AN EXTRACELLULAR COCCIDIUM, CRYPTOSPORIDIUM MURIS (GEN. ET SP. NOV.), OF THE GASTRIC GLANDS OF THE COMMON MOUSE.*

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(Plates XX. and XXI.)

The parasite about to be described is frequently found in large numbers in the gastric glands of the tame varieties of the common mouse, *Mus musculus*. In a brief preliminary description (Tyzzer[1]) it was given the name *Cryptosporidium muris*, and, although it was not at that time possible to classify it satisfactorily, the similarity of its development to that of sporozoa of the order *Coccidiomorpha* (*Coccidiidia* Calkins[2]) was noted. While it is possible that some forms of this organism have been already observed by certain investigators, they have in that case been mistaken for developmental forms of other parasites, and in no instance have they been recognized as belonging to a distinct species. This parasite undergoes the greater part of its development either attached to the surface of the epithelium or free in the lumen of the gastric glands so that its habit of life is similar in many respects to that of parasites of the order *Gregarinida*, of which no representative has been described hitherto in Vertebrates (Doflein[3]). Although it never penetrates the cells of the host, it appears, nevertheless, to be of the nature of a coccidium. It is described, therefore, not merely as a new species, but because it is to be hoped that new facts may be brought forth which may eventually throw light on the phylogeny of certain orders of the sporozoa, and thereby aid in their classification.

Certain forms of this parasite have probably been observed by other investigators, who have interpreted them as developmental forms of *Coccidium falciforme* (*Eimeria falciformis*, Eimer), a species which occurs in the intestine of the

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mouse. Thus J. Jackson Clark[4] states that he found free coccidia, many of which were encapsulated and filled with "swarm-spores" (merozoites?) in the glands of the cardiac end of the stomach of a mouse that sickened and was killed on the seventh day after it had been fed with material which contained the ripe spores of *C. falciforme*. He states that the striking feature was the presence of masses of "swarm-spores," lying free and on the surface of the mucous membrane and distending the ducts. He believed that these small organisms, some of which were in the epithelial cells of the gastric glands, represented the "swarm-spore" stage of *C. falciforme* which he claims to have found infecting the epithelium of the glands of Lieberkühn of the same mouse. From the description given it appears reasonably certain that the organisms observed in the gastric glands by this author were of the species about to be described, and not *C. falciforme* as he supposed. The bodies which he interpreted and pictured as spores within the encapsulated forms were probably specific granules, and the accuracy of the observation concerning the intracellular position of certain of the parasites in the gastric glands appears also questionable.

The presence of a parasite protozoon in the gastric glands of the mouse was evidently not noted by Wenyon,[5] who made a study of the protozoa of the intestine of the mouse. In a brief discussion of *C. falciforme* he calls attention to the occurrence in the intestines of free schizonts which were enclosed in cysts. The merozoites varied from three to twelve microns in length. Several of the figures illustrating this paper portray quite accurately the schizonts of the organism which occurs in the gastric glands, but, since Wenyon makes no mention as to whether his observations were made on sections or on smear preparations, it cannot be determined whether he dealt with coccidia that had been passed from the stomach into the intestine or with *C. falciforme*.

Attention was first attracted to this organism by the dilatation of the gastric glands of an old tame mouse in which a tumor had developed spontaneously. On superficial

examination of stained sections the dilated glands appeared to be filled with a granular material, but on further study this was found to consist of great numbers of minute organisms. The subsequent examination of the stomachs of other mice has shown this gland-infesting parasite to be of frequent occurrence in common tame mice. It occurs also in Japanese waltzing mice, but I have not yet found it in wild house mice, a small number of which have been obtained from different sources. Through the courtesy of Dr. Bashford of the Imperial Cancer Research Fund I was allowed to examine and identify this parasite in sections of the stomach of a mouse killed in his laboratory. Its occurrence in English mice as well as in those of this laboratory would indicate that it is not limited to any particular locality, but has a wide geographical distribution. I have found it either in a large proportion or in all of the mice of certain cages, and not at all in the mice of other cages. Such cage infection is due to the fact that the young mice become infected from ingesting food contaminated with the feces of the older mice which harbor the parasite. The daily scalding of all dishes in which food and water is served evidently keeps the infection confined to certain families of mice. The organism has been studied during different seasons of the year, and appears to occur at all times. It occurs in greater numbers in the gastric glands proper than in those of the pyloric end of the stomach. Infection of the mucous membrane ends abruptly at the pylorus, and only the resistant form, or oöcyst, is found ordinarily in the intestinal contents and in the feces.

Technic. — If a small amount of the gastric mucosa of an infected mouse is gently scraped off with a knife and mounted between slide and cover, the glands will be found more or less dilated and filled with the organisms which may be readily recognized either with the high dry or with the oil immersion lens. The spores are readily photographed in fresh preparations, and, if taken at a known magnification, the photographs will furnish an accurate measurement of the organism free from the shrinkage and the distortion

which result from various technical methods of preparation. A great variety of fixing and staining methods have been used for demonstrating the structure of the organism in both sections and smears. Sections were stained with eosin and methylene-blue, alum hematoxylin, Mallory's phosphotungstic-acid hematoxylin, and iron hematoxylin, after fixation in Zenker's fluid, and with the latter two stains after fixation in Flemming's fluid.

It would be quite impossible to follow the development or distinguish the structural features of this parasite from the study of sections alone. Sections are useful, however, in determining the relation of the parasite to the tissue of the host, and also the effect of the former upon the latter. The morphological study of the organism must necessarily be made from stained smears. Preparatory to making a smear, the stomach of a freshly killed animal is laid open, and all undigested food is swept to one side with the edge of the scalpel. The mucous membrane is then gently scraped with the point of the knife, and the soft material obtained in this way is thoroughly mixed on the cover-glass with a tiny drop of mouse serum before being smeared over its surface. The admixture of the scrapings with serum is of great aid in distributing the organisms and disassociating them from the gland epithelium. Smears prepared in this way take a better stain than those made from scrapings of mucosa without serum.

Various reagents were used for the fixation of spreads made in this way. The fumes arising from a gently heated, two-per-cent solution of osmic acid give good fixation. The best results have been obtained by allowing the osmic-acid fumes to act from fifteen to thirty minutes, during which the smears become dry. Both Wright's and Giemsa's stains may be used to advantage after treating the smears with osmic fumes. The fixed smear should be rinsed with methyl alcohol in order to remove any trace of acid before staining by Wright's method. The staining solution which consists of equal parts of Wright's stain and distilled water is allowed to act for eight or ten minutes. The smear is then washed, rinsed quickly with acetone, and cleared in oil

of turpentine. Giemsa's stain is used in the proportion of fifteen drops to ten cubic centimeters of distilled water. Smears were also fixed in Zenker's and Flemming's solutions, in methyl alcohol, absolute alcohol, and over formalin. Various methods of staining were found useful in bringing out special structural features of the organism, but these will be mentioned in the description of the latter.

The various forms presented by this parasite are taken to represent the different phases of its life history, and it has proved possible from a morphological study alone to follow it through its entire development in the gastric glands of the mouse. All forms are attached throughout their period of growth to the surface of the epithelium of the gastric glands, and in no instance has one of the parasites been found within a cell. Before proceeding to a description of the various developmental forms, certain structural features common to most of them may be taken up. Each organism as soon as it becomes attached to the surface of the epithelium develops a surface membrane which, except in those forms destined to pass from the body of the host, is extremely delicate, so that it requires special technical methods for its demonstration. It is brought out very clearly, however, in spreads fixed in osmic fumes, while in those prepared in the ordinary way the contour of the organism is not sharply defined, and its shrunken protoplasm appears to be situated within a clear unstained ring.

The attachment of this organism to the surface of the gland epithelium is made possible by a structural modification in the form of a knob-like projection at some point on its surface. The outer aspect of this projection is bluntly conoidal, and a delicate thread-like process is often found extending outward from its apex. This has been observed frequently in smear preparations and occasionally in sections. It evidently represents a slender protoplasmic process extending through the capsule of the organism at this point. That the protoplasm is specially modified within this organ of attachment is shown by its staining affinities. Whereas the cytoplasm of this organism is stained blue by the

Romanowsky stains, the interior of these projections stains a pale pink or red. Owing to its minute size no further details of its structure have been distinguished. This organ is, in function at least, comparable to the epimerite of gregarines. The parasite which would otherwise be of either an ovoid or ellipsoid form is made distinctly flask-shaped by the projecting attachment organ which is usually situated at one end.

Each organism contains one or more globules which stain with the usual fat stains such as Soudan III. or Sharlach R., and are blackened by osmic acid. In preparations which have been subjected to the action of alcohol or other fat solvents these globules may be represented by unstained vacuoles. That these globules vary in their chemical composition is shown by the fact that some are stained faintly and others intensely by the osmic acid, or they may become colored in a varying degree by the aniline stains. These globules are evidently of the nature of stored food material, and are generally used up during the process of segmentation.

This species presents a series of developmental forms which make it at once clear that it has both an asexual and a sexual mode of reproduction. In the light of what is already known concerning the life-cycles of other species of the sporozoa, it is not difficult to recognize the nature of the various forms presented by this organism in its development. On account of its minute size, however, it has not been found possible to follow each step in the processes of maturation and sporulation, both of which are followed to better advantage in larger species.

The schizonts (agametes), or those forms which show no sexual differentiation and in which multiplication is not preceded by sexual union, are devoid of any characteristic granules. They possess a cytoplasm which is stained intensely blue by either Wright's or Giemsa's stain. The small forms present a single rounded nucleus, the appearance of which varies with different technical methods of preparation. Thus, if Wright's method of staining blood

smears is followed, the nucleus appears as a clear vesicle within which is a rounded globule of deeply stained chromatin. In preparations which have been fixed over osmic acid and stained with Giemsa, the outline of the nucleus is fairly well defined and the granules of chromatin are distributed within it. The appearance of the nucleus varies according to the length of time the osmic acid or the methyl alcohol is allowed to act. During the growth of the schizont the nucleus divides into two daughter nuclei. There are subsequently two nuclear divisions so that eight separate masses of chromatin are finally formed. In stages taken during nuclear division the chromatin appears to be arranged first in one and later in two plate-like aggregations which resemble those found in dividing cells of higher organisms. It seems probable that there is a process of the nature of a primitive mitotic division. The small chromatin masses become grouped about the end of the organism farthest from its point of attachment, and cleavage of the cytoplasm commences by the formation of sharp depressions in the corresponding surface. By the extension of the cleavage depressions into the main mass of the cytoplasm, elongated, finger-like processes each containing a single mass of chromatin are formed. These elongated bodies develop at the expense of the material constituting the remainder of the schizont, and are finally transformed into banana-shaped elements, the merozoites. The number of merozoites produced in this process of schizogony is almost invariably eight. These elements extend from one pole to the other of the including membrane, and usually present a slight spiral twist. They are grouped about a globule of residual material which is usually situated at the proximal pole of the capsule, *i.e.*, near the organ of attachment. Portions of this residual material often color with osmic acid.

The merozoites vary from five to eight microns in length, and some are short and thick, while others are relatively slender. Each possesses an oval nucleus situated about one-third way from the thicker extremity. At some point between the nucleus and the thinner extremity a minute

granule is distinguishable in many merozoites. This granule is deeply stained with phosphotungstic-acid hematoxylin, and resembles to a certain extent the so-called blepharoplast of certain of the parasitic flagellates. I have not succeeded, however, in demonstrating a flagellum in any of the various forms of this parasite. The granule in question is not apparent in all merozoites, and whether it is absent in certain ones or whether the technical methods are inadequate for its demonstration in every instance has not been determined. The merozoites often occur in large numbers both free in the gastric glands and crypts and in contiguous portions of the stomach contents. In the free state they possess no limiting membrane, but this develops as soon as they become attached to the epithelium. They become attached by one extremity to the surface of the epithelium, so that their long axis is more or less perpendicular to the surface of the latter. They remain elongated for some time after attachment, but later take on a more rounded or oval form. It is quite possible that the deeply stained granule described above represents a structure concerned in the development of the organ of attachment. On becoming attached to the epithelium a fat globule appears within the merozoite.

The microgametocyte is quite similar in its morphology and its mode of development to the schizont. It is distinguished by its minute size, and the relatively large amount of chromatin which it contains. Like the schizont it possesses a thin limiting membrane, an attachment organ, and one or more fat globules. The dimensions of the entire organism never exceed five by three and a half microns. The nucleus divides in a manner similar to that described in the schizont, and the chromatin masses are often in pairs as though division had just taken place. The nucleus, however, divides into two, four, eight, and finally sixteen masses which come to be situated at the surface of the distal portion of the organism. These masses appear more dense and stain more intensely than the chromatin masses of the schizonts which are about to segment. They become more

and more dense and take on an elongated form, at the same time pushing out from the surface of the microgametocyte. Each chromatin mass together with a minute portion of the cytoplasm is eventually separated off to form a microgamete. Each microgametocyte thus gives rise to sixteen microgametes. A relatively large mass of residual material is left which often appears vacuolated. This is colored to a greater or less extent when treated with osmic acid. It has been with considerable difficulty that the exact form of the microgametes has been determined. As they lie upon the surface of the ripened microgametocyte, the chromatin is in the form of a slender rod slightly thicker at one end than the other, and, while it is possible to ascertain that each rod of chromatin is associated with a small amount of faintly staining material trailing off from its thinner end, the exact form of the microgametes is not readily made out. In preparations fixed in the fumes of osmic acid, and stained with Giemsa's solution, these elements may be distinguished free as well as upon the surface of the microgametocyte. The achromatic portion of the microgamete is approximately equal in length to the chromatin rod, and tends to broaden toward the extremity farthest from the chromatin, which is cut off abruptly. In a small portion of the microgametes the form is not so distinct and the achromatic portion appears as an indefinite shred, but in no case was there any indication of flagella.

The macrogametes, from the stored food material which they contain, present a structure much more complicated than that of either the schizonts or the microgametocytes. They are readily distinguished from the other forms by the specific granules which they possess. In life these appear as oval refractive granules, the size of which is quite constant for each organism, but varies in different ones according to the stage of their development. When treated with iodine solution — Lugol's solution was ordinarily used — these granules become colored, and appear in various tints from reddish brown to deep purple. In nearly all the methods of preparation employed, these iodophilic granules

disappear leaving the organism with a finely vacuolated or spongy appearance. Fixation in Flemming's fluid tends to fix these granules so that it is possible to stain them. They are also colored red with Giemsa's stain after fixation in formol vapor. The macrogametes like the schizonts and microgametocytes vary from a spheroid to an ellipsoid form with the attachment organ at one end making them typically flask-shaped. The limiting membrane of this form eventually becomes relatively thick and quite impervious, so that by ordinary methods such as are used for staining blood smears the more mature organisms remain unstained and appear as refractive bodies with a granular interior. In spreads fixed over osmic acid or in Flemming's fluid it is possible to stain a large proportion of these more resistant forms. The fixation over osmic acid appears to modify somewhat the tints produced by the Romanowsky stains, and the characteristic reddish color of the chromatin is not always obtained. What might be regarded as a poor Romanowsky stain, however, has been found valuable in differentiating certain substances found within this form of the organism. Thus in preparations stained by Wright's method after fixation over osmic acid, in which the chromatin of all forms stains a deep blue or a purplish tint, there appears in juxtaposition to the chromatin of many of the small macrogametes a mass of material which stains a bright red. This material is present throughout the entire subsequent development of the macrogamete, and, since it may readily be mistaken for the chromatin, the subsequent nuclear changes have proven difficult to follow. At first present in minute quantity it is approximately equal in amount to what has been ascertained to be the chromatin of the cell. This, together with the fact that it is often found in juxtaposition to the chromatin in the small forms, has suggested that it may be the product of a reduction division of the nucleus. The amount of chromatin in such forms appears to be of about one-half the amount of the chromatin of schizonts of similar size. The substance in question may occur in various parts of the young macrogametes, however, as well as associated with the

chromatin. Its subsequent fate and its physical features as well are not characteristic of degenerating chromatin. As the organism increases in size this material increases in amount, and is either in a single irregular mass or scattered throughout the organism. It becomes distinctly granular in character and usually appears refractive as compared with the chromatin. During the late development of the macrogamete the material in question appears to fuse into irregular masses or globules, the latter having the appearance of rings in the stained preparation. In the younger organisms it is colored red by Wright's stain, and is comparable in its refractive qualities to the eosinophile granules of leucocytes. Later in the development it may take either the blue or the red stain, but always stains intensely. In preparations fixed over formol and stained with alum hematoxylin this material stains red. This peculiarity thus differentiates it from chromatin. In Giemsa preparations it is always stained intensely, appearing almost black as compared with reddish-tinted chromatin. After prolonged study it appears most probable that this material is of the nature of a specific granulation which is peculiar to this species. It apparently first originates in the vicinity of the nucleus, and after increasing in amount is left as waste material.

In addition to the material just discussed there appears near the periphery of many of the small macrogametes a small rod of deeply-staining substance of the size and shape of the chromatin of the microgametes. The larger nucleus of the macrogamete is in some instances elongated and extends toward this object, which may be situated at any point between the surface of the macrogamete and its nucleus. Microgametes which are occasionally found upon the surface of the macrogametes are readily recognized, and their chromatic portion is identical in size, shape, and staining reaction to the rod which occurs in a small proportion of the macrogametes. The process of fertilization is undoubtedly shown by such forms, although it has not been possible to distinguish a preparatory reduction in the nucleus of the macrogamete. After the union of the male and

female pronucleus, the nuclear changes are not readily followed on account of the fact that they are obscured both by the food granules which continue to accumulate and by the increase in thickness of the limiting membrane which eventually takes on the character of a dense cyst-wall. In those preparations in which it is possible to follow subsequent nuclear changes, the nucleus appears to become irregular and spread out in the form of a chromidial net. Whether the chromatin becomes disseminated or whether there is definite nuclear division preparatory to the formation of the sporozoites has not been ascertained.

The protoplasm of the oöcyst or fertilized macrogamete becomes massed at the distal pole of the organism while the food granules occupy the remaining portion. The protoplasm segments into four masses which take on an elongated form, and eventually become sporozoites, leaving a mass of residual material in which iodophilic granules, lipoid globules, and the deeply-staining material already discussed may be distinguished. There is no secondary spore formation, such as occurs in most coccidia, but the whole organism is transformed into a single spore with a dense resistant membrane. The four sporozoites lie naked within this membrane. At first wide and flattened they become thin and rounded as they mature. Being much longer than the oöcyst they are bent in U shape over the surface of the residual material. They almost invariably come to lie parallel with one another and extend longitudinally or spirally in the ripened oöcyst.

Free sporozoites are often demonstrable in well fixed preparations, and they appear to become liberated from the spore when the material from the mouse's stomach is mixed with the serum of the mouse. They are readily stained after fixation over osmic acid. On escaping from the oöcyst they present in general a distinct boomerang shape with a thin, pointed anterior extremity, a short distance from which is a rod-shaped nucleus. They vary considerably in width and the wider ones appear flattened suggesting somewhat the body of a trypanosome. They present no flagellum,

however, and the wider ones have been considered to be the more immature forms. A large proportion of the oöcysts unquestionably become mature in the gastric glands, and it does not seem improbable that auto-infection may be effected to a small extent through the liberation of sporozoites from those oöcysts which do not happen to pass out into the intestine. In fact sporozoites have been found in sections both free on the surface of the gastric mucosa, and in large numbers in certain of the gastric glands. The sporozoite is readily distinguishable from the merozoite by its great length and by the character of its nucleus. The repeated observation in such preparations of four long slender organisms either in the vicinity of, or in the act of escaping from, an oöcyst indicates the frequent liberation in the stomach of sporozoites from oöcysts which have completed their development.

In order to ascertain whether the life-cycle of this organism was completed in the stomach or whether the development was continued elsewhere in the body the following experiments were performed, — the actual object in view being to determine if young uninfected mice could be artificially infected by feeding them the gastric mucosa and stomach contents of infected mice.

EXPERIMENT A.

The gastric mucosa and stomach contents of several infected mice were mixed thoroughly and kept at room temperature over night and then fed to four of a litter of seven young mice, the other three being taken for controls. In order to induce the mice to eat this material it was mixed with about an equal amount of cooked bread and milk, and fed to each mouse separately. The control mice were kept in a separate cage and fed thereafter on cooked food and boiled water as were the mice receiving the infected material. The fed mice together with the control mice were killed subsequently and the gastric mucosa examined both fresh and in section. The parasite was found in large numbers in two of the three controls, showing that the entire litter had been

previously exposed to infection. The uninfected mouse was much larger than the other six and its fur was more sleek and glossy. While this experiment failed as regards the artificial infection of mice with this organism, it tends to show that the nutrition of young mice may be somewhat affected by a severe infection with this parasite.

EXPERIMENT B.

In repeating the above experiment the stomach contents and gastric mucosa of three infected Japanese waltzing mice were fed to four of a litter of seven young common mice, while the remaining three were kept as controls. The material was eaten by the mice about twenty-four hours after its removal. One of the fed mice died within eighteen hours after eating this material, and presented no evidence of infection of the gastric mucosa. The other three, which were killed at longer intervals, all showed numerous parasites in the gastric glands while the three control mice showed none. Both fresh preparations and stained sections of the gastric mucosa were examined in each case. The results in detail are as follows:

Fed Mice.

No. 1.	Died within 24 hours.	No parasites in stomach.
No. 2.	Killed after 7 days.	Parasites in moderate numbers most of which are in the process of schizogony. Several oöcysts found after long search.
No. 3.	" " 14 "	Parasites in moderate numbers. Many macrogametes and ripe oöcysts.
No. 4.	" " 18 "	Parasites numerous. Ripe oöcyst predominating form. Free sporozoites numerous on surface of mucosa and in several glands.

Control Mice.

No. 5.	Killed after 7 days.	No parasites.
No. 6.	" " 14 "	" "
No. 7.	" " 18 "	" "

In the mouse killed seven days after feeding a large proportion of the organisms were undergoing schizogony although

a few oöcysts were present. The two mice killed fourteen days and eighteen days respectively after feeding showed a large number of oöcysts as compared with the other forms.

Experiment B shows definitely that the oöcysts which develop in the gastric glands are capable of infecting other mice so that it is probable at least that the development of this parasite is completed within the gastric mucosa of the mouse. It is quite possible that immature spores escaping from the gastric glands ripen as they pass onward in the alimentary canal.

A single attempt to infect a white rat with this organism was unsuccessful.

The pathogenicity of this parasite is slight. The only marked histological change is found in the dilatation of the gastric glands in severe infections. There may be a slight increase in the collections of lymphoid cells, but there is nothing of the nature of an acute inflammation. The dilatation of the glands is evidently brought about through the growth of large numbers of the organisms within the lumen, so that it is of a purely mechanical origin. It is effected in part by a flattening out of the epithelium, but the number of cells appears also to be somewhat increased as is shown in cross sections of the glands. Since all the growing forms of the organism possess a dense limiting membrane, it appears most probable that they assimilate food through the organ of attachment, and so live upon materials obtained directly from the cell rather than upon the products of gastric digestion. The influence of this parasite on the host is evidently much less deleterious than is the case with the intracellular parasites, and its adaptation to parasitic life is thereby more perfect than is that of the latter.

The sporozoon of the gastric glands although a true parasite and probably incapable of developing apart from its specific host, appears to be much nearer the free-living protozoa than is the case with most other species of the order Coccidiomorpha. Wenyon's statement, to the effect that in the mouse are to be found organisms which present all steps in the process of adaptation to life within the host

appears to be borne out by the discovery of this parasite which never enters the cells of its host. In this animal there occurs in the intestine a coccidium (*Eimeria falciformis*) which invades the epithelial cells, while in the stomach a parasite occurs having a similar life-cycle but which lives upon the surface of the epithelium. In its adaptation to a life on the surface of the secreting gland cells, it has developed a special organ of attachment comparable in its function to the epimerite of gregarines. The slender protoplasmic process which is often found protruding from this structure probably serves to enable the parasite to adhere to a surface after it has become dislodged from its original attachment. Evidence of the adhesive properties of these protoplasmic threads is found in the formation of rosettes in spreads made by mingling scrapings of the gastric mucosa with serum.

A peculiar differentiation of the cytoplasm (Tyzzer[6]) noted in the young and growing intracellular forms of the coccidium, *Eimeria stiedæ*, of the rabbit may be of the nature of a vestigeal attachment organ. It appears as a rather homogeneous concavo-convex projection or demilune at some point on the surface of the organism. In the forms which are still elongated, it is situated at the end, and it retains the eosin stain as does the attachment organ in the gastric sporozoon.

It has not been ascertained how the microgametes penetrate the limiting membrane of the macrogamete in order to accomplish fertilization. No micropyle has been found, and it is probable that the membrane in the unfertilized forms is of such character that these elements are able to actually pass through it to interior of the organism. The complexity of structure and the regularity of development presented by this parasite are remarkable when its minute size is taken into consideration.

Since a number of recognized species of parasitic protozoa occur in the mouse, the question arises as to whether the organism herein described does not represent a hitherto unrecognized phase of the life-cycle of one of the latter. It is quite certain that the stomach parasite is in no way

related to the sarcosporidium of the mouse, for the latter parasite has not occurred in this laboratory for a number of years. Although the intestines of a large number of mice have been examined, in no instance has an infection with *Eimeria falciformis* been found, so that the mice reared in this laboratory may be considered as exempt from this parasite also. It is possible that *Klossiella muris* (Smith and Johnson[7]), the coccidium which occurs frequently in the renal epithelium of the mouse, passes through certain phases of its development in the gastric glands, and that we are thus dealing with hitherto unrecognized developmental cycles of a previously described species rather than with a new species. Apart from the fact that the entire development of *Klossiella* appears not to occur in the kidney, nothing has been found in support of this hypothesis. Both *Klossiella* and the parasite of the gastric glands are often found in the same mouse, or either one without the other. In the mouse killed eighteen days after it had been experimentally infected (Experiment B) with the gastric parasite, *Klossiella* was not found, although from a study of sections it was found that large numbers of sporozoites were escaping from the oöcysts, which might possibly suggest that they were to continue development elsewhere in the same animal. Though present in large numbers both on the surface of the mucosa and in the gastric glands, none were found penetrating the tissues, and it seemed probable that they were to reinfect the gastric glands and possibly to again undergo asexual multiplication. In Experiment A, six of the seven mice used had been naturally infected prior to being fed material containing the gastric parasite. *Klossiella* was not present in any of these mice when killed ten, eleven, fifteen, thirty-two, and thirty-four days respectively after this feeding, although the gastric glands showed in each case great numbers of parasites. The settlement of the possible identity of species of the parasitic forms which occur in the stomach and in the kidney of the mouse must be left until the tissues of lots of mice, which have been kept for longer periods of time after artificial infection with each of the two forms in question,

are examined. It is possible that families of mice may be found which harbor one and not the other.

The gastric parasite cannot be identified with the intestinal flagellates or with amebæ, although these possibilities have been considered. The tortuous outlines of the sporozoites which escape from the oöcysts suggest the occurrence of a flagellate stage, but it has not been possible to demonstrate a flagellum. Furthermore, the intestines of mice killed seven, fourteen, and eighteen days respectively after artificial infection with this organism showed none of the three common intestinal flagellates, *Lamblia*, *Hexamitus*, and *Trichomonas*, while in another experiment young mice which were fed intestinal contents showed four days later great numbers of *Lamblia* and *Hexamitus*, but no parasites in the gastric glands. It is evident, therefore, that the organism under discussion undergoes practically its entire development attached to the surface of the epithelium of the gastric crypts and glands.

From what has been learned of this parasite of the gastric glands of the mouse, it has seemed best to consider it a species belonging to a new genus. The name *Cryptosporidium muris* (Tyzzer [1]) has been given it, the intention being to signify that it is a sporozoon in which spores are indistinguishable or absent in the oöcyst, and which is parasitic in the mouse. From a superficial observation it would appear that the mode of life of this species is essentially that of a gregarine. It never, however, enters the cells as do many species of the latter at some stage in their life history, and the large forms are incapable of motion. Its developmental cycle shows clearly that it belongs to that group of sporozoa known as *Coccidia*. The occurrence of the coccidia, *Eimaria mitraria* (Laveran and Mesnil [8]) and *Orchiobius herpopdellæ* (Kunze [9]), which are extracellular throughout their entire development, has led to a modification of the definition of this group, so that they are no longer defined as intracellular parasites.

Classification. — The organism under consideration clearly

belongs in the class Sporozoa, subclass Telosporidia, order Coccidiomorpha, suborder Coccidia, as defined by Doflein. Of the three families of this suborder it evidently should be included in the Eimeridæ (Lühe) which Doflein describes as follows: "Coccidia with macrogametes and microgametes of approximately equal size, which ripen apart from one another; no 'Syzygien' formation before copulation: each microgametocyte gives rise to numerous microgametes which move through the agency of two free flagella. The agametes divide directly into young agametes, the microgametocytes directly into microgametes." Although no flagella have been demonstrated in connection with the microgametes of this species, it fulfils all the other requirements of this family. It cannot be included, however, in any of the existing genera of the Eimeridæ, so that it becomes necessary to create a new genus.

The genus Cryptosporidium may be defined as follows: Sporocyst absent or united with oöcyst so that the entire organism becomes a single spore with four sporozoites.

For this genus *Cryptosporidium muris* is the type species of which the following is a brief description:

Cryptosporidium muris. Gen. et sp. nov. — The schizont after repeated binary nuclear division gives rise to eight merozoites. It is devoid of specific granules. Maximum size seven by six microns.

The microgametocyte in its early development resembles the schizont, but is somewhat smaller, never exceeding five by four microns in size. It gives rise to sixteen microgametes which consist of a rod of chromatin and a broader achromatic portion of about equal length. The microgametes measure from 1.5 to two microns in length.

The macrogamete possesses characteristic iodophilic (paraglycogen) granules, specific eosin-staining granules, and globules of lipoid and hyaline material. After fertilization a thick cyst membrane is developed, and the protoplasm divides into four masses which elongate and become sporozoites which lie naked within the oöcyst. They are usually bent in U shape over a relatively large mass of residual

material. The mature oöcysts measure approximately seven by five microns.

The mature sporozoites are long and slender and measure from twelve to fourteen microns in length. The anterior extremity is slender and pointed, and a short distance from it is the rod-shaped nucleus.

The entire development is extracellular. During growth the organism lives upon the surface of the gland epithelium, and all forms except the merozoite and sporozoite possess a thin limiting membrane and an organ of attachment. All forms also contain globules of lipoid material which may be situated in the residual body at the time of segmentation.

Since large numbers of sporozoites are set free from oöcysts which ripen before passing from the stomach, it appears probable that in this species autoinfection may be effected through sexual as well as through asexual reproduction.

Habitat, the gastric glands of the common mouse, *Mus musculus*. It evidently has a wide geographical distribution, but has only been found in the tame varieties of the common mouse. It occurs in the Japanese waltzing mouse as well as in common white and colored mice, and it is possible to infect the latter varieties with organisms obtained from the former.

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DESCRIPTION OF PLATES.

PLATE XX.

(All the figures shown in this plate were drawn with a camera lucida from stained smears using a two-millimeter oil immersion lens and a No. 6 compensating ocular. The microscope was elevated above the drawing so as to give a magnification of about two thousand diameters. Figures 1–7 and 10–18 inclusive were drawn from smears fixed over osmic acid and stained with Wright's blood stain; Figures 8 and 19 from smears fixed in Flemming's fluid and stained with Mallory's phosphotungstic acid hematoxylin; and Figures 20–26 inclusive from smears fixed over osmic acid and stained with Giemsa's solution in the proportion of fifteen drops to ten cubic centimeters of distilled water. Asexual multiplication (schizogony) is illustrated by Figures 1–8 inclusive, the development of the Microgametocyte and the formation of Microgametes by Figures 9–13 inclusive and by Figures 21 and 22, and Fertilization and Sporogony by stages shown in Figures 14–25 inclusive.)

FIG. 1. — A minute organism which has become attached to the surface of the epithelium. It presents a delicate limiting membrane and at its lower extremity the organ of attachment. Within it is a vacuole probably representing a globule of lipoid material. The cytoplasm stains intensely blue, and there is a dense mass of chromatin within the nucleus.

FIG. 2. — A similar organism of somewhat larger size. A delicate protoplasmic process extends from the organ of attachment.

FIG. 3. — In this there are two masses of chromatin which are apparently about to divide into four.

FIG. 4. — The four masses of chromatin are somewhat irregular and suggest nuclear division of the nature of that found in higher forms. Each is surrounded by a mass of cytoplasm which bulges slightly from the surface of the organism.

FIG. 5. — A form showing an early stage of asexual division. The chromatin is now situated within eight finger-like processes which project from the surface away from the organ of attachment.

FIG. 6. — A mature schizont about which there is no apparent limiting membrane. The eight merozoites remain in a compact mass with a small amount of residual material enclosed by their lower extremities.

FIG. 7. — Free merozoites showing variation in size.

FIG. 8. — Merozoites stained with phosphotungstic acid hematoxylin showing a minute deeply stained granule midway between the nucleus and the posterior extremity.

FIG. 9. — A minute organism with a relatively large amount of chromatin probably destined to become a microgametocyte.

FIG. 10. — A microgametocyte in which there are two masses of chromatin.

FIG. 11. — A later stage of nuclear division.

FIG. 12. — A microgametocyte in which eight masses of chromatin are in the process of division into sixteen.

FIG. 13. — A mature microgametocyte showing the characteristic grouping of the microgametes over the surface of the residual material away

from the organ of attachment. It is evident that the microgamete consists of a deeply stained rod of chromatin and of a less deeply stained material, but the shape of these elements apart from the chromatin is not brought out by this method of staining.

FIG. 14. — A small macrogamete showing nucleus and a small mass of red staining granules.

FIG. 15. — Macrogamete showing the outline of the iodophilic granules which are practically unstained.

FIG. 16. — A group of macrogametes, one of which presents a delicate process extending from the organ of attachment. All show the peculiar red-staining granules. The chromatin is deeply stained and arranged in irregular masses, but it is probable that the methyl alcohol present in the Wright's stain has caused more or less distortion. Vacuoles and hyaline globules are present in certain of the organisms figured.

FIG. 17. — Three oöcysts in which the sporozoites are not yet mature. In the upper and in the left hand figures these elements are coiled about irregularly within the cyst wall. In the lower right hand figure the sporozoites are more mature and lie parallel to one another and curved about the residual material. They are shown in this instance in optical section. The red-staining material has become fused into a homogeneous globule within the residual mass.

FIG. 18. — An oöcyst from which the mature sporozoites have escaped. Residual material is left, the greater portion of which contains iodophilic granules not shown by this method, but there is also a vacuole and a ring of eosin-staining material. The nuclei of the sporozoites are not clearly shown in this figure.

FIG. 19 — A ripe oöcyst showing the characteristic arrangement of the sporozoites which are bent over the centrally situated residual material and present a slight spiral twist.

FIG. 20. — Two macrogametes showing both the male and the female pronucleus. The deeply stained mass situated to one side of the vacuole is of the nature of a specific granulation which has been shown in red in the preceding figures.

FIG. 21. — Two free microgametes showing the rod of chromatin and a slightly wider achromatic portion.

FIG. 22. — A mature microgametocyte on the surface of which there are fifteen microgametes.

FIG. 23. — A large macrogamete in which the union of the male and female pronucleus has probably taken place. The cytoplasm is massed about a hyaline sphere at the pole distal to the organ of attachment.

FIG. 24. — A group of six parasites showing the characteristic clumping which takes place in the preparation of smears. No protoplasmic processes are in evidence. These organisms tend to adhere to one another through the peculiar properties of their attachment organs. The latter are stained red in this and in the four preceding figures. In the upper portion of this group is a schizont about to divide into eight merozoites. Two of the five macrogametes show both male and female pronucleus representing a stage in the process of fertilization.

FIG. 25. — An oöcyst from which the sporozoites are escaping. The

cyst wall is here stained a reddish purple. The rod-shaped nucleus is apparent near the sharper extremity of the sporozoites.

FIG. 26. — A merozoite showing at one extremity a rudimentary organ of attachment. This form is readily distinguished from the sporozoites by its size, shape, and the character of its nucleus.

PLATE XXI.

FIG. 27. — Photograph of a section of the stomach of a mouse with a severe Cryptosporidium infection with *C. muris*. The glands are dilated by great numbers of the organisms.

The photographs shown in Figures 28, 29, and 30 were taken from smear preparations stained with phosphotungstic-acid hematoxylin after fixation in Flemming's fluid.

FIG. 28. — A merozoite presenting a deeply stained granule in addition to the nucleus. To the right are two macrogametes. x 2,000.

FIG. 29. — To the left a merozoite similar to that in Figure 28. To the right a macrogamete showing nucleus, hyaline globule, vacuole, and attachment organ in the order named. The reticular appearance is due to the presence of iodophilic granules. x 2,000.

FIG. 30. — Two macrogametes showing deeply stained granular material. Nucleus not apparent. x 2,000.

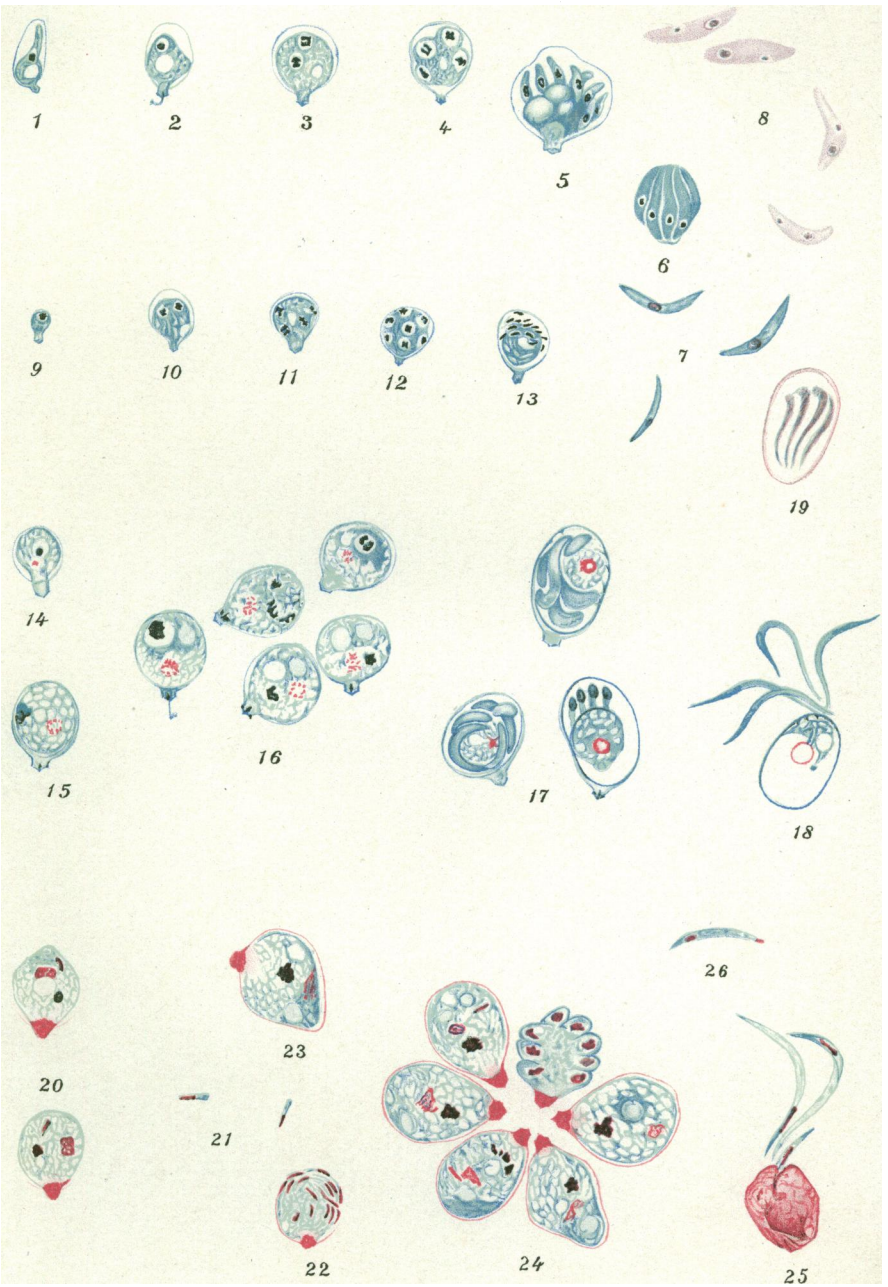
FIG. 31. — A section showing small organisms probably young schizonts attached to the epithelial surface to the left of the figure. x 1,000.

FIG. 32. — Several organisms attached to the surface of the epithelium. One schizont shows four masses of chromatin. x 1,000.

FIG. 33. — A group of merozoites lying free in the lumen of the gland. x 1,000.

FIG. 34. — Schizonts attached to the epithelium and lying free in the lumen of the gland. x 1,500.

FIG. 35. — Merozoites which have just become attached to the surface of the epithelium. x 1,500.



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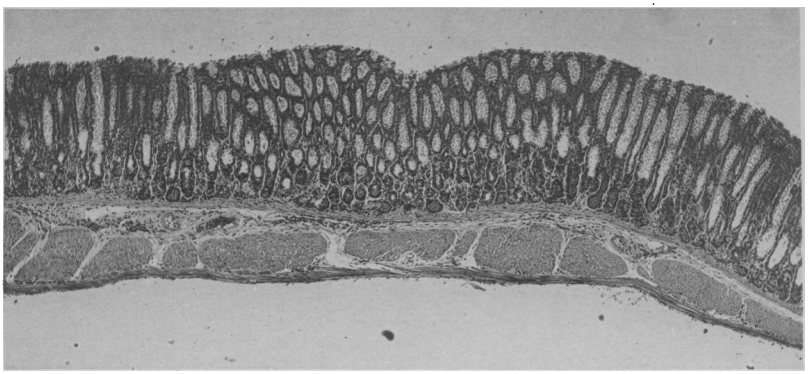

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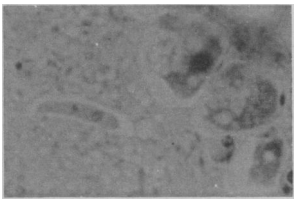

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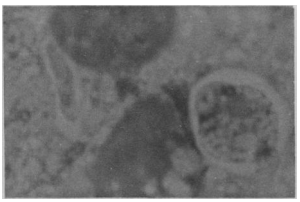

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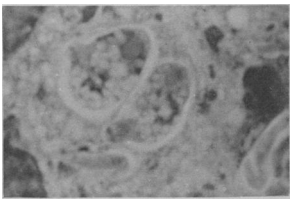

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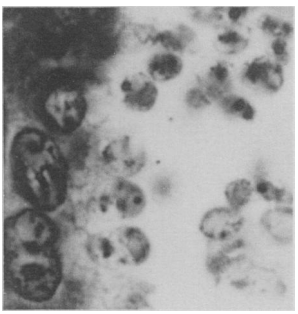

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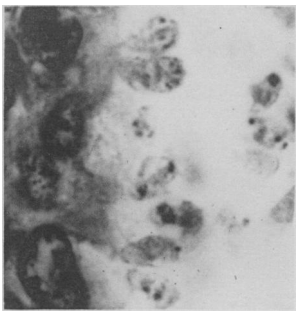

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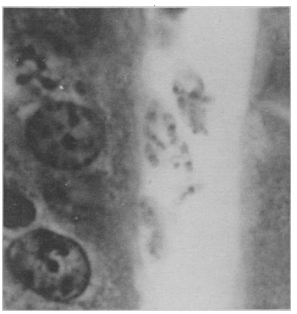

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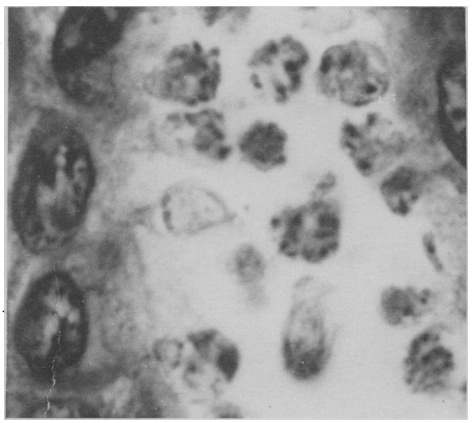

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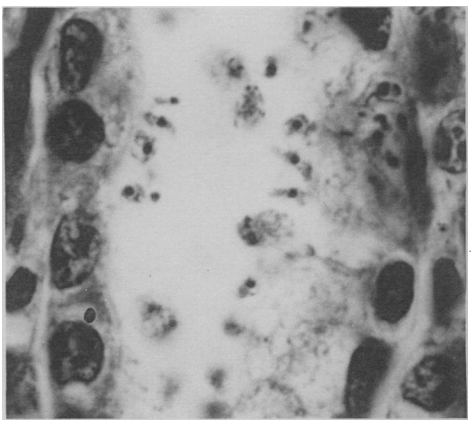

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