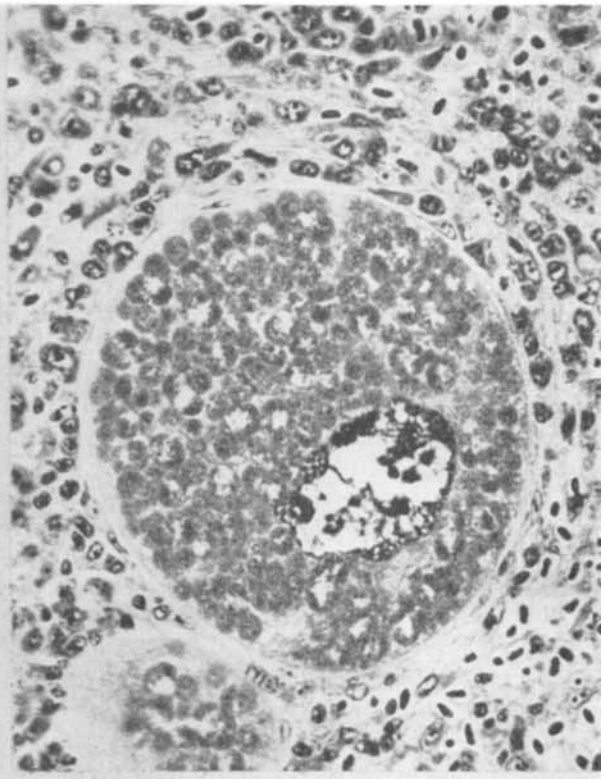

Figure 6

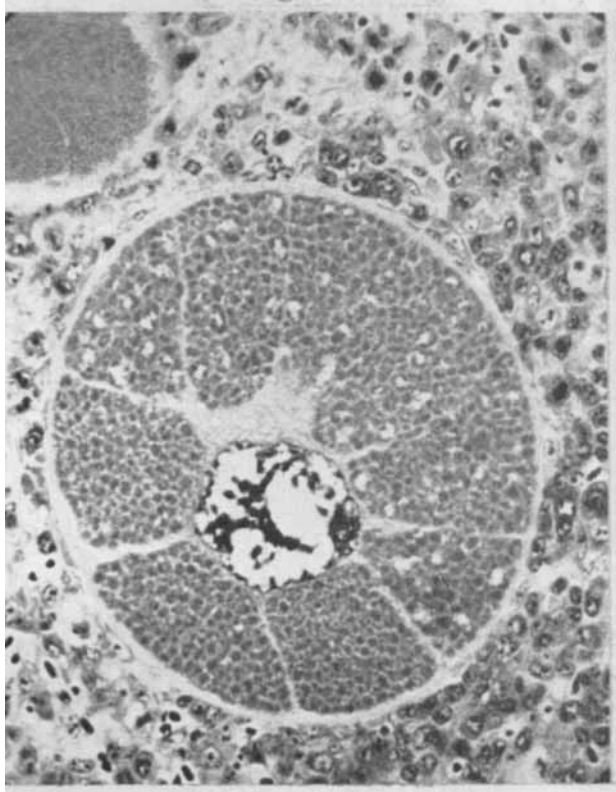

Figure 7

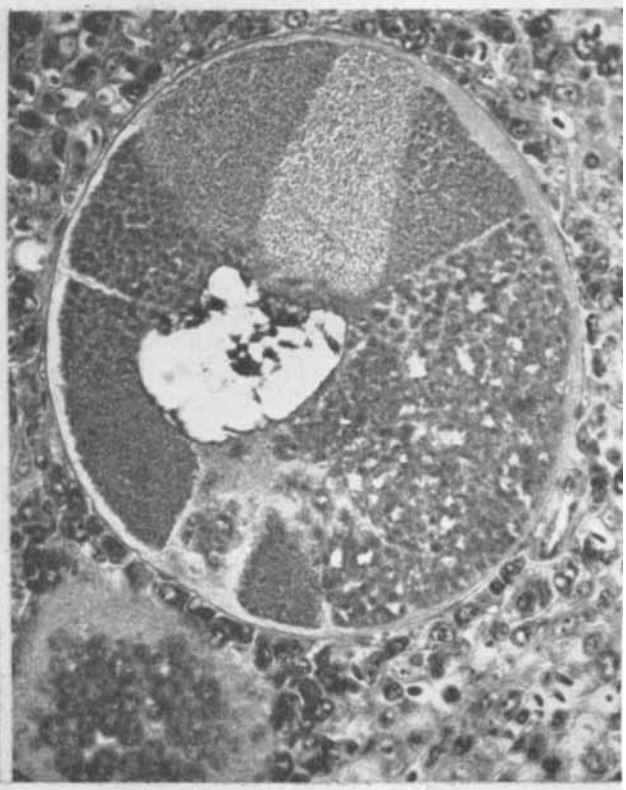

Figure 8

tions of cytomeres together so that they formed clusters resembling bunches of grapes (Fig. 5). Frequently seen were large cytomeres which duplicated in miniature the morphology of the central body (Fig. 6).

By the time or before cytomeres had filled the cytoplasmic area, the megaloschizont appeared to reach its maximum size, and subsequent development was limited to certain characteristic changes within the schizont. The cytomeres continued to increase in number, but became smaller, the chromatin more condensed and the products of division more granular in appearance (Fig. 7). There appeared to be a continuing synthesis and increase of chromatin. Sometimes a marked indentation of the central body occurred (Figs. 8 and 10); but in other instances there was no distortion (Fig. 9). A regular manifestation of increasing maturity was the formation of temporary septa between some of the original groups of cytomeres (Figs. 7 and 9). Development normally progressed uniformly throughout an entire megaloschizont. However, there were examples where three or four different stages were found in one schizont (Fig. 8). In such cases there was homogeneity within individual groups of cytomeres.

Ultimately, division by cytomeres terminated with production of small, round, clearly defined chromatin bodies, which may or may not have been merozoites (Figs. 9-10). The central body was reduced, in all mature megaloschizonts seen, to an amorphous mass of chromatin-staining material. And finally, the enveloping membrane was breeched and the merozoites were released into the surrounding host tissue.

Release of merozoites appeared to occur in either of two ways which might be termed "gentle release" (Fig. 11) and "explosive release" (Fig. 12). Where host tissues only adjoined the megaloschizonts the escaping merozoites appeared to have gradually filtered through the enveloping membrane at different places, thus the gentle release. Explosive releases occurred where an adjacent schizont was exerting pressure upon the mature schizont. In these cases there was a very marked outward rupture of the limiting membrane at a single point somewhere opposite the external source of pressure. The merozoites spread through the host tissue in a pattern indicating a forcible expulsion.

The fates of all merozoites produced in megaloschizonts were not clearly defined, but it has been well established that large numbers successfully invade cellular elements of the circulating blood and develop into gametocytes. In sections of spleen, merozoites were seen entering the circulation, having been carried from a ruptured schizont through a venous sinus into a venule and then into a vein. However, it is not necessary for merozoites which are produced in the spleen to enter the circulation to infect blood cells, because the marked hyperemia associated with infection of that organ places most of the spleen in nearly direct contact with blood cells.

*The Role of Macrophages.* A major interest of this study was to find, if possible, the earliest recognizable stage of parasitic development in the tissues. Both Huff(3) and Fallis *et al.*(2) indicated that macrophages appeared to be the principal host cells for megaloschizonts. Therefore, close attention was given to those cells and their contents. Macrophages were found to be most numerous in spleen tissues, upon which this phase of the study was then primarily centered. However, eventually other tissues and nearly all of their cellular elements were carefully examined. The ultimate findings were the same for all tissues.

In splenic tissues taken on the first 3 days of parasitemia the macrophages presented some interesting figures. They contained chromatin-staining bodies of varying sizes and configurations, many of which were comparable to some figured by Huff(3) in his description of early development of schizonts. But the suspected forms, instead of developing into a new generation of schizonts, had, along with the megaloschizonts, practically disappeared by the 4th day of parasitemia.

## DISCUSSION

*Role of the Central Body.* Because there is always a central body present in a developing megaloschizont, it is important that the nature of this body

Fig. 5. Primary groups of secondary cytomeres have been formed by division of primary cytomeres. Shrinkage has accentuated the separate identities of some of the groups. Note the cluster of buds on the lower right margin of the central body which are partially broken free. (Adrenal gland, 2nd day of parasitemia, 6 $\mu$ thick, Mallory triple stain, $\times$520.) Fig. 6. An almost completely filled megaloschizont. Separate identities of the primary groups are lost due to continued production of primary and secondary cytomeres. Note at the lower edge of the central body the large cytomere which has grown to resemble a miniature central body. Also in the upper left quadrant of the schizont observe how the chromatin-staining substance concentrates in hemispherical clusters on the periphery of the cytomeres and then rounds up as it separates. (Spleen, 1st day of parasitemia, 3 $\mu$ thick, Giemsa stain, $\times$510.) Fig. 7. Secondary multiplication has advanced to the point where internal pressure is evinced. The cytomeres are more crowded and much smaller than in Fig. 6. The central body is slightly compressed in places, and the identity of the primary groups is being reestablished. The three primary groups below and to the left of the central body are a bit more mature than the others. (Spleen, 3 $\mu$ thick, Giemsa stain, $\times$509.) Fig. 8. Multiple stages of development in one megaloschizont. At least four different stages are represented. Note the increased indentation of the central body and the distinct membrane along the right side of the most diffused group of merozoites. (Spleen, 1st day of parasitemia, 3 $\mu$ thick, Flemming's triple stain, $\times$510.)

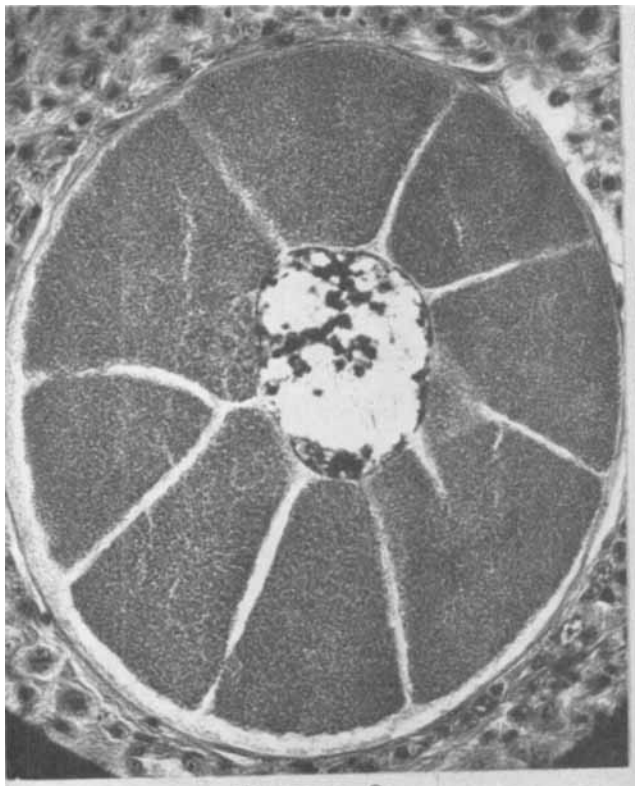

Figure 9

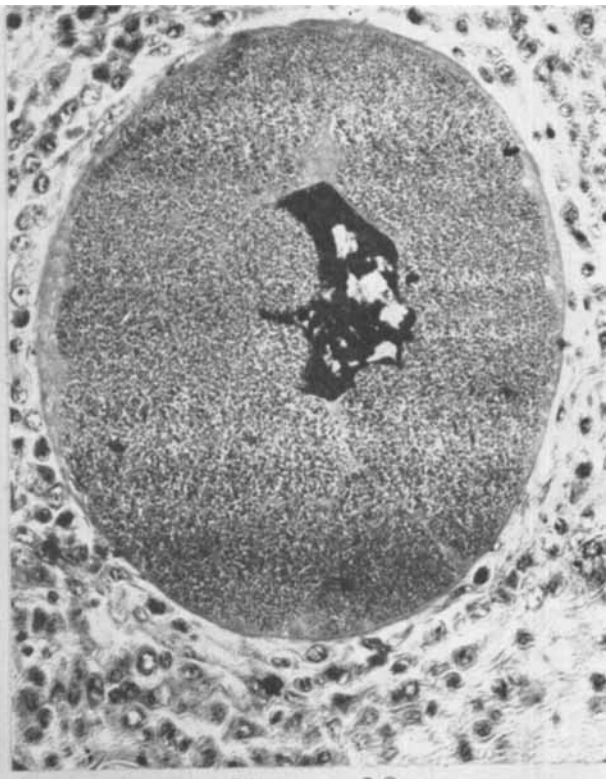

Figure 10

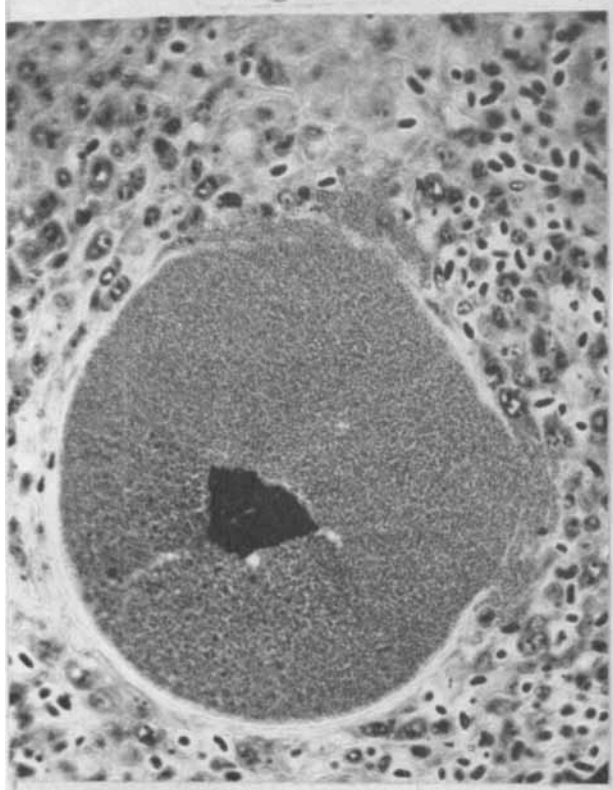

Figure 11

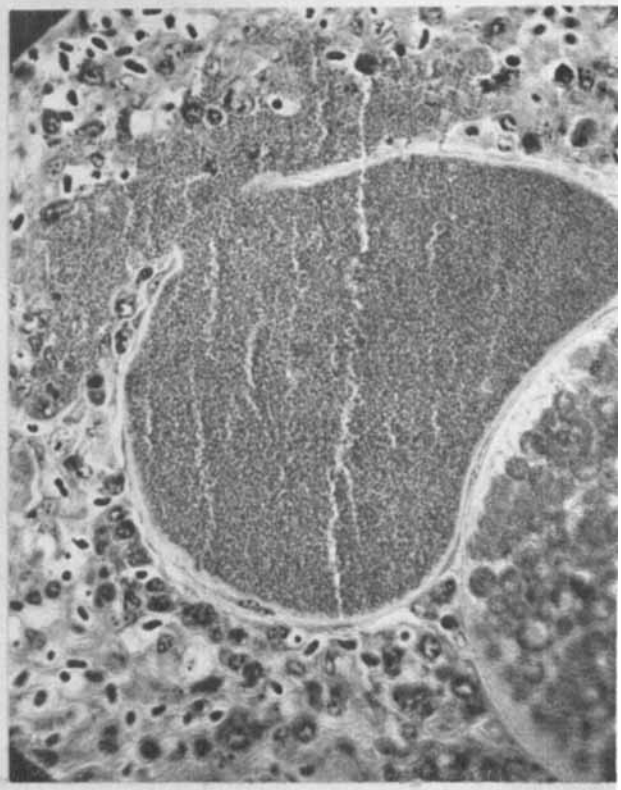

Figure 12

be determined. Huff(3) was of the belief "that this structure is probably the enormously enlarged nucleus of the original infected host cell, a belief shared by Brumpt." Observations made in this study indicate that it is an integral part of the parasite and it is assigned the important role of a primordium. These alternatives should be thoroughly explored.

Belief that the central body was the anlage of the merozoites produced in the megaloschizont within which it lay was strongly established before the smallest forms (Figs. 1 and 2) were found. This was based upon a series of observations made upon megaloschizonts which were well advanced in their development. First was the regular occurrence of bud-like evaginations, which will be referred to as buds in this section, from the periphery of the central body. Second was the similarity in cross-sectional morphology between these buds and some small cytomeres and between separate clusters of cytomeres and clusters of buds which were still a part of the margin of the central body (Fig. 5). Third, large cytomeres were often observed to resemble a central body in miniature (Fig. 6). And fourth, at certain stages of schizogony, megaloschizonts contained separate clusters of small closely associated cytomeres, indicating that the primordia of the individual clusters had originated independently of each other (Fig. 5). Finally there was the presence of two or more stages of development in a single megaloschizont (Fig. 8). Each stage was more or less precisely separated from the others. This seemed to indicate that the different stages had originated at different times due to intermittent eccentric production by, rather than simultaneous division of, a common ancestor.

When those considered to be the least mature forms were discovered (Fig. 1) they were carefully examined in their entirety, through serial sections, in an effort to determine whether they were hypertrophied host cells containing parasites. No parasites were found. Thus it appeared that the forms themselves were parasites, and that the nuclear structures belonged to the parasites instead of being hypertrophied host cell nuclei. Apparently their previous development had been wholly a matter of growth. Therefore following terminology used in reference to similar tissue forms of avian plasmodia by Coulston and Huff(1), Huff, Coulston, Laird, and Porter(5), and Huff(4), it is proposed that these be called trophozoites. If this interpretation were true, it was desirable to demonstrate the fate of the host cell nuclei. However, in all cases, the nuclei of the encompassing connective tissue cells made very difficult the separate identification of any other nucleus which might have been compressed against the membrane.

Subsequent to finding trophozoites in brain tissues taken on the 1st day of parasitemia, the entire series of brain tissue slides from all the infected ducks was carefully examined. A well integrated series of developmental stages, including the critically important early schizogony, was found. It was observed that a marked increase in the chromatin content of the trophozoite nucleus was associated with the occurrence of buds on its periphery (Fig. 2). Subsequently, formation of first cytomeres seemed to be concurrent with active budding by the nucleus, now the central body (Fig. 3). This all contributed to the interpretation that the central body sets off buds which become what this author calls primary cytomeres.

*The Formation of Cytomeres.* In addition to fundamental differences of opinion as to the origin of the cytomeres, evidence from this study points to a further divergence from Huff's(3) interpretation of the manner whereby cytomeres are formed. A study of his description, discussion, and illustrations gives the impression that in all but the earliest stages of development Huff found that chromatin-staining parasite substance filled all or nearly all of what he considered a greatly hypertrophied host cell, with the exception of the space occupied by the central body. Apparently, though he did not say so, the hypertrophying host cell was continually filled by the rapid expansion of the growing parasitic mass. Formation of cytomeres appeared to him to be the result of formation of U-shaped clefts which eventually cut off islands of parasite substances, thus forming the cytomeres. He also described and figured the occurrence of spherical and cleft-like vacuoles in the cytomeres of megaloschizonts.

The above theory has not been confirmed by the present study. Instead it appears that primary cytomeres multiply through a regular process of development and division to form secondary cytomeres. These repeat the multiplication through an undetermined number of generations until a final reduction produces merozoites or merozoite-like bodies.

But perhaps some of the observed differences can be rationalized. First, following the above interpretation and nomenclature, Figs. 2 through 5 demonstrate

Fig. 9. A nearly mature megaloschizont. Compression of the central body and redistribution of the merozoites will complete the maturation. (Spleen, 2nd day of parasitemia, 6 $\mu$ thick, Mallory's triple stain, $\times$510.) Fig. 10. Almost complete compression of the central body. Temporary septa are disappearing. (Spleen, 2nd day of parasitemia, 3 $\mu$ thick, Heidenhain's azan stain, approx. $\times$510.) Fig. 11. "Gentle release" of merozoites from a mature megaloschizont. The central body is completely compressed into an amorphous mass. (Spleen, 1st day of parasitemia, 3 $\mu$ thick, Giemsa stain, $\times$510.) Fig. 12. "Explosive release" of merozoites. Note how ruptured schizont has been compressed by the growth of an adjacent one, opposite the point of rupture. (Spleen, 1st day of parasitemia, 3 $\mu$ thick, Flemming's triple stain, approx. $\times$510.)

rather conclusively that at the beginning of cytomere formation the trophozoite is not filled with chromatin-staining substance. These figures show further that it is production of cytomeres which eventually fills the cytoplasmic area. Neither vacuoles in cytomeres similar to those figured by Huff, nor U-shaped clefts cutting off islands of parasite substance were found. However, there were planes of what appeared to be cytoplasm which separated individual cytomeres from each other. Separations persisted in closely packed clusters of cytomeres; but the cytomeres became hexagonal in cross section, just as any group of uniformly sized elastic spheres will do when placed under pressure and forced against each other (Fig. 6).

Some slides provided by Fallis had been prepared by the Maximow technique. In these, U-shaped clefts around cytomeres and cleft-like vacuoles within cytomeres were readily seen. But they appeared to be more artifacts of shrinkage than normal configurations. The large masses of parasite substance were noticeably more susceptible to shrinkage due to fixation than were host tissues. Therefore, it is believed that the clefts were formed as a result of closely packed cytomeres shrinking away from each other and becoming spherical, thus causing the separating planes to become wider and cleft-like in appearance. Cleft-like vacuoles within cytomeres could have been caused by shrinkage of clusters of chromatin-like material with resultant accentuation of the lines along which they were destined to separate during the next division.

One final aspect of the method of cytomere formation and multiplication may be found in the work of Wingstrand(8). In studying schizonts of *L. sakharoffi* in adult carriers he found small schizonts from which he described a method of cytomere reproduction somewhat similar to that presented here. The smallest parasite was described as rounded or oval with a large vacuole in the center. During subsequent development chromatin-like substance concentrated along opposite sides of the ring giving it a bipolar appearance. In some parasites Wingstrand found the chromatin-like substance divided into three or more parts. Multiplication occurred through separation of those parts. Wingstrand considered this manner of multiplication to be analogous to mitosis, and commented that the same process had been noted by Ivanic.

*The Formation of Merozoites and Final Schizogony.* Huff(3) described the process of merozoite formation in hepatic schizonts and likened it to the process of formation of merozoites from the cytomeres in megaloschizonts. His interpretation of schizogony in liver cells was that cytomeres, once they had filled the host cell, went through a process of breaking up into successively smaller bodies until merozoites were formed. This may be applicable to hepatic schizonts but does not seem to be fully applicable to what was observed in megaloschizonts during this study. The critical difference lies in the connotation of Huff's statement: "In Fig. 33 the large cytomeres have begun to break up into smaller bodies." This implies subdivision of parasite substance with no further increase in its quantity. The observations reported above indicate that there must have been a significant increase in the amount of parasite substance within the confines of the megaloschizont after it had reached its maximum dimension and in many cases even after what appear to be merozoites were formed.

In Fig. 9, although the cytomeres have apparently been completely reduced to merozoite-like bodies, the central body still retains its original identity. But no mature or rupturing megaloschizont was found to contain a central body which could be identified as anything except a deep-staining, amorphous mass of chromatin-like material (Fig. 11). A transitional phase between these two stages of development is shown in Fig. 10. Indentation of the central body may have been started by multiplying cytomeres, but final compression would have to be accomplished through multiplication by the merozoite-like forms. The evidence very strongly supports the interpretation that continued elaboration of parasite substance may occur within the megaloschizont even after merozoites have been formed.

In speaking of compression, exertion of pressures is inferred. All indications of the existence of pressures are only apparent, not tested or proven. But inasmuch as these apparent stresses seem to play an important role in the final stages of schizogony in megaloschizonts they should be considered further.

During its early development the megaloschizont apparently expands its size rapidly until either the surrounding host tissues are displaced to the limits of their elasticity, or the host encapsulates it within a connective tissue membrane of sufficient strength to restrict any further enlargement. Once development within the schizont is confined to a limited space, continued elaboration of parasite substance seemingly builds up increasingly greater internal pressure which is exerted equally against the encompassing membrane and the enclosed central body. The central body is eventually crushed and fluid which it apparently contained becomes distributed throughout the schizont between primary groups of cytomeres or merozoites and next to the limiting membrane. The continued multiplication of merozoites displaces more and more of the less dense fluids which appear to be finally forced through the connective tissue membrane and out of the schizont. The resultant breakdown of the encapsulating membrane, though it may be only local,

probably releases considerable of the internal pressure of the schizont. This release of pressure could cause rupture of the very delicate membranes which appear to maintain primary-group organization within the schizont. Merozoites, thus released, become equally distributed throughout the schizont and are free to be carried into the tissues with the remaining fluid content. Also, with rupture of the limiting membrane, the elastic host tissues apparently press inward and continue to force out merozoites after internal pressure is no longer in effect.

REFERENCES

1. Coulston, F. & Huff, C. G. (1947). The morphology of cryptozoites and metacryptozoites of *Plasmodium relictum* and the relationship of these stages to parasitemia in canaries and pigeons. *J. Infectious Diseases*, **80**, 209-217.

2. Fallis, A. M., Davies, D. M. & Vickers, M. A. (1951). Life history of *Leucocytozoon simondi* Mathis and Leger in natural and experimental infections and blood changes produced in the avian host. *Can. J. Zool.*, **29**, 305-328.

3. Huff, C. G. (1942). Schizogony and gametocyte development in *Leucocytozoon simondi*, and comparisons with *Plasmodium* and *Haemoproteus*. *J. Infectious Diseases*, **71**, 18-32.

4. ——— (1951). Observations on the pre-erythrocytic stages of *Plasmodium relictum*, *Plasmodium cathemerium*, and *Plasmodium gallinaceum* in various birds. *J. Infectious Diseases*, **88**, 17-26.

5. Huff, C. G., Coulston, F., Laird, R. L. & Porter, R. J. (1947). Pre-erythrocytic development of *Plasmodium lophurae* in various hosts. *J. Infectious Diseases*, **81**, 7-13.

6. O'Roke, E. C. (1934). A malaria-like disease of ducks caused by *Leucocytozoon anatis* Wickware. *Univ. Michigan, School of Forestry and Conservation Bull.*, **4**, 44 pp.

7. Shortt, H. E. & Cooper, W. (1948). Staining of microscopical sections containing protozoal parasites by modification of McNamara's method. *Trans. Roy. Soc. Trop. Med. and Hyg.*, **41**, 427-428.

8. Wingstrand, K. G. (1947). On some haematozoa of Swedish birds with remarks on the schizogony of *Leucocytozoon sakharoffi*. *Kg. Svenska Vetenskapsakad. Handl. Ser.* 3, 24(5), 1-31.

J. PROTOZOOL., 2, 167-177 (1955).

# Cytological Studies on *Pelomyxa carolinensis* with Special Reference to the Mitochondria*

REUBEN TORCH†

*Department of Zoology, University of Vermont, Burlington, Vermont*

SUMMARY. The mitochondria of living, unstained *Pelomyxa carolinensis* are homogeneous, nonrefractile bodies and occur as spheres, short rods, dumbbell forms or elongated rods. All forms are distributed uniformly throughout the cytoplasm, except for characteristic accumulations around the contractile vacuoles and small food vacuoles. Mitochondria were never observed within food vacuoles, nor in intimate contact with large food vacuoles containing recently ingested paramecia.

Aqueous solutions (1:200,000) of Janus green B, Janus blue, and Janus red stained the mitochondria. After 12 hours in Janus green B and Janus blue or 24 hours in Janus red, the mitochondrion appears as a differentiated structure with the stain localized in eccentric granules or crescentic areas. Aqueous solutions of Janus black (1:200,000), amethyst violet (1:200,000) and Rhodamine B (1:50,000) stained only food vacuoles within the organisms.

Dumbbell forms and elongated rods, often interpreted as division stages, were continuously observed in the living organism. These observations failed to reveal any division process.

Sectioned material was prepared from animals fixed in Champy's, Regaud's, Altmann's and Helly's fluids, as well as in a fixative composed of equal parts of 1% (w/v) chromic acid and 2% (w/v) osmium tetroxide. Excellent results were obtained with Champy and chrom-osmic fixation, while the other fixatives proved to be of little value.

MOST OF OUR knowledge concerning the morphology and function of mitochondria has evolved primarily from the tremendous amount of work done on vertebrate material. Unfortunately, information about these inclusions in invertebrates is very incomplete.

The amoeboid Protozoa offer certain advantages that make them ideally suited as experimental animals for mitochondrial investigations. Of the various Sarcodina, the giant amoeba, *Pelomyxa carolinensis*, was chosen for this study for several reasons: (1) their cultivation is simple and requires very little attention, (2) their slow movement makes possible detailed observations of living animals, (3) their large size facilitates experimental techniques, and (4) their increased use as experimental animals in radiation, biochemical and physiological studies warrants an accurate description of their cytology.

The purpose of the present study has been to identify and to describe the mitochondria of *P. carolinensis* as a step toward the determination of their possible significance within the cell. An attempt has been made to define their physical characteristics as accurately as

* A contribution from the Department of Zoology, University of Illinois, Urbana, Illinois. This paper is based on a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

† The author wishes to acknowledge the helpful advice and constructive criticisms of Prof. R. R. Kudo, under whose direction this work was accomplished.