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A COMPARISON OF THE LIFE CYCLE
OF *CRITHIDIA* WITH THAT OF
TRYPANOSOMA IN THE IN-
VERTEBRATE HOST

A THESIS ACCEPTED IN PARTIAL SATISFACTION OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
AT THE UNIVERSITY OF CALIFORNIA

BY

IRENE AGNES McCULLOCH

1919⁶

A Comparison of the Morphology and the Life
Cycle of the Genus Crithidia with that of
the crithidial stages of Trypanosoma.

by

Irene Agnes McCulloch, A. B., M. A.

University of Kansas, 1913.

A thesis submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy

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INTRODUCTION

In my work on the flagellate parasites of hemipteran insects during the past five years the evidence indicating a close relationship between *Crithidia* and the crithidial stages of *Trypanosoma* has been continuously accumulating and has become more and more convincing. The relationship is shown both in their morphology and in the stages of their life cycles. This paper presents a comparison of the morphology and the life cycle of the genus *Crithidia* with that of *Trypanosoma* in its crithidial stages.

Before taking up this comparison a brief discussion of the phylogenetic relations of *Leptomonas*, *Herpetomonas*, *Crithidia*, and *Trypanosoma* will be of value in clarifying the subject. In addition a short discussion concerning the hosts, their food, and their methods of infection will give a fundamental conception of the problem in hand.

The phylogenetic relation of these intestinal flagellates, *Leptomonas*, *Herpetomonas*, and *Crithidia*, to the haemoflagellates, or *Trypanosoma*, has received the attention of many investigators and consequently has been the subject of much controversy. Only a brief statement of the historical side of this controversy need be given here. Minchin (1908) discusses the possible sources from which the trypanosomes may have been evolved, namely, from the herpetomonad-like and the trypanoplasma-like ancestors. At that time as well as now the evidence pointed to the crithidial (herpetomonad-like) forms as the true recapitulative, developmental, primitive stage in the life life cycle of *Trypanosoma*. Minchin (1912) stated:

The types denoted by the generic names, *Leptomonas*, *Crithidia* and *Trypanosoma* form a perfect evolutionary series with monogenetic parasites of invertebrates culminating in digenetic blood parasites. It must be emphasized, however, that any such conclusions are of a tentative nature and can have no finality but are liable to modification with every increase of knowledge concerning these organisms.

Wenyon (1913) also discusses the whole question of the phylogenetic relationship of *Leptomonas*, *Herpetomonas*, *Crithidia* and *Trypanosoma*. If the trypanosome be regarded as the highest stage of development then his conclusion is that the phylogenetic order of these flagellates would be: *Leptomonas*, *Crithidia*, *Herpetomonas*, and *Trypanosoma*. He makes a distinction between the genera *Lepto-*

monas and *Herpetomonas*. *Leptomonas* is a flagellate having the non-flagellated and herpetomonad form in its life cycle; *Herpetomonas* has these two stages, together with a crithidial and a trypaniform stage. But such a distinction has not been generally accepted. His chief reason for placing *Herpetomonas* next to *Trypanosoma* is the presence of a trypaniform phase in the life cycle of a few herpetomonad flagellates, as, for example, those found in *Musca domestica* and *Drosophila confusa* (Chatton and Leger, 1911).

From the viewpoint of the present study of these flagellates the hemipteran insect hosts have been frequently divided, for convenience, into the plant-feeding and the blood-sucking types. According to Patton and Cragg (1913) some species of the three families of plant-feeding Hemiptera, the Pentatomidae, the Lygaeidae, and the Coreidae have been found to serve as hosts for either crithidial or herpetomonad flagellates. One more family should now be added to this list, namely, the Pyrrhocoridae, to which the "lupine bug" (*Euryophthalmus convivus*) belongs. Among the blood-sucking Hemiptera some species of the family Reduviidae and the family Cimicidae are parasitized by haemoflagellates having a cycle in a vertebrate host and in some cases by other flagellates having only the invertebrate or insect host. Among the hosts discovered in the family Reduviidae we find *Triatoma* (= *Conorhinus*) *megista* Burm., the invertebrate host of *Schizotrypanum cruzi* (Chagas, 1909) and *Triatoma protracta* Uhler, the invertebrate host for *Trypanosoma triatomae* (Kofoid and McCulloch, 1916).

In addition to the hemipteran insects the Diptera (flies, sheep ticks, Siphonaptera (fleas), and Anapleura (lice) also serve as hosts for some of the herpetomonad, crithidial, and trypaniform flagellates. the rat-flea (*Ceratophyllus fasciatus*) is one of the invertebrate hosts of *Trypanosoma lewisi*. Frequent reference will be made to this flagellate in the comparison of *Crithidia* with *Trypanosoma*.

The methods of infection of the above hosts have been described as casual, "cross," and hereditary. Of these three methods Patton (1908) proved that nymphs of *Lygaeus militaris* became infected by ingesting with their food the feces of infected adults. The feces contained encysted or spore forms. Porter (1910) described two additional methods for the infection of sheep ticks, the "cross," and the hereditary. The former occurs among insects with cannibalistic habits, the contents and parasites of the digestive tract of one host individual being eaten by another. The hereditary infection has been

pathogenic to its vertebrate host, the rat. The invertebrate host of this haemoflagellate is normally the rat-flea, which transmits the flagellate from rat to rat. Both the vertebrate and the invertebrate host of *T. lewisi* are widespread, abundant, easily procurable, and adaptable to laboratory conditions. To make certain that none of the stages of the life history of the so-called natural flagellates should be confused with stages of the life history of *T. lewisi* a stock of uninfected fleas was procured for the breeding cages. The rat-fleas have frequently been found to be infected with *Leptomonas pattoni*.

At the time of the publication of the Minchin and Thomson paper I was working on the morphology and life history of *Crithidia euryophthalmi* (McCulloch, 1917), a form which occurs in the alimentary tract of *Euryophthalmus convivus*. This material was of great interest; the intracellular process of multiple fission was found, and the initial infective spores were relatively abundant. Previously some time had been spent in studying the morphology and life history of *Crithidia leptocoridis* (McCulloch, 1915), which infects the digestive tract of the box elder bug, *Leptocoris trivittatus*. It had also been pointed out in a superficial way that the individuals of various forms, shapes, and structures found in a typical infection of this crithidia, *C. leptocoridis*, were apparently analogous to many of the corresponding figures of *Schizotrypanum cruzi* (Chagas, 1909) in the invertebrate host. As the investigation of *C. euryophthalmi* and *C. leptocoridis* proceeded it became increasingly easy to link the life history of *Crithidia* with the life history of *Trypanosoma* in the invertebrate host. This was especially true of the life history of *T. lewisi*. In order to demonstrate clearly the essential facts of the life cycle of one of these important flagellates a brief outline of the life history of *T. lewisi* will be given, based upon the work of Minchin and Thomson.

The developmental cycle of *T. lewisi* in the flea is divided into two phases, characteristic of the parts of the digestive tract in which the trypanosomes are found, viz., the stomach and the rectal phase. The cycle in the rat-flea requires a minimum of five days for its complete course. The trypanosomes enter the stomach of the flea with the blood of an infected rat. They show the characteristic structure of a trypanosome, the "kinetonucleus," or parabasal body, being posterior to the nucleus and the undulating membrane well developed. The change in the medium is probably responsible for the physiological changes which result in the bodies becoming more cylindrical. The trypanosomes then penetrate the epithelial cells of the stomach and

undergo the process of multiple fission, a process which characterizes the stomach phase. The initial steps in this process after the trypanosome has entered an epithelial cell of the stomach, is the formation of a "tailed" sphere. This is a spherical structure with the flagellum and some cytoplasm protruding at one point. A little later the the "tailed" sphere becomes "tailless," and shows internally the formation of a variable number of daughter individuals. In the formation of these daughter individuals, the nucleus, parabasal body, and blepharoplast each divide an equal number of times, forming merozoites of a crithidiomorph type, i.e., a long, free type of trypanosome whose external movements are crithidial but whose structure still shows the parabasal body posterior to the nucleus. The crithidiomorph flagellates may do one of two things: enter other epithelial cells and undergo the process of multiple fission, or collect at the pyloric opening of the stomach, to be carried down through the intestine into the rectum with the food. The intestine ordinarily serves as a passageway for the parasites from the stomach to the rectum, but under certain conditions rectal forms may migrate forward and attach themselves to the wall of the intestine in the postpyloric region.

The rectal phase is established by the entrance of the crithidiomorph forms into this region of the digestive tract, and this region becomes the permanent source of infection throughout the life of the flea. During the migration from the stomach to the rectum structural changes take place in the crithidiomorph trypanosomes. The posterior end of the body becomes club-shaped, and this shifting of the cytoplasm assists in the forward movement of the parabasal body to a position anterior to the nucleus. Following this condition, binary fission brings about the production of the smaller, minute crithidias which characterize this phase of the life history. The established rectal phase is described as consisting of: (1) the attached or haptomonad form, which is the multiplicative stage of the rectal development; (2) the free or nectomonad form; and (3) the final trypanosome form which is instrumental in infecting another host.

The above outline of the life history deals only with the developmental series; but in both the stomach and rectum there are present individuals which show degeneration and belong consequently to the degenerative series. I have found that the life history of *Crithidia euryophthalmi* can be correlated advantageously with the life history of *T. lewisi* in the invertebrate host, the flea. The life history of *C. euryophthalmi* is not the life history of a haemoflagellate but

apparently that of a more primitive flagellate (pls. 2-6) found living in the medium of the fluid contents of the alimentary tract of a plant-feeding insect. As such, *C. euryophthalmi* (fig. A, 2) lacks in the stomach phase the trypaniform characteristics, namely, the blepharoplast (*bl.*), the parabasal (*pb.*) (or kinetonucleus) located posterior to the nucleus (*n.*), and the well developed undulating membrane. In this connection it is interesting to note that, regardless of the contrast in the initial stages of the stomach phase in each life cycle, the process of multiple fission characterizes the stomach phase for both the haemoflagellate and the more primitive crithidial flagellate. In the stomach phase of the crithidial parasite there are also found the "rounding up" forms (pl. 3, figs. 24, 25), the spheres (pl. 3, figs. 26-32), and the final stages of multiple fission wherein the resulting merozoites are about to escape from the epithelial cell of the host (pl. 3, fig. 39). In addition to this I found numerous stages of an endogenous process of multiple fission (pl. 2, figs. 11-23).

Correlation of the life histories of these forms (*C. euryophthalmi* and *T. lewisi*) became more difficult in the stage following the process of multiple fission, on account of the structure of the digestive tract of the host, *Euryophthalmus convivus*. The digestive tract of the flea (cf. Minchin and Thomson, 1915, text-fig. 1) shows the stomach as a prominent enlargement of the tube, followed by a comparatively long, slender intestine, at the posterior end of which is the rectal enlargement. The digestive tract of *Euryophthalmus convivus*, or lupine bug, on the other hand, is quite unlike that of the flea (cf. McCulloch, 1917, text-fig. 1). The first enlargement of the mid-gut proper is followed by a second and a third enlargement, a narrow constriction of the digestive tract separating the three expansions. Posterior to these there is a relatively long intestine which passes through the center of a ruffled, ribbon-like gland. The intestine opens into the slight enlargement near the entrance of the malpighian tubules, which in turn opens into the rectum. Since the several parts of the digestive tract of the hemipteran and other insects have not been satisfactorily homologized as yet and the nomenclature used in describing the divisions is confused, it is exceedingly difficult to ascertain the homology of these several parts of the digestive tract. However, as indicated in my preliminary paper (McCulloch, 1917), all three enlargements anterior to the intestine were considered as parts of the stomach proper and were accordingly designated as the "crop," mid-stomach, and pyloric expansion. With this disposal of the three enlargements of the digestive tract, the

question immediately arose as to whether or not all the crithidial infections of these regions are a part of the stomach phase of the life history of *C. euryophthalmi*. A careful study of the crithidial infection of each of these portions of the digestive tract together with many examinations of the contents of the intestine, the gland, and the rectum, convinced me that food conditions do not permit the parasites to establish a permanent rectal phase in the rectum but that they are forced to remain anterior to the intestine, in the pyloric expansion. Preparations of the gland and intestine have as yet shown no infection posteriorly, and the rectum has contained an occasional infection of spore forms only.

To add to the difficulty in determining the extent of the stomach phase and the beginning of the rectal phase no structural changes of a striking nature occur in the stomach phase of *C. euryophthalmi* as is the case in *T. lewisi*; nevertheless, the behavior of the crithidias is of some assistance and the study of the serial sections and stained smears of the several parts have added materially to our knowledge of the phases. Taking into consideration the evidence from these sources the crithidial infection of the "crop" is interpreted as the stomach phase. The mid-stomach serves as a temporary location for the slowly migrating forms of the stomach phase, and the pyloric expansion becomes the region of the permanent location of the "rectal" phase during the life of the lupine bug. The established "rectal" phase of the life history of *C. euryophthalmi* in the pyloric expansion has three general types of individuals, the attached, or haptomonad, the free, or nectomonad, and the final spore forms, which probably serve to infect another insect. Only the last type of parasite has been found in the normal contents of the rectum.

With these brief explanatory outlines of the life histories of *T. lewisi* and of *C. euryophthalmi* a detailed discussion of the more important points concerning the comparative morphology of the crithidial stages of *Trypanosoma* and of *Crithidia* will now be given. The comparison of the life cycles which follows is based upon the work of Minchin and Thomson on the life cycle of *T. lewisi*, and upon the life history of *C. euryophthalmi* with special reference to the accompanying plates (pls. 2-6). For further details of the life history of *T. lewisi* the reader is referred to Minchin and Thomson's paper (1915).

THE COMPARATIVE MORPHOLOGY OF *CRITHIDIA* AND
THE CRITHIDIAL STAGES OF *TRYPANOSOMA*

The morphology of *Crithidia* and *Trypanosoma* has been the subject of careful investigation for a number of years, and our conception of the structure of these simple organisms has been modified from time to time by additional discoveries. This is especially true of the extranuclear organelles of these flagellates, owing to the recent investigations carried on in the Zoological Laboratory of the University of California by Dr. C. A. Kofoed (1916) and Dr. Olive Swezy (1916), the latter studying particularly the binuclear theory of Hartmann (1911). The work of these investigators has centered attention upon the extranuclear organelles of these flagellates, consisting of the blepharoplast (fig. A, *bl.*) at the base of the flagellum, the parabasal body (*pb.*), or kinetonucleus, the rhizoplast (*rh.*), the parabasal rhizoplast (*pb. rh.*), the flagellum (*fl.*), and the undulating membrane (*und. m.*). They have homologized the kinetonucleus of the Protomonadina (*Herpetomonas*, *Crithidia*, and *Trypanosoma*) with the parabasal body of the Polymastigina and the Hypermastigina. Bearing this in mind, it at once becomes clear that this extranuclear complex of organelles is the neuromotor apparatus of *Crithidia* and *Trypanosoma* (Kofoed, 1916). In a previous paper (Kofoed and McCulloch, 1916) the term *parabasal body* was used in place of *kinetonucleus* throughout and I shall employ this nomenclature in the present paper.

The position of this extranuclear complex of organelles determines largely whether the flagellate is a trypanosome or a crithidia. The trypanosome is characterized by the presence of the parabasal body and the blepharoplast posterior to the nucleus, and by a well developed, undulating membrane which passes forward laterally along the edge of the ribbon-like body. These characteristics are common to the flagellates found in the blood, and modifications of this structure take place as soon as the medium is changed, as in the transfer to the stomach of the flea. The transition stages between a trypanosome and a crithidia have been designated as crithidiomorphic trypanosomes by Minchin and Thomson, as previously noted. In the transition forms the parabasal body and the blepharoplast are still posterior to the nucleus at a greater or less distance, but the movement and shape of the body of the flagellate are distinctly like those of a crithidia. In

the crithidial forms the extranuclear complex of organelles is anterior to the nucleus (fig. A, 1, 2) and the body has a tendency to be cylindrical in cross-section and to show only a slight undulating membrane (*und. m.*, fig. A). These crithidial forms are common to the life cycle of both *Crithidia* and *Trypanosoma*. The next step in our

Fig. A. Typical crithidial forms of: (1) *Crithidia leptocoridis*; (2) *C. euryophthalmi*; (3) *Trypanosoma lewisi* (after Minchin and Thomson, 1915, pl. 37, fig. 66); (4) *T. triatomae*; (5) *Schizotrypanum cruzi* (after Chagas, 1909, pl. 13, fig. 16); showing the similarity of the several flagellates in size, form and structure. $\times 3500$. *bl.*, blepharoplast; *fl.*, flagellum; *n.*, nucleus; *pb.*, parabasal body; *pb. rh.*, parabasal rhizoplast; *n. rh.*, nuclear rhizoplast; *und. m.*, undulating membrane; *vac.*, vacuole.

discussion will be directed to the demonstration of the close resemblance of the crithidial forms of the genus *Crithidia* to those of *Trypanosoma* in the invertebrate host.

A series of typical crithidial flagellates (fig. A) has been selected from the stomach phase of the life histories of both *Crithidia* and

Trypanosoma to show in detail the comparative morphology in this particular stage. *C. leptocoridis* (fig. A, 1) and *C. euryophthalmi* (fig. A, 2) have been selected to represent the structure of *Crithidia* and *Trypanosoma lewisi* (fig. A, 3, after Minchin and Thomson, 1915, pl. 37, fig. 66), *T. triatomae* (fig. A, 4), and *Schizotrypanum cruzi* (fig. A, 5, after Chagas, 1909, pl. 13, fig. 16) that of *Trypanosoma*.

Each crithidial flagellate of this series has an elongate body, cylindrical in outline but slightly flattened at the anterior portion, which forms the undulating membrane. At the edge of this membrane there is a sharply defined flagellum of variable length (fig. A, 1, 3). The length of the flagellum has no particular significance since considerable variation exists within each species. Posteriorly, however, there is in the stomach phase a consistent difference between the crithidial form of *Crithidia* and the crithidial form of *Trypanosoma*. The posterior ends of the bodies of the true crithidias are more attenuate (fig. A, 1, 2) in the stomach phase, and do not show the slight tendency to become club-shaped until the rectal phase is reached. In figure A, 4, *T. triatomae* is quite blunt at the posterior end, and the shifting of the cytoplasm into this region is increasing the width of the body at the expense of the length. Another difference almost as consistent as the one just noted is the variation in the nucleus (*n.*). In *Crithidia* (fig. A, 1, 2) it is usually anterior to the center, and in the crithidial forms of *Trypanosoma* it is posterior to the center (fig. A, 3, 4, 5).

Internally the similarity of the structure of the organelles and their relationship in general is quite marked. The nucleus (*n.*), the blepharoplast (*bl.*), and the parabasal body (*pb.*) are common to each of these flagellates. The nuclear rhizoplast (*rh.*) and the parabasal rhizoplast (*pb. rh.*) are found in all the above flagellates with the exception of *T. lewisi* (fig. A, 3). The absence of these organelles in the crithidial form of *T. lewisi* is questionable since they are present in all the others. They may have been overlooked because of the delicacy of their structure and the faintness with which they stain.

The nucleus and the extranuclear organelles will now be taken up in detail.

Nucleus.—This organelle in each of the above flagellates may be described as round or slightly oval in shape and of the vesicular type. It varies somewhat in size from 1μ in figure A, 3 to 1.7μ in figure A, 5, but it is usually about two-thirds of the width of the body in diameter. In *Crithidia leptocoridis* the nucleus shows clearly the ves-

icular type of structure, having a relatively large karyosome, a light area outside of this, and a clearly defined nuclear membrane. In figure A, 3 a slightly different condition exists; the nucleus of *C. euryophthalmi* is of the vesicular type, but the nuclear membrane is encrusted with considerable chromatin, and the central karyosome is somewhat reduced as compared with that of *C. leptocoridis* in figure A, 1. An examination of plate 5 indicates that the nucleus of this flagellate (*C. euryophthalmi*) is characterized by the heavily encrusted nuclear membrane, the small central karyosome, and the broad, clear area between the membrane and karyosome.

In the trypanosomes (fig. A, 3, 4, 5) the nucleus is slightly posterior to the center of the body, which has a tendency to be shorter and more club-shaped at the posterior end than is that of the crithidias (fig. A, 1, 2). The nucleus of *Trypanosoma lewisi* (fig. A, 3) shows a peculiar structure. In this particular form it is difficult to demonstrate the nuclear membrane, since the light area encircling the more deeply stained portion could be interpreted as either intranuclear or extranuclear. However, taking into consideration other figures of *T. lewisi* (cf. Minchin and Thomson, 1915, pls. 37, 42) this light area has been regarded as being extranuclear and the diameter is 1μ instead of 1.4μ . Within this relatively deep-staining nucleus the chromatin is divided unequally into a large, irregularly shaped granule and a smaller one. The nucleus of *T. triatomae* (fig. A, 4) resembles that of *C. euryophthalmi*. It is also characterized by the chromatin-encrusted nuclear membrane, the wide clear area, and the small central karyosome, as in *C. euryophthalmi*. A nucleus of a somewhat more complex type is found in *Schizotrypanum cruzi* (fig. A, 5). A faint nuclear membrane limits the clear nuclear space which surrounds the central karyosome containing a centriole at the base of the rhizoplast. This nucleus is 1.7μ in diameter and somewhat larger than that of any other flagellate in the series.

Blepharoplast.—This structure is of great interest since it is the center of the extranuclear organelles, or neuromotor apparatus. In the crithidias this structure is not a sharply defined basal granule at the base of the flagellum. For instance, in *Crithidia leptocoridis* a slight enlargement at the base of the flagellum is noted (fig. A, 1). Since this slight enlargement stains with almost the same intensity as does the flagellum it is exceedingly difficult to get a satisfactory conception of its structure. By careful focusing of the binocular microscope with Watson's no. 20 holoscopic oculars, however, the small enlarge-

ment of slightly greater staining intensity can be observed at the junction of the parabasal rhizoplast with the nuclear rhizoplast. In *C. euryophthalmi* (fig. A, 2) even greater difficulty is experienced in endeavoring to find the blepharoplast. In this form there is no enlargement of the base of the flagellum, but the area at the junction of the rhizoplast is darker in appearance, and frequently a small granule at one side can be seen by careful focusing.

Among the trypanosomes the blepharoplast is apparently a more clearly defined structure. Our investigations of *T. triatomae* (fig. A, 4) have yielded more tangible results. We found in this trypanosome a slight enlargement of the base of the flagellum but no differentiation in the staining capacity of the flagellum and basal granule. In *T. lewisi* (fig. A, 3) and in *Schizotrypanum cruzi* (fig. A, 5) the figures of these trypanosomes show what is evidently a well defined basal granule, or blepharoplast. This is particularly true of *T. lewisi* (fig. A, 3). In both the crithidias and the trypanosomes the blepharoplast frequently divides independently of the nucleus. In the binary fission of *C. euryophthalmi* the blepharoplast and parabasal body may divide before the nucleus divides (pl. 3, fig. 4), or the nucleus may divide first (pl. 3, fig. 33). In figure 25, plate 3, the nucleus is dividing but there are no indications of the division of blepharoplast and of the parabasal body. A similar condition exists among the trypanosomes, indicating that the blepharoplast is the kinetic center of the extranuclear organelles, or neuromotor apparatus. But this is not the only kinetic center of these simple organisms. The nucleus also contains a kinetic center which initiates division in the form of a centrosome, at the base of the nuclear rhizoplast in those flagellates containing this organelle. In the nucleus of *S. cruzi* (fig. A, 5) this centriole, or centrosome, at the base of the nuclear rhizoplast is clearly defined within the central karyosome of the nucleus. The origin of the blepharoplast is still the subject of investigation and as yet no conclusive evidence is at hand to establish beyond doubt the way in which this structure originates. In the endogenous buds, which are exceedingly small and difficult to interpret, the blepharoplast seemingly originates from the single nuclear structure as an outgrowth rather than by a mitotic process. The outgrowth forms a second deep-staining mass anterior to the nucleus, and at this stage the nonflagellated forms have the appearance of "binucleated" spores. As development proceeds the kinetic center of the blepharoplast is probably established as the center of the neuromotor apparatus, and

from this blepharoplast the flagellum grows anteriorly and the parabasal body to one side. Connecting the parabasal body with the centrosome, or blepharoplast, is the parabasal rhizoplast (*pb. rh.*). Parallel cases indicating such an origin of the blepharoplast can be found among other flagellates.

Parabasal body and rhizoplasts.—In all of these flagellates the parabasal body (*pb.*) presents the same general appearance with respect to its location, size and shape. In *C. leptocoridis* (fig. A, 1) the parabasal body is a bar-shaped structure made up of two deeply stained granules lying in close proximity. Immediately surrounding this deep-staining bar is a clear area, limited by a sacklike covering of dense cytoplasm, evidently continuous with the contractile cytoplasmic sheath of the flagellum. Between the parabasal body and the blepharoplast region the cytoplasmic sheath takes on the appearance of a cone-shaped mass of contractile fibers (*pb. rh.*), the apex of which is found at the base of the flagellum and continuous with the outer contractile cytoplasm of the flagellum. The axial or central part of this parabasal rhizoplast is presumably of blepharoplastic origin and connects the blepharoplast and parabasal body.

The parabasal body of *C. euryophthalmi* is similar to that of *C. leptocoridis*. It is not so large comparatively (fig. A, 2) and it seldom has the bilobed appearance. In *C. euryophthalmi* the most interesting and valuable evidence has been found concerning the relation of the parabasal body to the blepharoplast. In text figure B, 1–5 a series of flagellates has been drawn from good iron-haematoxylin preparations to show clearly that the position of the organelle is lateral and not axial. In figure B, 1 the end of the bar-shaped parabasal body, which lies lateral to the nuclear rhizoplast, extends outward to the periplast. From another angle the entire length of this organelle is visible (fig. B, 2) peripheral to the nuclear rhizoplast, which passes behind and underneath the parabasal rhizoplast. Still another aspect of the possible position of this structure is indicated in figures B, 3, 4. In each of these figures it is medial to the nuclear rhizoplast and behind or beneath it as it passes from the centrosome of the nucleus to the centrosome, or blepharoplast, at the base of the flagellum. In such a figure as B, 5 the relationship of this organelle to the others of the neuromotor apparatus can be seen most clearly. The cytoplasm has become exceedingly clear and does not conceal the other structure. The parabasal body in the flagellate stains very deeply and has the appearance of a chromotoidal mass suspended in

a sacklike structure from the region of the blepharoplast. Unlike the suspensory apparatus of *C. leptocoridis*, *C. euryophthalmi* does not show a fan-shaped mass of contractile cytoplasmic fibrils covering this axial portion, which is of blepharoplastic origin (figs. A, 1, 2; figs. B, 1-5). The outline of this cytoplasmic envelope in *C. euryophthalmi* is definite, slightly opaque, and continuous with the cytoplasmic sheath of the flagellum.

The parabasal body of the trypanosomes (figs. A, 3, 4, 5) is similarly a bar-shaped structure, which stains deeply, located to one side of the nuclear rhizoplast and blepharoplast. In *T. lewisi* (fig. A, 3) the parabasal body is relatively small while the blepharoplast is correspondingly large. As previously noted, no parabasal rhizoplast has been figured by Minchin and Thomson in *T. Lewisii*. Here and there suggestions of a connection might be pointed out in their figures. While it is possible that *T. lewisi* is exceptional in this respect, yet since the structure is found in other flagellates of this group, we may infer that a critical study of the preparations with a binocular microscope will reveal the presence of such a connection in the crithidial stages of this flagellate.

Trypanosoma lewisi (fig. A, 3) is likewise the only flagellate here figured in the crithidial stages without a nuclear rhizoplast. Here again *T. lewisi* is either exceptional or the structure has perhaps been overlooked, since it is usually discerned with difficulty. Chagas (1909) has figured a nuclear rhizoplast in *Schizotrypanum cruzi* and we have found it also in *T. triatomae*. Therefore we may expect that it will be found in *T. lewisi*.

The parabasal body in *Trypanosoma triatomae* (fig. A) is a relatively large structure, its width approximately equal to one-half of its length. It is located a short distance anterior to the nucleus, and is suspended from the blepharoplast by a fan-shaped parabasal rhizoplast like that of *Crithidia leptocoridis*, i.e., a suspensory apparatus with a fibrous appearance. In the crithidial stage of *T. triatomae* a nuclear rhizoplast was observed connecting the blepharoplast with the centriole of the karyosome, but such a connection was not found in the trypaniform individuals. If a nuclear rhizoplast be present in the trypaniform stage, in which we did not find it, it is possible that we may have been prevented from observing it because of the density of the cytoplasm in this stage and a tendency in this delicate thread in the trypanosomes to stain lightly. In the crithidial stages of this trypanosome, on the other hand, the cytoplasm is more or less vacuolated in appearance thus making the nuclear rhizoplast more evident. When a try

panosome rounds up for the process of multiple fission no nuclear rhizoplast is in evidence, but after the complete transition into the crithidial form the nuclear rhizoplast can be readily demonstrated with the high power binocular microscope and Watson's holoscopic eyepiece. It is also possible that further work will show that the centrosomic structure of the trypaniform flagellate differs from that of the crithidial form, and consequently that the rhizoplasts are absent in the former.

In *Schizotrypanum cruzi* (fig. A, 5) the parabasal body is slightly bilobed in appearance, and is suspended from the blepharoplast by a clearly defined, fibrous rhizoplast. The suspensory apparatus of the parabasal body is here relatively larger and apparently more highly developed than in any of the other flagellates. A few of the trypaniform stages also show a similar parabasal rhizoplast, according to the figures of Chagas (1909). In the crithidial stages of this trypanosome, as shown in figure A, 5, there is a sharply defined nuclear rhizoplast passing from the centriole of the nucleus to the blepharoplast. The position of the parabasal body in *S. cruzi* is similar to that of *Crithidia euryophthalmi* and is situated apparently to one side of the nuclear rhizoplast.

One of the most important discoveries in connection with the study of these organelles is the fact that the parabasal body, or the so-called kinetonucleus, is not axial in position in *Crithidia euryophthalmi* (figs. B, 1-4) and apparently not in the other flagellates (figs. A, 1, 3-5). With the evidence at hand to show definitely that this body is not axial but a lateral appendage of the blepharoplast in *C. euryophthalmi* it is necessary to look upon this organelle as something other than a second nucleus or a kinetonucleus. Reference has already been made to the work of Dr. Kofoed (1916) concerning the homology of this organelle with the parabasal body of other flagellates. The observations concerning the origin of this structure, the location and relation of the parabasal body to the other organelles as found in the study of *C. euryophthalmi* offer some of the best evidence against the binuclear conception of these flagellates (Hartmann, 1911).

Flagellum and undulating membrane.—Owing to the relationship which exists between the flagellum (*fl.*) and undulating membrane (*und. m.*) it is convenient to describe these two organelles together. The flagellum consists of an outgrowth from the blepharoplast and is surrounded by a cytoplasmic sheath which is continuous with the sack-like sheath covering the parabasal body. In the ordinary preparations no distinction can be made between this central portion originating

from the blepharoplast and the cytoplasmic sheath surrounding it. The entire flagellum stains as a single heavy line, almost chromatoidal in appearance. It does not stain so deeply as does the nucleus, but much deeper than the cytoplasm of the body and of the undulating membrane. As the flagellum forms and lengthens, there is an accompanying elongation of the protoplasm which forms the undulating membrane (*und. m.*). Both endoplasm and ectoplasm enter into the formation of this organelle. Usually there is a thin, narrow band of clear ectoplasm lying parallel to the flagellum (fig. A, 5). The length of the membrane, and consequently of the intracellular part of the flagellum, is greater among the crithidias of the stomach phase than of the rectal phase owing to the shifting of the cytoplasm of the body in the transition. This is usually true also of the crithidias as compared with the crithidial stages of the trypanosomes (fig. A, 1, 2, 3, 4). As previously noted, the length of the free flagellum (fig. A, 3) has no significance from the viewpoint of comparative morphology, since there is a wide variation in the length of this organelle for each species of *Crithidia* and of *Trypanosoma*.

THE LIFE CYCLE OF *CRITHIDIA EURYOPHTHALMI*

The life cycle of *C. euryophthalmi* in *Euryophthalmus convivus* begins, so far as known, with the casual ingestion with food of spores from the fecal matter of infected insects. *E. convivus* commonly feeds upon the sap from the growing tips of the lupine, which show many indications of excreta. In these same regions of the lupine galls and other abnormal growths occur in great abundance. The possibility that these insects were getting their infection of *C. euryophthalmi* from the sap of the lupine was suggested by the work of França (1914). Examinations of the sap of the lupine were accordingly made. Nematodes, numerous yeast-like spores, and bacteria were found. No organisms were discovered, however, of any description which could be linked to the known stages of *C. euryophthalmi* in the digestive tract of the host.

The large number of parasites in the life cycle of *C. euryophthalmi* can be grouped readily into two series: the developmental, or infective, and the degenerative, which are comparable to the developmental and degenerative series of *T. lewisi* in the flea, as described by Minchin and Thomson. That the correlation between these two life cycles is marked will become clear in following the discussion of the life cycle

of *C. euryophthalmi*, notwithstanding the fact that the initial stages of the two life cycles are entirely different in the insects. The initial stages of the crithidial life cycle are small, oval spores, which develop into elongate crithidial flagellates, whereas the initial stages of the trypanosome are trypanosomes from the blood of a rat, which indirectly by a process of multiple fission, produce very similar elongate crithidial flagellates. Subsequently all of the rectal stages in each life cycle are very similar.

Fig. B. Five figures of *Crithidia euryophthalmi* to show the relation of the parabasal body to the flagellum, blepharoplast, rhizoplast, and nucleus. $\times 3500$. Abbreviations same as in figure A.

The developmental or infective series of *Crithidia euryophthalmi* consists of the stomach phase or the crithidias of the "crop," mid-stomach and upper part of the pyloric expansion. The degenerative series includes the late rectal phase or the attached crithidias of the pyloric expansion.

THE DEVELOPMENTAL SERIES

STOMACH PHASE

The stomach phase of *Crithidia euryophthalmi* is apparently initiated when the small, oval spores (pl. 2, figs. 1, 2) casually ingested with food begin to develop in the "crop." Such initial infective spores develop rapidly into a swarm of multiplying crithidias (pl. 2, figs. 2-7). Later as mature flagellates (pl. 2, figs. 9, 10) the crithidias

may be carried immediately to the pyloric expansion through the mid-stomach by the current of food, or they may undergo a process of multiple fission extracellularly or intracellularly in the "crop." The process of multiple fission may be similar to that of the spheres of *Trypanosoma lewisi* (Minchin and Thomson, 1915), which have certain characteristics in common with the somatella in the multiple fission of some of the Polymastigina (trichomonad flagellates) described by Kofoid and Swezy (1915). In addition to this type of multiple fission there is a second and entirely different process of multiple fission, the internal or endogenous budding (pl. 2, figs. 11-23). These two processes of multiple fission have been described briefly in a preliminary communication (McCulloch, 1917). Endogenous budding has been emphasized in this paper and described in detail because of its interest and importance.

EXTRACELULAR CRITHIDIAS

Oval spores.—The initial infective spores (pl. 2, fig. 1) are ovoid and stain deeply. They are found in small numbers in the "crop," and present several distinguishing marks which serve as a means of identification. They average 1.7μ in width and about 3.4μ in length. The nucleus stains diffusely and forms a solid mass of chromatin at the extreme posterior end of the body. The parabasal body lies within the anterior half of the spore, about equally distant from the nucleus and forward end. One end of this bar-shaped structure, or parabasal body, lies close to the thick periplast. On all the other sides of this organelle there is the characteristic light area, which quickly destains in iron-haematoxylin preparations. Careful focusing has revealed a faint nuclear rhizoplast passing from the nucleus toward the region of the parabasal body.

Developing crithidias.—When the initial infective spores begin to develop in the "crop" a change occurs in their shape and staining capacity (pl. 2, fig. 9). The forward outgrowth of the flagellum assists in the elongation of the anterior end and the formation of the undulating membrane (pl. 2, figs. 6-9). The posterior region elongates less rapidly, but in time it frequently attains an even greater length than the anterior end (pl. 2, fig. 9). The length of the free flagellated crithidias which result from the developing forms varies greatly at all times regardless of their location in the digestive tract. In figure 9, plate 2, the crithidial flagellate has reached the extreme

length, comparatively, of 32μ . At the other extreme, a short crithidia of 7μ (pl. 2, fig. 12) is evidently mature, since it is undergoing a process of multiple fission of the endogenous-budding type. Between these extremes is a series of intergrading forms. The sizes and shapes of a swarm of free crithidial flagellates from the life cycle of anyone of these species of flagellates under discussion show great diversity, and *Crithidia euryophthalmi* is not an exception, as indicated in plates 2 and 4. The change in the appearance of the nucleus as development proceeds is noticeable. The deeply staining mass of chromatin (pl. 2, figs. 1, 2) becomes a nucleus containing a prominent central karyosome and a chromatin-encrusted nuclear membrane (pl. 2, figs. 3-10). Between the karyosome and membrane is a clear area, which destains very readily after iron-haematoxylin.

MULTIPLE FISSION

Endogenous budding.—In looking over a large number of preparations of the digestive tract of *Euryophthalmus convivus*, in the early part of 1916, I chanced upon a splendid preparation of a "crop" from a nymph which contained an exceedingly heavy infection of crithidias of all shapes and sizes. The smear was well fixed and well stained.

Among other things the nuclear structure was studied in detail to determine whether the nucleus of these crithidias divided by a mitotic process or by a more primitive method of mitosis. This search led to the discovery of a flagellate which apparently contained two nuclei (pl. 2, fig. 12), in a linear arrangement with respect to the long axis of the body. Shortly after another crithidia (pl. 2, fig. 13) was found in the same preparation, containing apparently three similar nuclei, which were arranged in a like linear series. In the latter (pl. 2, fig. 13) careful focusing revealed the outline of a fourth partially concealed beneath the most anterior nuclear structure. In each of these flagellates (pl. 2, figs. 12, 13) no indications of any ordinary process of binary fission were detected. The blepharoplast, parabasal body, and the rhizoplasts in each were still intact in so far as could be determined. The alternative hypothesis that these nucleus-like structures, which are relatively small, are internal parasites of a bacterial or protozoan nature naturally was given full consideration. Drawings of these flagellates were made with the camera lucida and the readings were taken for future reference. Owing to the abun-

dance of the parasites in this preparation of the "crop" ample material was at hand for an extensive study of the morphology of this phase of the life cycle of *Crithidia euryophthalmi*. From time to time more flagellates were observed, apparently multinucleated, but little additional light was thrown upon their peculiar nuclear structures until a large flagellate, such as shown in figure 22, plate 1, was observed. This crithidia was relatively large and contained approximately twelve "binucleated" spore-like forms. The structure of the sporelike forms within the periplast of the large flagellate was similar to that of the numerous small, oval spores in the immediately surrounding field, which were, beyond doubt, stages of the life cycle of *C. euryophthalmi*. Another large flagellate containing six nuclear structures within the periplast (pl. 2, fig. 21) was also drawn. The flagellum and parabasal body were clearly outlined, as in the former, multinucleated flagellates. Some of the enclosed nuclear-like structures were deeply stained, owing to the thickness of the preparation, but the majority presented the same appearance as did the nuclear-like structures in figures 12 and 13.

Investigation of the smear revealed more and more evidence of a possible endogenous, or internal budding, in the life cycle of *Crithidia euryophthalmi*. Past experience indicated that the preparations of the "crops" of young nymphs furnished the best smears for the study of the initial infections, of which the endogenous budding forms were evidently a part. An effort was accordingly made during the next breeding season of *Euryophthalmus convivus* to collect as many young nymphs as possible in order to obtain additional preparations of the digestive tract, with reference to the "crop" in particular.

Out of a large number of preparations of the "crops" prepared during the following season, only two contained additional stages of the process of endogenous budding. The percentage of infection of the "crops" of young nymphs was found to be approximately twenty per cent as compared with two per cent among adult insects.

In the two preparations there were numerous small, "binucleated," spore-like forms, grouped near discarded flagella, with or without blepharoplasts, and with parabasal bodies still attached (pl. 2, fig. 23). It was easy to conceive of the degeneration of the cytoplasm surrounding the spore-like forms, leaving a field covered with the internal spores, or zooids, and the extranuclear organelles of the parent cell. In the earlier stages of degeneration presumably the parabasal bodies were still attached by the parabasal rhizoplasts to the blepharoplasts

at the bases of the flagella. Later stages showed only flagella among the zooids. This additional evidence from the two smears at once suggested the work of Moore and Breinl (1907), in which "latent bodies" were described in the life cycle of a haemoflagellate, *Trypanosoma gambiense*.

According to these investigators *T. gambiense* in the blood of a vertebrate host formed latent bodies in the nuclear region, under certain conditions. These latent bodies are pictured and described as being small nucleated structures having a nuclear membrane and a single mass of chromatin in the center. The exact method of the formation of the bodies in the nucleus or from the nucleus is not clear, since none of the early stages in the formation of the latent bodies are figured or described in detail. Apparently only the results of an endogenous process in the life cycle of *T. gambiense* were observed by these investigators. The final stages of the process in the life cycle of *T. gambiense* are the presence of large, degenerating trypanosomes with minute, nucleated latent bodies in the nuclear area. The flagellum, kinetonucleus, or parabasal body and cytoplasm are in various stages of degeneration. Their figures of the latent bodies (Moore and Breinl, 1907) should be compared with figure 23 of this paper.

The latent bodies in the life cycle of *Trypanosoma gambiense* and the endogenous zooids of the life cycle of *Crithidia euryophthalmi* are not the only instances of an endogenous budding in the life cycle of the Protomonadina (*Trypanosoma*, *Crithidia*, *Herpetomonas*, *Leptomonas*). Minchin and Thomson (1915) searched for evidence of an endogenous budding in the life cycle of *T. lewisi* but failed to find any indications of the process. However, in the life cycle of *Leptomonas pattoni*, one of the so-called natural flagellates frequently found in the digestive tract of the flea, they found several crithidial-like flagellates containing a nucleus and an endogenous bud. These authors suggested that the endogenous buds found therein were doubtless comparable to the latent bodies of Moore and Breinl.

The early stages of the endogenous, or internal budding of *Crithidia euryophthalmi* are shown in figures 11 and 12, plate 2. In figure 11 there is a relatively short flagellate undergoing multiple fission in this way. The nucleus has budded off two circular, nucleus-like buds, each showing a chromatin-encrusted nuclear membrane. The parabasal body and the flagellum of this flagellate are still intact and have no indications of binary fission. Somewhat similar to this flagellate

is the one shown in figure 12, plate 2. The anterior end of the body is slightly shortened but the nuclear structure has the same general appearance. Only one bud has been given off, and this lies directly posterior to the nucleus proper. As in the former case, the chromatin is collected on the inner surface of the nuclear membrane of both the nucleus and endogenous bud. In figure 13, plate 2, a more complex organization was observed. The nucleus and two clearly defined nuclear buds are arranged in a linear series, the two buds being posterior to the nucleus proper. Partially concealed by the nucleus is a third bud, which is being constricted off from the nucleus. This particular view of the process is in all probability an end view and only a portion of the bud is visible. If the observations and interpretation of this structure be correct, the nucleus, with all of its chromatin collected on the inner surface of the membrane, repeatedly constricts or buds off a portion, forming a series of nuclear buds (pl. 2, figs. 13, 20, 21). There is at hand at present no evidence of a central karyosome being present in the nucleus when endogenous buds are formed. The nuclear buds, therefore, in so far as our observations have gone, are due neither to a clearly defined amitosis nor to a primitive form of mitosis of the chromatin material which normally occurs in a central karyosome.

Although evidence of a mitotic process in this nuclear division is wanting, nevertheless some evidence of a promitosis has been found in the division of the nucleus in an early stage of the formation of a somatella (pl. 3, fig. 25). The formation of the somatella involving the "rounding up" of an elongate flagellate into a sphere will be described shortly; it will suffice for our purpose here to point out salient features of the internal structure of the "rounding up" flagellate (pl. 3, fig. 25). The blepharoplast, parabasal body, and the rhizoplast of this flagellate have not yet begun to divide but the nucleus is beginning to form two daughter-nuclei. The central karyosome which is normally present in *C. euryophthalmi* has formed two unequal masses of chromatin connected by a centrodosome. No critical evidence was found showing that there is present at each end of the centrodosome a centriole or centrosome differentiated from the chromatin material in this minute form. Peripherally there is the nuclear membrane still present and intact, but it is constricting in the center to form two nuclei. If a similar division of the chromatin material occurs in the nuclear budding it has thus far escaped observation. The number of early stages of nuclear budding studied has been small, owing to the rarity of

preparations showing the process. In every instance of nuclear budding so far observed the chromatin has been peripheral, with a more or less unequal distribution on the nuclear membrane. Frequently there is one heavy mass with a uniform amount elsewhere on the membrane (pl. 2, fig. 15).

A somewhat larger, elongate flagellate, with sharply defined endogenous buds, is shown in figure 14, plate 2. Unlike the endogenous forms just described the buds here are not posterior to the nucleus. In figure 14, opposite the parabasal body, there is a bud decidedly anterior and lateral in position. A second endogenous bud is also anterior between the nucleus and parabasal body. Within all of these nuclear structures the chromatin is massed irregularly upon the nuclear membrane.

In addition to these elongate flagellates undergoing multiple fission of this type there are also pear-shaped crithidias (pl. 2, figs. 15-18) containing several or numerous endogenous buds. Possibly it is the greater thickness of these flagellates, with consequent decrease in destaining capacity, which makes the buds within stand out so distinctly. Another marked characteristic of the pear-shaped flagellates is the lack of differentiation between the nucleus and the buds. There is no evidence of a parent nucleus which has given rise to the buds. The distribution of the chromatin within these buds presents some interesting variations. In figure 15 the buds contain a clearly defined chromatin encrusted nuclear membrane together with one distinct mass or chromatin granule. In figure 17 the massing of the chromatin may mean a more advanced stage since it is no longer on the entire nuclear membrane but has been segregated into two masses, which give the buds a bipartite appearance. Proceeding to figure 18 in this pear-shaped individual more steps in advance are portrayed, namely the unused portions of the flagellate are beginning to degenerate around the endogenous buds. The parabasal body has already disappeared, and only a portion of the discarded flagellum remains near by. The structure of the endogenous buds of this spherical crithidia is remarkably uniform. In each bud the chromatin granule is adherent to the nuclear membrane.

Other elongate flagellates undergoing multiple fission are observed in figures 19, 20, and 21. Some interesting variations can be pointed out in these crithidias. In figure 19 there is a nuclear rhizoplast, which can be traced from the edge of the nuclear membrane to the blepharoplast. Of greater interest are the variations shown in figures

20 and 21. In figure 20 the binucleated appearance of the nucleus and buds is very prominent. The chromatin granules have a paired effect which is difficult to interpret. In figure 21 a more complex structure is represented. Anterior to the nucleus there is a group of five endogenous buds. Two of these, owing to their position, are not destained sufficiently to make clear their nuclear structure. This large flagellate has a sharply defined parabasal body and flagellum, similar to those in figures 19 and 20. The large size of such forms has aided materially in the interpretation of the endogenous buds, which, upon developing, form the so-called zooids.

The most mature stage of endogenous budding yet found, wherein the resulting zooids are still within the periplast, is pictured in figure 22. There are approximately twelve clearly defined zooids massed near the central part of this large flagellate. The structure of the zooids could be studied readily. The nucleus and parabasal body in each, because of their deep stain, helped to distinguish the several zooids. The other organelles were not visible. Another interesting feature concerning these zooids is the difference in the stages of their development. Some are larger and more mature and were doubtless budden off from the nucleus first. Probably the zooids of the flagellate have been retained within the periplast of the parent for a longer period than usual. If the size of these zooids be compared with that of the free zooids shown in figure 23 the former, on the whole, are larger and more fully developed. There are, moreover, no marked signs of degeneration of the parabasal body, of the flagellum, or of the cytoplasm. The parabasal body is almost hidden by the zooids in that region.

The final step in the endogenous-budding process comes with the degeneration of the body-plasm of the parent flagellate, leaving a mass of zooids, together with the flagellum and parabasal body of the parent. Portions of smears have been observed to be literally covered with zooids and discarded flagella. As degeneration proceeds the parabasal bodies next disappear, and finally the flagella, leaving only numerous zooids of various sizes and structure. The last phase is the one most frequently observed in preparations of *Crithidia euryophthalmi*. I had been working with *C. euryophthalmi* more or less for a period of almost two years before the clue as to the origin of these small zooids was found. It had been most puzzling to find so many of these small, non-flagellated, binucleated forms (pl. 4, fig. 40), which were obviously unlike the initial infective spores (pl. 2, figs. 1, 2). They

were smaller in size, their periplast was thinner, and they destained much more rapidly after iron-haemotoxylin. Serial sections of the mid-stomach showed them grouped in pockets between the epithelial cells. Possibly the endogenous process occurred there or they were lodged there as the current of food carried the others down into the pyloric expansion. They could be arranged in a series, beginning with those averaging about 1.7μ in length (pl. 4, fig. 40) and gradating to the size of the spores which were regarded as the initial infective spores (pl. 2, fig. 1; pl. 4, figs. 40-54).

The development of the endogenous buds into binucleated zooids is not easy to interpret. In figure 23, plate 2, the numerous zooids present variations in both size and structure. Unlike the latent bodies of *Trypanosoma gambiense* of Moore and Breinl (1907) and the endogenous buds of *Leptomonas pattoni* of Minchin and Thomson (1915), these endogenous buds show no central chromatin granule within the nuclear membrane. Their chromatin is distributed at the periphery of the nucleus usually in one of three ways. In the elongate flagellates (pl. 2, figs. 11, 14, 19) the buds have their chromatin material massed irregularly on the nuclear membrane. A second form of peripheral chromatin in the nucleus is a noticeable mass or granule at one point. This is characteristic of the buds within the pear-shaped flagellates (pl. 2, figs. 15-18). The third type of nuclear structure in the buds is possibly only a slight modification of the others. In figure 20, plate 2, the internal or endogenous buds appear to be binucleated because of the peculiar segregation of the chromatin material on the nuclear membrane. At present these several modifications of the nuclear structure are not regarded as having any special sequence or significance. In some of the smallest zooids of figure 23 there are still other modifications wherein a single, deeply staining mass is frequently observed. Owing to the size of these zooids it is extremely difficult, even with a binocular microscope, to form any adequate conception of their structure.

A diligent search has been made among these small forms to find a series of developing zooids which would clearly show the whole process of the formation of the several organelles. While *Crithidia euryophthalmi* undoubtedly furnishes the necessary material for such a study, the size of the endogenous buds makes the interpretation exceedingly difficult. It is therefore disappointing that a complete series has not yet been accumulated showing the steps which are thought to take place in the development of a bud into a typical

erithidia. The results of the study now indicate that the extranuclear organelles are apparently formed as outgrowths from the nucleus. It is conceived that the centrosome of the nucleus divides, giving rise to the extranuclear centrosome, or blepharoplast. From the blepharoplast, which is the dynamic center of the nuclear outgrowth, two other organelles are formed. The flagellum grows forward anteriorly and the parabasal body to the side. The connection which persists between the blepharoplast and the nucleus is the nuclear rhizoplast. Between the blepharoplast and the parabasal body is the parabasal rhizoplast. Careful observation of some of the larger forms reveals a cytoplasmic sheath around the flagellum, which is continuous with a similar sheath around the parabasal body. The latter sheath is like a sack, in which the deeply staining, bar-shaped parabasal body is suspended. The variation in size of the parabasal body may explain the light area which is frequently observed to surround this organelle. The fan-shaped appearance of the parabasal rhizoplast is due in all probability to this sacklike sheath. Any general conclusions concerning the origin of these organelles would, at this time however, be premature, although the observations of the zooids under the binocular microscope tend to give this conception of their origin. It is necessary to keep in mind constantly the fact that the material with which we are dealing is the complex life cycle of a flagellate and that several stages of the life cycle have not yet been followed, step by step, in the living material. The possibility of confusing two life cycles is always present, and the fact that all of the work is done near the limits of microscopical magnification adds further possibility of misconception.

Aside from these difficulties and doubtful points, however, the discovery of all of these stages of what has been interpreted as a process of endogenous budding, opens up further problems for investigation in the life cycles of these flagellates. The origin of the parabasal body in the endogenous bud is a big problem in itself. In addition, the light thrown upon the probable origin of the numerous binucleated spores or *Leishmania*-like bodies, which occur so abundantly in the life cycles of such flagellates as *Schizotrypanum cruzi* (Chagas, 1910), *Crithidia melophagia* (Porter, 1910), and *C. leptocoridis* (McCulloch, 1915), is very suggestive.

Somatella.—Previous to the discovery of the multinucleated flagellates which were undergoing a process of internal or endogenous budding, another type of multiple fission had been studied in prepara-

tions of the "crop" of *Euryophthalmus convivus*, namely, that which leads to the formation of the somatella (pl. 3, figs. 28-32). These spherical crithidias have certain characteristics in common with the tailed and tailless spheres described in the life cycle of *Trypanosoma lewisi* by Minchin and Thomson (1915). In a general way they resemble also the somatellas in the life cycle of some of the Polymastigina described by Kofoed and Swezy (1915). Beginning with the earliest stages of this type of multiple fission a series of flagellates can be arranged which parallels the successive stages of multiple fission of *T. lewisi* (cf. Minchin and Thomson, 1915, pl. 37). At the beginning of this series such flagellates as shown in figures 24 and 25 of plate 3 are to be found. In figure 24 the elongate flagellate is beginning to round up. The attenuate ends are being drawn up to the central part of the body and the flagellum has become intracellular throughout its length. A somewhat different formation of the sphere is shown in figure 25, plate 3. The long, attenuate ends are being wrapped about the body and the flagellum is likewise entirely intracellular in this flagellate. Other important features to be observed in connection with this flagellate are the first indications of nuclear division in the spherical formation. The centriole or centrosome within the nucleus of the rounding-up flagellate is initiating the division of the organelles. It has divided into two daughter-centrosomes, which are still connected by a centrodesmose, and with each daughter-centrosome there is present a varying amount of chromatin material. No chromosomes are present at this stage of the division, and their presence at any period throughout the process in *Crithidia euryophthalmi* has not been established. Indications of chromosomes in the ordinary binary fission have been observed, but no definite number has been determined. The nuclear membrane has begun to constrict on either side of the centrodesmose. The entire division of the chromatin material takes place within the nuclear membrane, and the process is apparently a primitive type of promitosis. The centrosome within the karyosome is not always the first to divide; on the contrary, in many instances the first indication of the division of the organelles is shown by the blepharoplast or extranuclear centrosome. When the blepharoplast divides the division of the parabasal body follows immediately. A repetition of the division of the several organelles occurs and the spheres finally break up into a number of merozoites, or daughter individuals.

The spherical formation is completed in figure 26, plate 3. A flagellum is protruding beyond the surface of the sphere. Internally

division of the blepharoplast, the parabasal body, and the nucleus has occurred. A second flagellum growing out from the daughter-blepharoplast cannot be observed. Another more advanced stage of multiple fission is shown in the somatella in figure 27. The number of nuclei and of the parabasal bodies is the same as in figure 26, but the outgrowths of the daughter-flagella are clearly shown in this sphere. Spheres without protruding flagella are to be found in figures 28 and 29, plate 3. The formation of the merozoites within the spheres, as presented in figure 29, has advanced to the point where they are clearly defined. In figure 28 another important observation can be made, namely, that the divisions of the blepharoplast and the nucleus do not occur simultaneously. In this particular sphere there are three parabasal bodies present but only two nuclei. In figure 29 there are four merozoites visible, each of which shows the outgrowth of a flagellum, and a similar spherical formation is shown in figure 30, wherein the four merozoites are somewhat larger and more developed. In the latter somatella, however, the thickness of the sphere prevented the usual amount of destaining necessary to show the nuclear structure. In each of these merozoites the body is elongating and the anterior end is becoming attenuate.

Another thick sphere is found in figure 31, and all the nuclei therein have the appearance of being a solid mass of chromatin. In this somatella the number of merozoites which could be counted is twelve. The irregular outline of the sphere indicates that the breaking up or plasmotomy of the sphere is about to occur. Possibly some of the merozoites have already escaped. In the investigation thus far the number of merozoites in a somatella has been exceedingly variable. In some of the Polymastigina, Kofoid and Swezy (1915) found the number of merozoites to be eight, which is apparently constant for the somatellas of these flagellates. Minchin and Thomson (1915) report a variable number of merozoites in the spheres of *Trypanosoma lewisi* but they found the average number to be approximately ten. In the spherical mass of flagellates shown in figure 31, plate 3, the number is twelve, but in a still larger sphere (pl. 3, fig. 32) the number is probably double that, or twenty-four. The denseness of the latter may have obscured some of the parabasal bodies and nuclei. Protruding from the surface of this sphere are numerous flagella which are outgrowths from the daughter-blepharoplasts. In the somatellas of the Polymastigina the nucleus and the extranuclear organelles may divide simultaneously, but in the spheres of *T. lewisi*

and in the somatella of *Crithidia euryophthalmi* either the extra-nuclear or the nuclear organelles may divide first. The ultimate number of nuclei is equal to the number of parabasal bodies and of the blepharoplasts.

The stage of development of the merozoites when plasmotomy occurs, is variable. Usually the rupture of the somatella occurs when the merozoites are just beginning to elongate, as in figure 31. All the organelles of these merozoites are definitely outlined, and the flagella are free for a certain distance of their length. In a few instances extremely elongate flagellates have been observed wriggling about within the spherical structures. There is no evidence of a residual mass of cytoplasm.

A comparison of these two methods of multiple fission in the life cycle of *Crithidia euryophthalmi* reveals the fact that they are fundamentally different. In the first place, the former method, endogenous budding, involves only one organelle of the crithidia, namely, the nucleus. In the latter method a somatella results from a repeated division of the nucleus, blepharoplast, parabasal body, and, in all probability, the nuclear and parabasal rhizoplasts. The flagella, however, in each case are new outgrowths from the newly formed blepharoplasts in endogenous buds or from the daughter-blepharoplasts in the somatella. Secondly, the flagellates undergoing endogenous budding retain their normal shape as elongate or pear-shaped crithidias. In some instances their size increases as the buds develop. The flagellates forming somatellas round up into spheres. The comparative efficiency of the two processes from the standpoint of the multiplicative phase in the host cannot be estimated. The length of time necessary to complete each process and the conditions under which each occurs are at present unknown. On the whole, the number of individuals resulting from an equal number of endogenous flagellates or of somatellas is approximately the same. Our conclusion, however, concerning the two methods is that the endogenous budding is of greater importance in the life cycle of *C. euryophthalmi* since in the preparations there are relatively many more evidences of the endogenous budding than of the somatella.

BINARY FISSION

Another method of multiplication of *Crithidia euryophthalmi* in the "crop" is binary fission. This process has also been observed in the pyloric expansion. While the number of crithidias found dividing

in this way is relatively small, on the whole, yet crithidias of almost any stage of development apparently may thus increase their number. Some conception of the prevalence of the process throughout the life cycle may be gained by studying figures 33 to 37. In addition to these smaller crithidias in various stages of development many instances of binary fission have been observed among the elongate or mature flagellates.

The blepharoplast or the centrosome of the nucleus may initiate the division of the several organelles. In figure 33, plate 3, the centrosome of the nucleus is in the process of binary fission and there are present two daughter-nuclei. In each of the nuclei the chromatin is peripheral on the nuclear membrane. The blepharoplast and the parabasal body have not yet begun to divide. The general appearance of this binary fission form is very similar to that of the smaller somatella. In the unflagellated crithidia shown in figure 34, plate 3, the blepharoplast and parabasal body have divided but the nucleus is still intact. A more advanced stage of binary fission is found in figure 35. In this small, unflagellated crithidia the blepharoplast, parabasal body, and the nucleus have divided, and a light, thin area, which is preliminary to the cleft in the cytoplasm, extends longitudinally between the two sets of organelles. The flagella can also be observed. The longer, more clearly defined flagellum is evidently that of the parent since the second is shorter and less distinct. The last stages of binary fission are shown in figures 36 and 37. In figure 36 the cleft in the cytoplasm can be traced from the anterior to the posterior end, and the daughter flagellum has also attained a greater length. The two flagella in figure 37 are the same length; the separation of the two daughter individuals is more marked and almost complete in figure 38. The posterior ends are the last to remain attached and the lashing about of the anterior ends assists efficiently in the final tearing apart.

One of the most interesting problems in connection with this method of reproduction has always been the origin of the daughter-flagellum. Is it due to the division of the flagellum of the parent or to a new outgrowth from the daughter-blepharoplast? A review of the work already done on this genus indicates that with the exception of Porter (1909, 1910) all authors regard the daughter-flagellum as a new outgrowth and consider that the parent-flagellum does not split to form two daughter flagella. My work on *Crithidia leptocoridis* and *C. euryophthalmi*, unlike that of Porter, is in agreement with

the results of other investigators. For some time the evidence of the new outgrowth was difficult to obtain (McCulloch, 1915), but very clear evidence of such an outgrowth from the blepharoplast has finally been discovered. Text-figure C gives a very clear picture of the origin of the daughter-flagellum in *C. leptocoridis*. The larger size and the greater abundance of crithidias undergoing this process make *C. leptocoridis* the most desirable material to illustrate this point.

The importance of binary fission in increasing the number of parasites in the multiplicative phase in the "crop" is not great. At the present time no evidence of the process has been found in the mid-stomach, which serves chiefly as a passage way for the crithidias mi-

Fig. C. Flagellate stage of *Crithidia leptocoridis* to show the outgrowth of the new flagellum in binary fission. $\times 3500$. Abbreviations the same as in figure A.

grating from the "crop" to the pyloric expansion. In the pyloric portion of the digestive tract, however, binary fission is of great importance in increasing the number of crithidias, which attach themselves to the epithelial lining of the pyloric expansion.

In connection with the process of binary fission in *Crithidia euryophthalmi* another problem of interest has occurred, namely, the nature of the difference, if there be any, between the division of the several organelles in binary fission and the division of these same organelles in the somatella. Minchin and Thomson (1915) have regarded the increase in the number of nuclei and kinetonuclei in the spheres of *Try-*

panosoma lewisi as being due to a repeated binary fission wherein the resulting individuals failed to separate immediately. Kofoed and Swezy (1915) have described in detail the process of binary fission and of multiple fission for the trichomonad flagellates, and these authors found the increase of organelles in multiple fission to be due to thrice repeated mitosis. The larger size of those flagellates together with the correspondingly increased size of the organelles presents better material for the study of binary and multiple fission than do the trypanosomes or the crithidias. In *C. euryophthalmi* the minute size of the flagellates undergoing either process have made accurate interpretation thus far impossible. For this reason the relation of binary fission to multiple fission in a somatella must remain an open question for the present.

INTRACELLULAR CRITHIDIAS

Another salient similarity between the life cycle of *Crithidia euryophthalmi* and the life cycle of *Trypanosoma lewisi* in the invertebrate host is the appearance in each life cycle of a stage of intracellular multiple fission. In *C. euryophthalmi* there is figured for the first time an epithelial cell in the life cycle of a crithidia taken from a "crop" containing numerous crithidial parasites. Careful examination of this infected cell shows that there are three distinct groups (pl. 3, fig. 39, *a*, *b*, *c*) of parasites and a number of scattered crithidias. Altogether there are approximately seventy parasites in this one epithelial cell. In figure 39*a* the crithidias are of two sizes: long, slender flagellates, and short, non-flagellated forms. Since multiple fission of either the endogenous or somatella type produces zooids or merozoites of approximately the same size, it appears that several infections have occurred in this cell. It is conceivable that all the elongate flagellates are due to one infection while the short, non-flagellated crithidias are due to a second infection. In figure 39*b* there are ten small oval forms. In structure they show a diffuse nucleus, as do the other parasites within this host-cell, which is probably due in part to the thickness of the cell. A nuclear rhizoplast can be observed passing from the nucleus to the blepharoplast, and a short intracellular flagellum extends forward to the anterior end of the body. The parabasal body of each is a relatively small and deeply staining structure. At *c* and *e* of figure 39 are more of the small oval forms. In the former region the cytoplasm of the host-cell has been

completely destroyed within a certain radius of the parasites. In the latter the small forms are scattered in the cytoplasm of the host-cell. At *d* there is still another group of elongate flagellates, which are probably the results of another process of multiple fission. They are approximately the same size and shape, and are in the same stage of development. Whether they are merozoites from a somatella or zooids from endogenous buds is not clear, but they have broken apart somewhat and are making their way to the periphery of the host-cell. At *f* a flagellate is protruding through the cell wall and the posterior end is directed forward to penetrate the tissue. In the study of the living material of both *C. leptocoridis* and *C. euryophthalmi* crithidias have been observed to direct their posterior ends forward and to use the flagellated ends as propellers in penetrating tissues.

The early stages of the intracellular multiple fission have not yet been found within the epithelial cells of the crop and the more advanced stages, such as shown in figure 39, do not indicate definitely the method of multiple fission. These intracellular crithidias had been described as the results of multiple fission within somatellas before the discovery of the stages of the endogenous process of multiple fission in this life cycle. The evidence is not absolutely convincing either way, but there is at the present state of the investigation a preponderance of evidence in favor of their probable origin by the plasmotomy of a somatella. The circular outlines of the vacuoles in which the groups of parasites are found in figure 39 suggest the formation of the spheroidal somatella. Further there are no evidences of discarded flagella within these cells which suggests the possibility that endogenous budding might have occurred with the formation of circular cavities in the cytoplasm of the host-cell. On the other hand, if the numerous small oval spores are due to a process of multiple fission within a somatella the subsequent plasmotomy has taken place very early. Usually the breaking apart of the merozoites does not occur until they have become elongate flagellates. Moreover, the exact method of the process of intracellular multiple fission is not so important as the fact that under certain conditions crithidias become intracellular and destroy the epithelial lining of the digestive tract which they penetrate. Each destruction of a host-cell thus also means a tremendous increase apparently in the number of the parasites. The intracellular crithidias are not found frequently in the preparations. We have no proof that it is an obligatory phase, though it might well be so. Nor have we evidence that it follows the forma-

tion of a zygote, as does the somatella of *Plasmodium* in the wall of the digestive tract of a mosquito. There is no evidence that the zygote precedes the somatella in the Polymastigina. In the life cycle of *Trypanosoma lewisi* the process of intracellular multiple fission is apparently obligatory. The trypanosomes, or haemoflagellates, penetrate epithelial cells of the "crop," undergo multiple fission, and crithidiomorphie merozoites are produced. This phase brings about the early stages of transition from a trypanosome to a crithidia. A similar need for such a phase is not present in the life cycle of *Crithidia euryophthalmi*. At present our knowledge of intracellular multiple fission in the life cycle of *C. euryophthalmi* is too meager to permit of extended correlation between the life cycle of this more primitive flagellate with that of the more highly developed haemoflagellate *T. lewisi*. It is conceivable that in the evolution of trypanosomes from the crithidial-like flagellates the intracellular multiple fission was carried over and became more specialized and more important in the life cycle of the haemoflagellate.

RECTAL PHASE

The stomach phase of *Crithidia euryophthalmi*, beginning with the initial infective spores and ending with the great swarm of parasites resulting from binary, extracellular, and intracellular multiple fission in the "crop," is followed by the established rectal phase of the life cycle in the pyloric expansion.

Owing to the structure of the digestive tract of *Euryophthalmus convivus*, with its three divisions separated only by a narrow constriction, which allows a possible intermingling under normal conditions of the crithidias in the "crop" with those of the mid-stomach and pyloric expansion, it is extremely difficult to say where the stomach phase ends and the rectal phase begins. In *Trypanosoma lewisi* the transition between the stomach and rectal phase, as has already been pointed out, is marked by definite structural changes. The trypanosomes, brought into the stomach of the flea with blood from a rat, enter epithelial cells and undergo multiple fission, producing merozoites of a crithidiomorphie type. The merozoites thus produced may do one of two things, either enter other epithelial cells of the stomach or collect at the pyloric opening and be carried down the intestine to the rectum with food. As they pass through the intestine the structural

changes which convert crithidiomorphs into crithidias are taking place.

The frequency with which we find preparations of the "crop" free from infection with *C. euryophthalmi*, the less frequency of infection of the mid-stomach, together with the almost invariably heavy infection in the pyloric expansion, lead us to the conclusion that the crithidias of *Euryophthalmus convivus* have the same tendency to migrate posteriorly as does *T. lewisi* in the flea. In *C. euryophthalmi*, however, the movement is less marked than in *T. lewisi*. This tendency of slow progression posteriorly doubtless depends upon the movement of food contents from the "crop" into the mid-stomach and pyloric expansion.

The migrating mass of crithidias from the "crop" soon establishes three distinct types of parasites in the pyloric expansion: the nectomonads or free flagellates, the haptomonads or attached flagellates, and the infective spores which serve for transmitting *C. euryophthalmi* to another host. These types or classes of parasites are comparable in almost every way to the nectomonads, or free flagellates, the haptomonads, or attached flagellates, and the final trypaniform stage of the rectal phase of *T. lewisi*. The nectomonads and haptomonads of each life-cycle are almost identical in structure and behavior.

NECTOMONADS

Smear preparations of the mid-stomach and of the pyloric expansion show little difference in the morphological structure of their crithidial infection. The serial sections of these two parts, however, show a sharp distinction in the structure of haptomonad forms and the nature of the epithelial lining to which they are attached, and a slight distinction in the structure of the nectomonads in the mid-stomach and in the pyloric expansion. The nectomonads of the former are usually of the elongate, slender type (pl. 5, figs. 73-80). The zooids, the result of the processes of multiple fission (pl. 4, figs. 40-54) are abundant in the smear preparations of both regions. The series sections thus far have shown numerous zooids (pl. 4, figs. 40-50) in the anterior portion of the mid-stomach. These zooids (pl. 4, fig. 40) are frequently grouped together in the grooves between epithelial cells. For this reason it is possible that the current of food in passing down the digestive tract failed to carry them on into the pyloric expansion. The zooids are small forms,

(pl. 4, figs. 40–47) from 1μ (pl. 4, fig. 40) to 1.5μ (pl. 4, fig. 47) in length and from 0.7 to 1μ in diameter. In comparing them with the initial infective oval spores (pl. 2, figs. 1, 2) it is noticed that they are smaller, stain less densely, and do not show a heavy periplast. The nuclear structure also is unlike that of the initial spore forms, and the location of the nucleus within the zooid adds another distinguishing character. These zooids have always been found in the anterior portion of the mid-stomach in the serial sections while the sections immediately posterior contain developing crithidias (pl. 4, figs. 59–64). Groups of elongating forms are frequently found (pl. 4, fig. 59). With the elongation of the body of the zooids, especially of the anterior end, the nucleus instead of being filled with chromatin is now vesicular and contains a distinct karyosome. This group (pl. 4, fig. 59) shows the parabasal bodies in close proximity to the nuclear membranes. Judging from the conditions found within the great majority of forms, figure 59 is probably an exceptional case in this respect. The parabasal body normally moves anteriorly in the early development of the zooid (pl. 6, fig. 40) before the flagellum and the anterior end grow out (pl. 4, figs. 41–53). In the figures just noted the flagellum is not yet visible. The nuclear rhizoplast, extending from the nucleus forward to the region of the blepharoplast and parabasal body, is found by focusing carefully. In figure 54 the flagellum is growing out from the blepharoplast but there is no noticeable lengthening of the anterior end of the body. In figures 55 to 58 the flagellum and anterior end of each are lengthening simultaneously. In these same figures the nuclei are like the nuclei of the zooids, being completely filled with chromatin. Farther on posteriorly are found crithidias such as are shown in figures 63 to 72. Beginning with figure 65 there is also an elongation of the posterior end, which is equal to that of the anterior.

The majority of these developing crithidias have the vesicular type of nucleus. Figures 65, 67, and 68 are exceptions, but no significance can be attached to them since the position, relative thickness of the body, or the technique, could explain these exceptions in this region of the digestive tract. The study of the serial sections leads us to think that the time necessary for the stomach crithidias to reach the rectum is approximately the amount of time required for the zooids to develop into mature flagellates. Not all of the developing zooids become mature in the mid-stomach. Under certain conditions the food current probably carries many of the non-flagelated stages or zooids back into the pyloric expansion before they have

scarcely begun to develop. As the developing forms and the fully matured forms enter the pyloric expansion, they become part of the permanent crithidial infection therein. The content of the crithidial infection of this region varies from time to time. Nectomonads may predominate or all nectomonads may become haptomonads, or attached forms. Usually the nectomonads and haptomonads are both present in large numbers. Mature nectomonads in the mid-stomach are usually the elongate, slender crithidias (pl. 4, figs. 73-79). In figure 74 the body size is approximately 25μ in length and 1.7μ in width. Figures 75 to 78 are yet narrower, averaging about 1μ in width. The nectomonads of the pyloric expansion are frequently like figures 73 to 79, long, slender forms together with numerous shorter and stouter crithidias (pl. 4, figs. 80-90). It is conceivable that the elongate slender forms give rise to the shorter, stout forms. The length of the body is decreased and the width is increased. Both the anterior and the posterior ends of the body become less attenuate.

The structure of the nucleus of the elongate forms varies considerably. In figures 73 and 80 there are distinct central karyosomes with chromatin-encrusted membranes. In figure 74 the chromatin is in five granules scattered within the nuclear membrane. The chromatin is broken up into granules in figures 76 and 79. The breaking up of the chromatin material in these nectomonad forms doubtless means the beginning of a degeneration, which will be discussed shortly. The nuclear structures in figures 77 and 78 are unique. In figure 77 the nuclear membrane is rather densely encrusted with chromatin and is elongate and irregular in outline. In figure 78 the nuclear membrane is oval, chromatin-encrusted, with two masses of chromatin at the anterior and posterior regions of the nucleus. In the series of figures 81, 83 to 86 a karyosome of a variable size is found in each and its location is not always central. In figure 82 a distinct chromidial fragmentation of the chromatin has taken place, which is another indication of degeneration. The short, stout forms (pl. 4, figs. 87-90) are transition forms from the nectomonads to haptomonads. While these flagellates are still free forms they resemble the haptomonads of the pyloric expansion, which are attached in the mid-region of this division. In the anterior part of the pyloric expansion the haptomonads are relatively long, slender flagellates while posterior to the middle portion they are still short and more pear-shaped. The nuclear structure of these transition forms indicates no degeneration as yet. They all have a central karyosome and a more or less encrusted nuclear membrane.

HAPTOMONADS

One of the most characteristic features of the life cycle of a crithidial flagellate is the great mass of attached forms which line definite parts of the digestive tract of the host. Minchin and Thomson found three regions of the digestive tract of the rat-flea where the crithidias of *Trypanosoma lewisi* might attach themselves, namely, the prepyloric, post-pyloric, and the rectal regions. In the lupine bug there are likewise three regions where the haptomonads may attach themselves: (1) in the posterior part of the "crop," where they are possibly comparable to the prepyloric crithidias in the posterior part of the stomach of the flea; (2) in the posterior half of the mid-stomach, where they are probably comparable to the post-pyloric crithidias of the rat-flea, attached to the anterior part of the intestine; and (3) in the pyloric expansion, where they are comparable to the haptomonads of the rectum of the rat-flea. In *Trypanosoma lewisi* in the flea these investigators regard the prepyloric haptomonads as being due to a forward migration from the rectum, possibly as a result of the food conditions. The prepyloric haptomonads are not found frequently in the flea, and in only one preparation of the "crop" of the lupine bug were haptomonads found. In the lupine bug haptomonads were found in a number of preparations of the mid-stomach, but they were commonly present in preparations of the pyloric expansion. The haptomonads observed in the single preparation of the "crop" were of the rectal type, small oval forms similar to those from the pyloric expansion shown in plate 6, figures 107 and 117. The serial sections of the digestive tract, however, showed no haptomonads in the "crop."

From the serial sections of the mid-stomach abundant material was obtained for the study of the haptomonad of this region. The attached forms here (pl. 6, figs. 93-96) are relatively small, slender flagellates. They are uniform in size and shape, on the whole, and form a definite fringe on the inner surface of the epithelial lining of the sections of the mid-stomach. These flagellates attach themselves to the epithelial cells by means of the flagella. They frequently almost surround the elongate, columnar epithelial cells, which project into the lumen of the digestive tract. The grooves between masses of epithelial cells evidently afford a particularly good place for the haptomonads, since they are found in compact layers in such places. Haptomonad crithidias of this type are the only ones found in the mid-stomach and they continue to line the digestive tract posteriorly

into the pyloric expansion. Considering the attached crithidias the most anterior sections of the pyloric expansion are almost identical with the sections from the posterior part of the mid-stomach. The lumen of the narrow constriction separating the mid-stomach from the pyloric expansion is frequently almost blocked with the bodies of the flagellates extending out into the passageway between these two enlargements of the digestive tract.

The structure of these haptomonads (pl. 6, figs. 93-96) is also relatively uniform. The majority of the crithidias show the vesicular type of nucleus, with a central karyosome and a nuclear membrane containing an inner lining of chromatin material. In figure 94 the nuclear membrane is slightly distorted in appearance and the karyosome is excentric, being posterior. In figure 96 the nuclear membrane is not distinct and the enlarged mass of chromatin material is in the form of two granules. These haptomonads are usually characterized by their attenuate anterior and posterior ends. The posterior ends of these crithidias more than of any others show relatively extreme attenuation.

Beginning in a region just posterior to the mid-stomach end of the pyloric expansion a series of transition haptomonads is found (pl. 6, figs. 97-106), lining the middle part of this division of the digestive tract, these transition haptomonads, which vary considerably in size. Figure 97 shows a broad, stout form. Such forms undergo binary fission, producing two smaller, more slender individuals. Possibly figures 100 and 101 are the products of such a division. The binary fission of the broad, stout forms rapidly increases the number of haptomonads which make up the dense and compact layer of attached crithidias. The nuclear structure of these forms shows comparatively little variation. They have the vesicular nucleus with the small central karyosome and chromatin-encrusted nuclear membrane.

At the posterior portion of the pyloric expansion are found the normal rectal forms (pl. 6, figs. 107-124) which are common to the life cycles of so many of these flagellates. One of the interesting things observed in connection with these forms is that the wall of the pyloric expansion becomes exceedingly thin. There are few indications left of the epithelial cells lining this part of the mid-gut. Sections of this portion of the tract previous to any infection by the flagellate are not at hand, unfortunately, and consequently it is difficult to estimate the total amount of destruction incurred. The pyloric expansion of an infected adult bug, however, is extremely weak and

becomes torn very readily; it has every appearance of having been almost entirely destroyed by the crithidias. Among these small haptomonads numerous variations of size and shape are noted. The length of the free flagellum (pl. 6, figs. 109, 110) is exceedingly variable. In other crithidias whatever flagella are present are intracellular throughout their length. The changes brought about in the flagella are probably due to their absorption. In every case the flagellum attaches the crithidia to the wall of the digestive tract. The nuclear structure of the haptomonads in this region, with the exception of the forms in figure 91, shows no indication of degeneration. The round haptomonads finally become free forms. They can be seen to drop off in the living preparations and to roll up into round or oval forms (pl. 6, figs. 118, 119, 122). The cytoplasm of these forms is vacuolate and stains lightly. These round crithidias then degenerate along with the nectomonads, which are constantly degenerating. The degeneration of the haptomonads and nectomonads will be described under the degenerative series.

FINAL SPORE FORMS

The structure of the digestive tract of *Euryophthalmus convivus*, including three portions of the stomach proper and the intestine with its gland, differentiates very clearly between the degenerative series of crithidias and the final spore forms which can be transmitted to another host. In the anterior part of the digestive tract is found the developmental series which becomes the degenerative series together with the final spore forms. Posterior to the gland only the final spore forms have been observed. The preparations of the rectum show only these final spore forms (pl. 6, figs. 125-131). These are oval, non-flagellated forms containing a thick periplast, within which are the nucleus and parabasal body. In figure 126 the characteristics of these final spore forms may be noted. They are approximately 2.8μ in length and 1.4μ in width. The nucleus lies in the extreme posterior end of the body and stains deeply. The parabasal body is sharply outlined within a vacuolate area. A faint nuclear rhizoplast may be visible, extending from the nucleus toward the parabasal body. As previously indicated in a preliminary paper (McCulloch, 1917) it was some time before the true rectum was discovered, and consequently the significance of these final spore forms was not entirely clear in the beginning of the investigation. However, with the discovery of the true rectum and the fact that it contained only these

final spores the characteristics of these rectal forms became clear and it was relatively easy to find them in both the mid-stomach and pyloric expansion. In the "crop" of the lupine bug the multiple fission gives rise to numerous small zooids. Some few of these zooids, on reaching a certain stage of development, become the well protected final spore forms. Their development into mature flagellates is arrested for an indefinite time, perhaps in response to some unfavorable internal or external chemical condition. The periplast becomes thickened and these zooids stain deeply and retain the stain much better than the unprotected zooids. Since only a few of the zooids become thus encysted the change cannot be regarded as a general change in response to some general stimulus. In the infections of the mid-stomach and pyloric expansion some of the final spore forms can be found at almost any period in the life history of the flagellates. Considering that these are the only stage of the life cycle of the flagellate which have been found in the rectum they have been regarded as the final spore forms, which upon being ingested with food by another insect host become the initial infective spores. Our observations upon these cannot be regarded as conclusive as yet, owing to the fact that experimentally we have not produced infection with these spores.

THE DEGENERATIVE SERIES

The degenerative series includes all individuals of the life cycle other than the final spore forms and their antecedents which have just been discussed. It has already been pointed out that the rectal phase of *C. euryophthalmi* in the pyloric expansion of the lupine bug is comparable to the rectal phase of *T. lewisi* in the rectum of the flea. In the lupine bug the degenerating forms abound in the posterior portion of the pyloric expansion and their number is apparently not decreased by constant elimination under normal conditions of such parasites from the intestinal contents, which pass through a relatively long intestine before reaching the colon and rectum. If any flagellates succeed in passing out with the intestinal matter into this long intestine they are evidently destroyed by the new chemical medium before they reach the rectum. In the flea the degenerating forms are in the rectum and it is possible that their number is repeatedly being decreased with each discharge of the feces.

There is little danger of confusing the developmental and degenerative series in the life-cycle of *C. euryophthalmi* since the differentia-

tion of the parasites into the final spore forms and the ordinary propagative forms takes place apparently in the stomach phase in the "crop." The majority, in fact, nearly all of the parasites become ordinary propagative forms which develop and increase their numbers in the digestive tract of the lupine bug. After passing through the various processes of the life cycle they degenerate in the late rectal phase in the pyloric expansion. The permanent rectal phase persists through the life of the lupine bug after the first infection; the degenerative series is soon formed, and likewise persists throughout the life of the host.

The individuals of the degenerative series are distinguished among the mass of living crithidias by a sticky periplast, to which bacteria frequently adhere by virtue of the tendency of crithidias to adhere to each other, by slow sluggish movements, and by odd sizes and shapes. The degenerating forms are detected in stained preparations by nuclei with diffused chromatin or by a vesicular nucleus breaking up into a number of chromatin granules, and by the vacuolated cytoplasm. The size, shape, and location of the crithidias also assists in distinguishing between the developmental and degenerative crithidias.

CONCLUSIONS

1. The crithidial flagellates of the life cycle of *Trypanosoma* are structurally like the crithidial flagellates of the life cycle of *Crithidia*. The extranuclear organelles, the blepharoplast, parabasal body, parabasal rhizoplast, nuclear rhizoplast, and the flagellum are all common to the crithidial flagellates of both *Trypanosoma* and *Crithidia*.

2. From the viewpoint of comparative morphology the differences existing between the crithidial forms of *C. euryophthalmi* and the crithidial forms of *T. lewisi* are less marked than are the differences between similar stages of *T. lewisi* and *Schizotrypanum cruzi*.

3. Using the life cycle of *T. lewisi* as a standard for comparison of the life cycle of a haemoflagellate or a trypanosome and the life cycle of *C. euryophthalmi* as the standard of the life cycle of a more primitive crithidial flagellate, there are more parallel stages and phases in these two life cycles than exist between the life cycle of any trypanosome and the life cycle of any herpetomonad or of any leptomonad now known. Furthermore the close correlation between these two life cycles of *T. lewisi* and of *C. euryophthalmi* affords new evidence that

the evolution of a trypanosome has probably taken place from a crithidial flagellate rather than from a herpetomonad or leptomonad flagellate.

4. The process of multiple fission in the somatella of *C. euryophthalmi* is fundamentally like the multiple fission (sphere formation) of *T. lewisi* and also like the multiple fission (somatella) of the trichomonad flagellates. In each of these flagellates the nucleus, parabasal body, blepharoplast, and flagellum, or flagella, are concerned in the process. In each after multiple fission plasmotomy occurs.

5. The process of multiple fission by endogenous budding in the life-cycle of *C. euryophthalmi* tends not only to establish another link in common between the life cycles of *Trypanosoma* (e.g., *T. gambiense*) and the life cycle of *Crithidia* but also to link the life cycle of *Crithidia* more closely to the lower protozoan forms which contain numerous *Leishmania*-like bodies in their life cycles.

6. The endogenous buds in the life of *C. euryophthalmi* are comparable to the latent bodies in the life-cycle of *T. gambiense*.

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EXPLANATION OF PLATES

All figures were outlined with a camera lucida using a $\frac{1}{12}$ objective on the binocular microscope and a Watson no. 20 holoscopic eye-piece. The magnification is in all cases approximately 3500. Unless otherwise stated all figures were made from iron-haematoxylin preparations.

PLATE 2

Crithidia euryophthalmi from the "crop" of *Euryophthalmus convivus*.

Fig. 1. Small, oval infective spore which has been casually ingested with food. This spore shows the structure common to these phases, thick periplast, deeply staining nucleus in extreme posterior end, heavily stained parabasal body. Faint nuclear rhizoplast.

Figs. 2-8. A series of developing crithidias showing successive stages of development. Both anterior and posterior ends are becoming attenuate. The nucleus changes from a solid mass of chromatin to a vesicular nucleus, chromatin-encrusted membrane and central karyosome. Flagellum grows forward and carries anterior end of body along with it, which forms undulating membrane.

Figs. 9-10. Two mature flagellates illustrating extremes in length, width, and shape, common to *C. euryophthalmi*. A whole series of intergrading forms occur between these two extremes.

Figs. 11-23. Multiple fission; endogenous budding. (Fig. 11). Flagellate with a nucleus, two endogenous buds posterior to nucleus. Chromatin of both nucleus and buds massed on nuclear membrane. Blepharoplast and parabasal body intact, no indications of division.

Fig. 12. A short flagellate; one endogenous bud; chromatin peripheral in each nuclear structure.

Fig. 13. Flagellate with three clearly defined nuclear structures and the beginning of a third bud from the most anterior, the nucleus proper. Chromatin peripheral in each of these nuclear structures.

Fig. 14. Elongate flagellate nucleus; two endogenous buds anterior to the nucleus; chromatin peripheral.

Figs. 15-17. Pear-shaped crithidias with endogenous buds. If nucleus be present in each there are no differences in structure between nucleus and bud. Buds in these flagellates are always sharply defined. Chromatin material on membrane but most of it is in definite granules.

Fig. 18. Pear-shaped form with numerous endogenous buds but no definite nucleus. First stages of degeneration present, parabasal body has disappeared and only a fragment of discarded flagellum is near. Chromatin peripheral but also in form of one granule.

Fig. 19. Elongate flagellate; two endogenous buds posterior to nucleus. Nuclear rhizoplast still present. Chromatin distributed irregularly on the nuclear membranes.

Fig. 20. Elongate flagellates with nucleus and two endogenous buds; chromatin massed in the anterior and posterior portion of each nuclear structure. Parabasal body has taken no part in this multiple fission.

Fig. 21. Large endogenous flagellate with five buds, all anterior to nucleus proper. Two of the buds are not destained sufficiently to see their structure. The organelles other than nucleus have taken no part in the process of endogenous budding. Flagellum, blepharoplast, parabasal rhizoplast and parabasal body are still intact and clearly shown.

Fig. 22. Late stage in endogenous budding. Buds have formed zooids, each of which has a nucleus and a second, deeply-staining structure anterior to nucleus. Flagellum, parabasal body, parabasal rhizoplast, and the blepharoplast are still intact. No signs of degeneration are to be observed in this flagellate.

Fig. 23. Drawing made from a field literally covered by discarded flagella and small zooids. The blepharoplast and parabasal body are still attached to some of the flagella. Zooids show various stages of nuclear structure. Some have a single mass of chromatin, others two granules within a chromatin-encrusted membrane. Some few of the zooids contain a nucleus, nuclear rhizoplast, and a second mass of chromatin anterior to these.

PLATE 3

Figs. 24-32. Multiple fission; somatellas. (Fig. 24). An early stage in the formation of a somatella. The flagellate is rounding up, and the flagellum is entirely intracellular. No indications of division of the nucleus, parabasal body, or the blepharoplast are present.

Fig. 25. A somewhat different type of rounding up. The attenuate anterior and posterior ends are being wrapped about the body. The nucleus has begun to constrict or to divide by a process of primitive promitosis. The blepharoplast and parabasal have not yet begun to divide.

Fig. 26. A more advanced stage in the formation of the somatella. The flagellum of the rounded up flagellate is protruding and both the nucleus and blepharoplast, together with the parabasal body, have divided. The new daughter-flagellum is not yet visible.

Fig. 27. A sphere, or somatella, still more advanced in its development. Not only have the blepharoplast, parabasal body, and the nucleus divided but the new outgrowth of the second flagellum from the daughter-blepharoplast is clearly visible.

Fig. 28. A spherical crithidia showing a repeated process of division on the part of the nuclei and parabasal bodies. Three parabasal bodies and two nuclei are present. There are possibly indications in one of the nuclei wherein the chromatin has been divided that another division was about to occur.

Fig. 29. A sphere, or somatella, without protruding flagella, containing at least four definitely outlined merozoites. The nuclei, parabasal bodies, nuclear rhizoplasts, and the outgrowths of the flagella are clearly visible.

Fig. 30. A densely stained, small somatella containing four relatively large merozoites which are beginning to elongate.

Fig. 31. A sphere, or somatella, breaking up and the merozoites about to escape. The destruction of the sphere has occurred later than usual and the merozoites have become almost mature flagellates. All nuclear structures are deeply stained, owing to the thickness of the sphere.

Fig. 32. An exceedingly large sphere, comparatively, showing many protruding flagella. Here again the nuclear structures are deeply stained because of the thickness of the sphere. The exact number of merozoites cannot be determined, but approximately twenty-four nuclei and parabasal bodies can be counted.

Figs. 33-38. Binary fission. (Fig. 33.) A small spherical crithidia undergoing binary fission. The nucleus has divided but the blepharoplast and parabasal body show no indications of fission.

Fig. 34. Binary fission, in which the blepharoplast and parabasal body have divided but the nucleus has not yet divided. A flagellum from the daughter-blepharoplast has already grown forward.

Fig. 35. Binary fission taking place in a developing crithidia. Both the nucleus and parabasal body have divided, and a new flagellum can be observed growing from the daughter-blepharoplast.

Fig. 36. Simple binary fission; blepharoplast, parabasal body, and nucleus have divided. The chromatin in the nuclei is peripheral, about the membrane.

Figs. 37, 38. A more advanced stage of binary fission, showing in addition to the division of the blepharoplasts, parabasal bodies, and nuclei a cleavage in the cytoplasm to form two crithidias in each case.

Fig. 39. Intracellular multiple fission: one of the many infected cells from the "crop" of *Euryophthalmus convivus*. This cell is in a degenerating condition. The nucleus stains a blue-gray color in iron-haematoxylin. There are at least three and possibly five intracellular infections by *C. euryophthalmi* in this cell. There are approximately seventy parasites within this cell: (a) A group of parasites of two sizes, small oval forms, non-flagellated and elongated *Crithidia*. Nuclei of all crithidias are diffuse, possibly due to thickness of smear. Nucleus and parabasal body readily observed but the other organelles are not always clear. (b) Another group evidently the result of a process of intracellular multiple fission. (c) Similar to b. Circular cavity about these non-flagellated crithidias may indicate the outline of a former somatella wherein plasmotomy has occurred early. (d) Elongate merozoites probably resulting from another intracellular somatella. Plasmotomy has occurred and the merozoites are about to make their way out of the host cell. (e) A scattered group of oval merozoites. Considerable variation in size is noted. Parabasal body, nucleus, and intracellular portions of flagellum clearly shown. (f) A mature merozoite making it way out of the host cell. The non-flagellated, or posterior, end directed first.

PLATE 4

Crithidia euryophthalmi from mid-stomach of *Euryophthalmus convivus*.

Figs. 40-92. Nectomonads (Figs. 40, 41). Small oval forms at the beginning of the developmental series of merozoites or zooids in the early rectal phase, showing nucleus, parabasal body, nuclear rhizoplast, and light area around the parabasal body. Nucleus at posterior end of the body. Anterior end pointed or blunt.

Figs. 42-47. More advanced stages in the developmental series; width of body and distance between nucleus and parabasal are increasing.

Figs. 48-54. Size of the oval forms increasing; nucleus still diffuse and located at extreme posterior end of body. Nuclear rhizoplast present, but no indication of the flagellum except in figure 54.

Figs. 55-58. Developing forms. Anterior end elongating and extending out along the flagellum. Nucleus still diffuse. In figure 58 the posterior end of the body is slightly elongated.

Fig. 59. A group in upper part of mid-stomach. Note vesicular type of nucleus, with the centriole-like karyosome. Both ends of the body are more or less pointed. The position of the parabasal body near the nucleus is unusual.

Figs. 60-62. Both anterior and posterior ends are elongating. Vesicular nucleus, nuclear rhizoplast, parabasal body, parabasal rhizoplast, and flagellum very distinct.

Fig. 63. Posterior end blunt; vesicular type of nucleus. Parabasal body near the nucleus. Anterior end of the body well developed; a slight undulating membrane present.

Fig. 64. Form a little more developed. Posterior end of the body is blunt or round, and nucleus is diffuse.

Fig. 65. Almost mature flagellate; nucleus diffuse; posterior end elongated and quite broad.

Fig. 66. Free form, showing vesicular nucleus and a distinct undulating membrane.

Fig. 67. Nectomonad developing into elongate flagellate; nucleus diffuse.

Figs. 68-72. Nectomonads of mid-stomach, long, slender, flagellates showing the common variations in the nuclear structures. The diffuse type in figure 68; vesicular in figures 69 and 71; chromatin-encrusted nuclear membrane in figures 70 and 72.

PLATE 5

Figs. 73-92. Nectomonads from pyloric expansion (Figs. 73-80). A series of elongate, slender flagellates from crithidial infection of the pyloric expansion. Vesicular types of nuclear structure in figures 73, 75, 80. Chromatin broken up into granules in figures 74, 76, and 79. Chromatin of nucleus on the membrane in figures 77, 78.

Figs. 81-86. Another group of nectomonads from pyloric expansion, illustrating the changes which take place in shape of body, length decreases, width increases. These are shorter, broad types of crithidias, and probably come from the longer, more slender forms. Nuclear structure usually shows the central karyosome. Figure 82 shows the chromatin broken up, a condition which may indicate an early stage of degeneration of the nectomonads.

Figs. 87-90. Transition flagellates from nectomonads to haptomonads. By a process of binary fission these transition forms become reduced in size. Certain nectomonads become attached to wall of the pyloric expansion or they form haptomonads. Others degenerate before reaching this stage.

Figs. 91-92. Free flagellates. Their size is doubtless due to a process of binary fission and they resemble the attached haptomonads of the pyloric expansion. They are either nectomonads which are about to become haptomonads or *vice versa*. If the latter, the haptomonads upon becoming free again usually degenerate at once.

PLATE 6

Figs. 93-96. Haptomonads from mid-stomach. Haptomonads of this type may be found attached to wall of the "crop," posterior part of mid-stomach or anterior part of pyloric expansion. Small, slender flagellates usually with a vesicular type of nucleus are shown in figures 93-95. Figure 94. Nuclear membrane distorted, karyosome excentric. Figure 96 may show degeneration since there is no nuclear membrane and the chromatin is breaking up.

Figs. 97-124. Haptomonads from pyloric expansion (Figs. 97-106). Transition haptomonads lining mid-portion of the pyloric expansion. Bodies becoming pear-shaped; flagella largely absorbed and intracellular throughout length. Nucleus usually vesicular. Binary fission is common among these forms. Their size is reduced in this way.

Figs. 107-124. Small haptomonads, found in the extreme posterior portions of the pyloric expansion. They form a dense compact layer of parasites attached to epithelial lining. Reduction in size shown, beginning with figure 107. Degeneration is shown in the nuclear structure, as in figure 114, and in the cytoplasm of figures 117, 118. Figures 118-124 are all haptomonads which show advanced stages of degeneration; the cytoplasm is vacuolate and the nuclear structure is no longer normal. Most of these have become detached from the wall of the pyloric expansion.

Figs. 125-131. A series of final spore forms from rectum, showing thick periplast, deeply stained cytoplasm, and two chromatin bodies. Nuclear rhizoplast not visible in these deeply stained spores.

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