TREASURY DEPARTMENT

Public Health and Marine-Hospital Service of the United States

HYGIENIC LABORATORY.—BULLETIN No. 46

JUNE, 1908

HEPATOZOON PERNICIOSUM (N. G., N. SP.); A HAEMOGREGARINE PATHOGENIC FOR WHITE RATS; WITH A DESCRIPTION OF THE SEXUAL CYCLE IN THE INTERMEDIATE HOST, A MITE (*LELAPS ECHIDNINUS*)

BY

W. W. MILLER

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SUMMARY.

Hepatozoon perniciosum, n. g., n. sp., is a hæmogregarine found in white rats in Washington, D. C., and the cause of an epizootic observed among these animals. The protozoon is conveyed by a mite (*Lelaps echidninus* Berlese), which is the true intermediate host. Infection is transmitted to the rat when the mite is swallowed by the rat.

The mites are ecto-parasites upon the rats, from which they receive infection by sucking the blood.

Multiplication of the hæmogregarine in the rat takes place in the liver. In the stomach of the mite the hæmogregarines conjugate and form an ookinet, which penetrates the stomach wall and completes its development in the body tissues of the mite.

The illustrations in this bulletin have been prepared from drawings made by the laboratory artist, Prof. Leonard H. Wilder.

HEPATOZOON PERNICIOSUM (N. G., N. SP.)—A HÆMOGREGARINE PATHOGENIC FOR WHITE RATS; WITH A DESCRIPTION OF THE SEXUAL CYCLE IN THE INTERMEDIATE HOST, A MITE (LELAPS ECHIDNINUS BERLESE).[a]

By William Whitfield Miller,
Assistant Surgeon, U. S. Public Health and Marine-Hospital Service, Washington, D. C.

INTRODUCTION.

Although the first of the Hæmocytozoa to be discovered (*Lankesterella ranarum*, by Lankester in 1871) was a hæmogregarine, our knowledge to-day of this important group is far from complete. A great number of hæmatozoa have been described in cold-blooded animals, in nearly all of which there is a more or less close resemblance to the gregarine type. The most noteworthy peculiarity of these parasites is the wormlike form assumed during at least some portion of the life cycle. They are, at some period, endoglobular; but they lack the ameboid motion, and pigmentation so characteristic of the hæmamœbæ. Multiplication occurs by division into two or into many individuals.

Classifying them according to the vertebrate host, the following is a brief description of the known species.

HÆMOGREGARINIDÆ OF AMPHIBIA.

About ten species of hæmogregarines in batrachians are known, all of which affect the red blood cells of frogs and toads. Until quite recently the belief was unquestioned that sexual as well as nonsexual multiplication occurred in the same host. In fact, Hintze[b] interpreted certain appearances as a conjugation of the

[a] Manuscript submitted for publication June 25, 1908.

[b] Hintze, R.: Lebensweise und Entwicklung von *Lankesterella minima* (Chaussat). Zoolog. Jahrb., Abt. f. Anat. u. Ontog., Bd. 15, Heft 4, 1904, pp. 693–730.

parasites and the formation of an ookinet which entered the intestinal epithelium, became encysted, was cast off in the feces and served, when ingested by another frog, to propagate the parasite.

Lühe believes this observation to be in error and regards the oocyst as coccidial in nature. The multiple infections of protozoa (flagellates, coccidia, and hæmogregarines) found in frogs, often in the same animal, renders it very difficult to determine positively to which parasite certain phases in the life cycle belong.

A recent observation by Durham[a] upon the life history of *Drepanidium* in a South American toad is of special interest. He found that nearly all the toads were infested with a species of tick. In the stomach of the tick were seen forms of the *Drepanidium* apparently in copulation, also peculiar cysts 60 micra in diameter, which he believed to be some stage in the life cycle of the parasite. Durham regards the tick as the probable intermediate host, although this is not definitely established.

The studies of Billet[b] and also some recent observations of Laveran and Nègre[c] seem to indicate that trypanosomes may play a part in the life cycle of *Lankesterella* and other hæmogregarines. Beyond question, a great deal of work is necessary upon this group before many doubtful points can be clarified.

HÆMOGREGARINIDÆ OF SNAKES AND LIZARDS.

Lühe[d] mentions nineteen species affecting the red blood corpuscles of snakes and lizards. He divides them into two genera, *Karyolysus* and *Hæmogregarina*. A noteworthy peculiarity of the former is the existence of large and small trophozoites (without intermediate stages)—the macro-merozoites and micro-merozoites. Schizogony takes place in red blood cells in the capillaries of internal organs. The membrane remaining after destruction of the red cell forms a sort of a cyst wall. The schizonts are of two types, macro-schizonts and micro-schizonts, corresponding to the two varieties of merozoites.

[a] Durham, H. E.: *Drepandium* in the toad. Liverpool School of Tropical Medicine, Memoir VII—Report of the yellow-fever expedition to Para, Liverpool, 1902, pp. 78–79.

[b] Billet, A.: Sur le *Trypanosoma inopinatum* de la grenouille verte de l'Algérie et sa relation possible avec les *Drepanidium*. C. R. Soc. Biol., Paris, vol. 57, No. 27, 1904, pp. 161–165.

[c] Laveran, A., & Nègre: Sur un protozoaire parasite de *Hyalomma ægyptium*. C. R. Soc. Biol., Paris, vol. 58, no. 21, 1905, pp. 964–966.

[d] Lühe, M.: In Handbuch der Tropenkrankheiten, C. Mense, Leipzig, 1906, Bd. III, pp. 209–210.

Lutz[a] regards the small forms as male and the large as female. He has never seen evidence of conjugation of the trophozoites in the blood of the host. The intermediate host is unknown.

HÆMOGREGARINIDÆ OF TURTLES AND CROCODILES.

A large number of hæmogregarines have been described in turtles. *Hæmogregarina stepanovi* Danielewsky has been carefully studied by Danielewsky, and in its sexual development by Siegel.[b]

In *Hæmogregarina stepanovi* the gametocytes, according to Siegel, are the elongated trophozoites which are bent into a U shape on account of their length being greater than that of the red blood cell which they inhabit. Schizogony occurs as in other hæmogregarines. There is no dimorphism of the schizonts. The invertebrate host is a leech, in the gut of which the gametocytes develop and fertilization takes place. The ookinet which develops from the zygote invades the intestinal wall and enters the esophageal glands, where elongated sporozoites are formed. Infection of the turtle, to which the leech next attaches itself, is said to take place in a manner similar to the malarial infection from mosquitoes. Siegel believes that the ovary and eggs of the leech may be invaded by the sporozoites, and the next generation of leeches thus infected.

HÆMOGREGARINIDÆ OF FISHES.

About twelve species are known. *Hæmogregarina bigemina*, from the blood of blennies (*Blennius pholis*), has been described by Laveran and Mesnil.[c] In the nonsexual multiplication of this parasite by binary fission a resemblance to the manner of multiplication in piroplasma is observed, and incidentally to that in trypanosomes. In this connection it is interesting to note that trypanosomes have been found very frequently in the blood of fishes, which also harbor hæmogregarines. The group is as yet imperfectly known. The mode of transmission of the infection has not been determined.

In all hæmogregarines of cold-blooded animals which have been studied, nonsexual multiplication or schizogony takes place in the red blood corpuscles, usually in some internal organ. The merozoites, when set free, invade other red blood cells, and increase considerably in size before reaching maturity.

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[a] Lutz, A.: Ueber die *Drepanidien* der Schlangen. Ein Beitrag zur Kenntniss des Hamosporidien. Centblt. f. Bakt., 1. Abt., vol. 29, 1901, pp. 390–398.

[b] Siegel: Die geschlechtliche Entwicklung von *Hæmogregarina stepanovi* in Rüsselegel *Placobdella catenigera*. Arch. f. Protistenkde., 1903, Bd. 2, H. 3, pp. 339–342.

[c] Laveran, A., & Mesnil, F.: Deux hémogrégarines nouvelles des poissons. C. R. de l'Acad. des Sci., vol. 133, 1901, pp. 572–577.

HÆMOGREGARINIDÆ OF MAMMALS.

Although the first protozoan blood parasites to be observed, with the exception of trypanosomes, were hæmogregarines, and since that time a great number have been discovered in cold-blooded animals and more or less completely described, knowledge of such forms in warm-blooded animals dates back only three or four years.

In 1905 James[a] published a description of a new parasite found in the white blood cells of pariah dogs in India, their occurrence having been previously noted by Bentley.[b] James did not regard the parasite, which he named *Leucocytozoon canis*, as a hæmogregarine, but considered its relationship to parasites in the blood of birds, certain forms of which appear to infect the white blood corpuscles.

These so-called "leucocytozoa" were first described by Danielewsky[c] in the blood of owls.

Quite recently Schaudinn,[d] Laveran,[e] and Berestneff[f] have shown that the forms of this parasite (now known as *Leucocytozoon ziemanni*), which resemble parasites in leucocytes, are in reality macrogametes to which are attached portions of the nucleus of the red cell originally invaded. The name is somewhat misleading.

James found *Leucocytozoon canis* present in the blood of 6 out of 45 dogs examined. The polymorphonuclear cells were the only variety affected. The largest percentage of infected corpuscles recorded was 9 per cent. In one single instance only was a parasite found free in a stained blood smear. On one occasion a free form was seen in a fresh preparation, having escaped from the leucocyte by reason of its continuous vermicular movements. The wormlike body remained in contact with the host cell, but continued its movements for an hour or more.

The intracorpuscular forms, when stained by Romanowsky's method, are described as oblong bean-shaped bodies enveloped by the protoplasm of the leucocyte and apparently surrounded by a delicate cyst. In addition to the bean-shaped bodies, James describes parasites, almost round in shape, with a very indistinct capsule. The leuco-

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[a] James: On a parasite found in the white corpuscles of the blood of dogs. Sci. Mem. Officers Med. & San. Dept. Govt. India, 1905, n. s., no. 14.

[b] Bentley: Preliminary note upon a leucocytozoon of the dog. Brit. med. journ., May 6, 1905, p. 988.

[c] Danielewsky, B.: La parasitologie comparée du sang. Kharkoff, 1889. Also, Developpement des parasites malariques dans les leucocytes des oiseaux. Ann. de l'Inst. Pasteur, 1890, p. 427.

[d] Schaudinn, F.: Generations- und Wirtswechsel bei *Trypanosoma* und *Spirochæte*. Arb. a. d. Kaiserl. Gesundheitsamte, vol. 20, 1904, p. 387–439.

[e] Laveran, A.: Contribution à l'étude de *Hæmamœba ziemanni*. C. R. Soc. Biol., vol. 55, 1903, p. 620–623.

[f] Berestneff, N.: Ueber das Leucocytozoön Danielewsky. Arch. f. Protistenkde, vol. 3, 1904, p. 376–386.

cytes affected are of the polymorphonuclear variety. The nucleus in 86 per cent of the parasites is situated near one extremity and is oval or round in shape, and stains like the nuclei of the leucocytes.

The manner in which nonsexual multiplication takes place in the bone marrow is described by Christophers.[a]

Since the discovery of *Leucocytozoon canis* by Bentley a number of somewhat similar parasites have been observed in the blood of mammals, all in tropical regions. In some instances they affect only the white corpuscles, in others only the red cells, but never both. Certain peculiarities and their similarity to hæmogregarines in cold-blooded animals are sufficient to identify them in the Hæmogregarinidæ.

In 1905 Balfour[b] described a new parasite in the red blood cells of *Jaculus jaculus,* the jerboa, or kangaroo rat, of Sudan. This organism, according to Laveran, is a hæmogregarine.

The trophozoite, which is either free or lies in the remains of a red blood cell, is sausage shaped, nonpigmented, and nonmotile. It measures 5.5 to 7 micra in length and from 1.4 to 2.8 micra in breadth. When stained by the Leishmann-Romanowsky method, the nucleus is large, oval, and centrally located. In the peripheral blood all forms are in about the same stage of development. The animal host does not suffer in health.

The parasite (*Haemogregarina balfouri*) of the jerboa is bent slightly upon itself and its length is a little greater than that of the red corpuscles. In addition to the form described, Balfour observed on one occasion a free motile vermicule form without flagellum. In a stained specimen which showed this stage the total length was 15.5 micra, one extremity being finely tapered to a point, the other rounded and containing chromatin granules. Multiplication occurs by schizogony. The schizonts are found in the liver, kidney, and bone marrow, in the form of cysts. The cyst walls appear to be formed of the remnant of the cell of the organ invaded. In the liver the schizonts appear quite similar to those of certain coccidia. The manner of invasion of the liver cells has not been determined. No sexual cycle or mode of transmission is known. Balfour states that no naked-eye indications of disease are present in an infected animal. The spleen is not enlarged. He also mentions the fact that he has discovered what seems to be the same parasite (*H. balfouri*) in the mononuclear leucocytes of a rat (*Mus decumanus*) in Khartoum.

In 1905 Christophers[c] discovered a parasite in the red blood cells

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[a] Christophers, S. R.: *Leucocytozoon canis.* Sci. Mem. Officers Med. & San. Dept. Govt. India, 1906, n. s., no. 26.

[b] Balfour, A.: A hæmogregarine of mammals. Journ. Trop. Med., vol. 8, 1905, p. 241; vol. 9, 1906, pp. 81–84.

[c] Christophers, S. R.: *Hæmogregarina gerbilli.* Sci. Mem. Officers Med. & San. Dept. Gov. India, 1905, n. s., no. 18.

of the Indian field rat (*Gerbillus indicus*) which resembles *H. balfouri*. The affected corpuscles (somewhat enlarged) contained a minute motionless vermicule with a short, sharply flexed tail (trophozoite). A distinct space surrounded the parasite. In some fresh preparations there were free parasites similar to the above, except that the pointed extremity was no longer bent. They exhibited active gliding movements, also twisting and turning motions. In stained specimens the trophozoites were in the red cells and appeared to be enveloped by a capsule. A large chromatin body was situated in the "tail" of the parasite, also occasional scattered granules.

No certain sexual differentiation of the trophozoites could be made out. Although all the organs of infected rats were carefully studied, no evidence of nonsexual multiplication could be found. Infected rats showed no constitutional disturbances. Differential leucocyte counts indicated that the percentage of different kinds of leucocytes differed but little from the normal.

The only ecto-parasites found upon the rats were insects—a new species of louse (*Hæmatopinus*) measuring 1.4 by .5 mm. Certain structures were encountered in the dissection of these lice which Christophers regarded as phases of the sexual cycle of *H. gerbilli*. Although experiments were made to infect lice from infected animals, no recorded attempts were made to transmit the infection from one animal to another by means of lice. In his experiments a number of lice were placed upon a young heavily infected rat. These were removed at stated intervals and examined. On the fourth day two lice were removed and examined. In the midgut and intestine vermicules in active motion were observed. On the seventh day large cysts were observed lying free in the body cavity of the insect. They were of large size, measuring as much as 350 micra in diameter. When dissected out they appeared as minute white spheres which, with the microscope, were observed to be filled with minute cysts containing six or eight crescentic bodies. Younger cysts measuring 10 micra in diameter were also seen, also intermediate ones varying in size between the smallest and the largest. No satisfactory stained specimens of these intermediate stages were obtained. No abnormal appearance was observed in the salivary glands of any of the lice examined. When ruptured cysts were mixed with the blood of the Indian rat and examined with the microscope, the crescents became active vermicules.

No forms suggesting sexual elements or conjugation were observed by Christophers. In a recent work this writer[a] states, regarding the life cycle of *H. gerbilli* in *Hæmatopinus stephensi:* "Vermicules

[a] Christophers, S. R.: The sexual cycle of *Leucocytozoon canis* in the tick. Sci. Mem. Officers Med. & San. Dept. Govt. India, 1907, no. 28.

are freely liberated in the midgut and in the body cavity large cysts are found; but, as a result of work on *L. canis* in the tick, I am doubtful whether the cysts have anything to do with the hæmogregarine."

Patton [a] has described a parasite in the large mononuclear leucocytes of palm squirrels. Forms measuring 10 micra in length were seen, one extremity being bent and somewhat pointed, resembling a tail. Free vermicules exhibited slow "streaming" movements. Patton made a number of leucocyte counts upon blood from infected and noninfected squirrels and found both a relative and numerical increase of the large mononuclears in the former. Smears were made from all the organs of the squirrel, but no evidence of multiplication was observed.

Lice (a species of *Hæmatopinus*) were found upon many of the squirrels examined. Upon dissection active vermicules measuring 15.5 micra were seen in the gut of the lice which had been placed upon infected squirrels. No further development was observed, nor were any parasitic bodies found in the ovaries or salivary glands.

Christophers [b] has recently published his observations upon the sexual cycle of *Leucocytozoon canis* in the tick. He had previously described the schizogony, which takes place in the bone marrow, but never in the liver or kidney. The merozoites are formed in cysts. One cyst may contain 30 or more merozoites.

The sexual cycle in the dog tick (*Rhipicephalus sanguineus*) is described as follows: In the gut of ticks examined immediately after removal from infected dogs free vermicules are seen; after twenty-four hours they have penetrated the cells of the gut wall and are seen in various stages of fission, which precedes the formation of the sexual elements. On the third day conjugation bodies and the first stages of the development of the fertilized oocyst are seen. Mature oocysts are observed on the fourth day. According to Christophers, the vermicules, after entering the intestinal cell, divide into two, four, or even eight vermicules. Two of these vermicules then conjugate and an oocyst is formed, which, when fully developed, measures 14 micra in diameter and splits up into 12 to 14 sporozoites, without an investing capsule. The entire process takes place in the epithelium of the gut wall and not in the cœlomic cavity of the tick.

From the description of Christophers it seems difficult to understand his interpretation of some of the stages observed. Especially it would seem difficult to distinguish the parasites formed by fission of the vermicules from the free sporozoites. He mentions no feature

[a] Patton, W. S.: On a parasite found in the white corpuscles of the blood of palm squirrels. Sci. Mem. Officers Med. & San. Dept. Govt. India, 1906, n. s., no. 24.

[b] Christophers, S. R.: The sexual cycle of *Leucocytozoon canis* in the tick. Sci. Mem. Officers Med. & San. Dept. Govt. India, 1907, no. 28.

which distinguishes the male elements (microgametes) from the female (macrogametes).

The method of inoculation of a dog with *L. canis* by the tick is unknown. Christophers states that since the female tick does not suck blood again after repletion, the passage of the sporozoite into the ova seems a necessity if the parasite is to regain entrance into the mammalian host. He has been unable to find sporozoites in the ova or, in fact, anywhere except in the gut of the ticks.

Adie,[a] in India, has observed in blood smears from *Mus rattus*, in each instance accompanied by trypanosomes, a leucocytozoon with an oval nucleus and without cytocyst, and about one-third the length of the leucocyte in which it was inclosed. Further details regarding it are lacking.

Summary.—A critical examination of the literature dealing with the Hæmogregarinidæ reveals the fact that almost nothing is definitely known concerning their sexual cycle and manner of transmission.

HEPATOZOON PERNICIOSUM.

Material and Technique.

An examination of a blood smear from a white rat which had died from an unknown cause revealed a large number of protozoan parasites somewhat resembling *Leucocytozoon canis*. It was found upon inquiry that the stock of white rats kept for laboratory use, in cages, in a wooden building adjoining the stable was being greatly depleted by disease. Many of the animals became anæmic and emaciated and after a longer or shorter period, usually a week or more, died. Upon examination it was found that some of the cages were infested with mites, which were present in large numbers upon the rats. Some of these mites, taken from an infected rat, were found to harbor the leucocytic parasite found in the blood of the rats. A more extended study revealed the interesting fact that the sexual phase of the parasite occurs in the mite.

Seventeen rats, four of which died later, were made the basis of a study of the parasite in the rat. In addition, a large amount of material was obtained from rats experimentally infected. Material in a perfectly fresh condition was taken from all organs and preserved in fixing fluids. All the customary fixatives were employed, but the most satisfactory reagent for preserving the parasites in tissues was found to be formalin (formalin = 40 per cent formaldehyde). In strengths of 4 to 10 per cent of formalin in normal salt solution the tissues were immersed for twenty-four hours, then washed out in water and preserved in 70 to 80 per cent alcohol. Sections varying

[a] Adie, J. R.: Note on a leucocytozoon found in *Mus rattus* in the Punjaub. Journ. trop. med., vol. 9, 1906, p. 325

in thickness from 5 to 15 micra were made from paraffin-embedded tissue. The stains used for sections were Weigert's iron hematoxylin, Delafield's hematoxylin, and Giemsa's solution for Romanowsky staining. Smears from blood and the various organs were prepared, both dry and fixed in alcohol, formol alcohol, and methyl alcohol. Giemsa's and Wright's stains were found the most satisfactory for blood smears.

In drawing blood from living rats some difficulty is usually experienced. If a portion of the tail (about 1½ inches) is removed, sufficient blood for smears is readily obtained. If the blood examination is to be repeated daily, a slight manipulation causes the granulation tissue formed upon the stump to bleed freely. The increased number of leucocytes on account of the slight inflammatory reaction from the wound does not interfere with the detection of the parasite. Obviously blood obtained in this manner could not be used for differential or total counts of the white blood cells, in which instance it is necessary to remove another small piece from the extremity.

In handling the rats a pair of long curved surgical dressing forceps, with which the animal is grasped by the loose skin at the back of the neck, are very convenient.

Hepatozoon perniciosum in the Blood of White Rats.

When stained smears made from the blood of infected rats are examined with the microscope, large oval nucleated bodies are seen embedded in the protoplasm of leucocytes. The white cells affected are almost invariably large mononuclear lymphocytes, seldom transitional leucocytes, and very rarely polymorphonuclear leucocytes. The mononuclear and transitional cells of the rat's blood resemble very closely cells of the same type in human blood. The nuclei stain strongly with basic stains, and the protoplasm is free of basic staining granules. The nuclei of the former variety are almost always excentrically located in the cell protoplasm, and the parasite, when present, is usually situated beside the nucleus, where the protoplasm is most abundant. In transitional cells the parasite is often wedged in the angle formed by the bending of the nucleus. The white cells which contain parasites appear to suffer in no way by reason of their presence. Evidence of degenerative or other changes is lacking.

A marked increase in the number of leucocytes, especially of the large mononuclear variety, is invariably observed when parasites are present. This increase varies in direct proportion to the number of parasites in the blood. The blood in severe infections, shortly before death, strongly suggests that seen in human lymphatic leukemia, both in the fresh and stained condition. Nucleated red cells, which are present in small numbers in normal rat's blood, are often greatly increased in numbers. A series of actual and differential leucocyte counts of the blood of rats, with infections of varying severity, is given below.

Rat No.	Large lymphocytes containing parasites.	Number of parasites free.	Total white blood cells.	Large mononuclears.	Transitional.	Polymorphonuclears.	Eosinophiles.	Small mononuclears.
	Per cent.			*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	72.	2	36,000	62.	6.2	20.9	1.1	9.8
2	16	4	32,600	48	5.5	31.1	2.1	8.3
3	53.5	6	102,000	65.1	9.6	18.3	.8	7.2
4	30	0	16,000	51.3	4.5	31.2	.6	12.4
5	82.5	6	128,000	78.7	6.5	11.6	.3	2.9
6	39	0	28,500	45.8	6	39.6	.5	8.1
7	48	1	64,000	50.1	8.6	35.5	1.8	4
8	2	0	12,600	30.2	8.5	51	2	8.3
9	12	0	10,800	35.6	10.5	48	1.5	4.4
10	20.5	0	15,200	30.5	7	50.7	1.6	10.2

A number of red blood cell counts made upon the blood of heavily infected rats gave from 1,900,000 to 3,200,000 per cubic millimeter. Hemoglobin, with the Tallquist scale, from 20 to 60 per cent.

PARASITES IN FRESH BLOOD.

When a drop of blood is spread out thinly between cover slip and slide the parasites are readily made out in the leucocytes. They appear as large rod-shaped bodies with rounded extremities and are slightly more refractile to light than the leucocytes. No nucleus can be detected. In the fresh condition the parasite appears longer and more slender than when stained. It is often longer than the leucocyte in which it is contained, the protoplasm of the latter appearing to be stretched somewhat in order to encompass the parasite.

The first studies made upon *H. perniciosum* in the blood were limited to material obtained from naturally infected rats, in all of which the infection had existed for some time before the discovery of the microorganism. In the blood of these rats nearly all the parasites were in leucocytes, and very few were observed free. In rats artificially infected, and in which daily blood examinations were made from the beginning, conditions were found to be somewhat different at the inception of the disease.

The first parasites observed in recently infected animals were free in the blood plasma, few in number, and actively motile. Later, the common encysted forms appeared and the motile forms—the "vermicules"—disappeared. In a few rats, vermicules were found during the course of the disease and especially just before death. The vermicules, as observed, are slender bodies 15 to 16 micra in length, with clear delicately striated nongranular protoplasm, somewhat refractile to light. The anterior extremity, bluntly pointed, is always the one directed forward when in motion. The posterior extremity is narrower, rounded, and nearer the nucleus. The movements, size, and appearance of the vermicules are identical with those of vermicules formed from sporozoites, which will be described later in detail.

PARASITES STAINED.

In blood smears stained by Giemsa's or Wright's stain the parasites appear as elongated oval bodies, broader and shorter than in the fresh blood, measuring on the average 12 by 6 micra. The nucleus stains almost the same color as the nucleus of the leucocyte in which it appears. The protoplasm of the parasite stains pale blue (more strongly at the periphery), is uniform in character, and is free of granules. The nucleus usually occupies the middle third of the parasite; occasionally it is located at the extremity. In outline the common form is somewhat quadrilateral with rounded corners or is irregularly oval. Instead of being made up of chromatin granules the nucleus is composed of skeins or bands of chromatin, often arranged in a more or less parallel fashion at right angles to the parasite. It is probable that the nucleus is quite thin and flat, since parasites are frequently seen in which the chromatin is arranged in a single narrow band near and parallel to one side. Surrounding each parasite and separating it from the protoplasm of the leucocyte is a delicate cyst wall, which appears usually as a clear, unstained, narrow area of even thickness. The superficial layer of protoplasm of the parasite seems condensed. That the cyst is an integral part of the latter and not of the leucocyte is shown by the fact that free encysted forms are occasionally seen.

The behavior of the parasites toward stains is an additional evidence of the presence of a cyst wall. When less powerful stains are employed than those mentioned the parasite stains with great difficulty, although the leucocytes are strongly stained. In faintly stained specimens the parasites appear as colorless, refractile bodies, the interior being unstained.

It is noteworthy that in *H. perniciosum*, as in other hæmogregarines of mammals, the forms met with in the circulation—the trophozoites—are approximately equal in size. Moreover, the dimensions do not vary greatly in different animals or in the same animal at different times. This peculiarity is explained, as will be shown later, by the fact that the parasites are discharged only when fully grown into the circulating blood.

In stained specimens the free vermicules are slender, curved or straight, with blue staining protoplasm, devoid of granules and without a capsule. The nucleus is formed of several rounded chromatic bodies (pl. II). One extremity is often pointed, and the vermicule is longer and slenderer than the encysted parasite. The latter unquestionably originates from the vermicule and all gradations between the two are observed, especially in smears from the liver.

In the first stage the vermicule becomes broader and shorter, retaining the granular nucleus; next, the latter is changed to the bandlike or homogeneous form, either before or after being engulfed by a phagocytic mononuclear or transitional white cell; and finally a capsule is developed. The ultimate fate of the parasites is uncertain. In rats with a mild grade of infection the encysted trophozoites may be found in moderate numbers six to eight weeks or even longer after the onset of the disease; yet when the livers of such animals are examined no schizonts are found. From this it is evident that the trophozoites after becoming encysted may continue in the circulation, acting merely as foreign bodies, for some time after their formation. The pathogenic effects appear, as in other hæmatozoa, to be caused by toxic substances set free during the stage of multiplication.

In addition to the forms described, others are sometimes observed. These consist of slight variations from the usual motionless type, the trophozoite. Large oval or even spherical parasites are seen, with banded nuclei, sometimes free, more often in leucocytes, and rarely showing signs of a cyst wall (pl. II). Such parasites are common in liver smears, but rare in the circulating blood. No evidence of sexual differentiation of the trophozoites has been observed, unless the forms just described be regarded as sexually different from the usual type. It seems more probable that these large parasites are very young schizonts, which occasionally escape from the liver after partial development, into the blood stream.

NONSEXUAL MULTIPLICATION OF *H. PERNICIOSUM* IN THE RAT.

It has already been mentioned that the trophozoites found in the large lymphocytes are practically uniform in size and appearance. No form is seen which may be construed as young or partly developed. The explanation of this lies in the fact that multiplication and development into mature parasites takes place in the cells of a single organ, the liver. At an early stage of infection, when parasites are numerous in the blood, a section of the liver shows many parasites in various stages of schizogony. Similar forms have been found in no other organ, although carefully sought for. The trophozoites are especially abundant in the spleen, kidney, and brain, but there is no evidence of multiplication in these organs.

In the capillaries of the liver are many large lymphocytes containing encysted parasites similar to those encountered in the peripheral circulation. In addition, there are parasites whose contour blends with the protoplasm of the leucocyte and appears to be unprovided with a cyst (pl. II). Free parasites, some of which are large and oval in shape (pl. II), are also abundant.

The multiplication of the parasite takes place in a liver cell. The earliest stage is a small spherical form 10 micra in diameter, with a large vesicular nucleus composed of chromatin granules in a fine mesh work, and a large karyosome. It is embedded in the protoplasm of the liver cell, the nucleus of the latter being pushed to one side. The cavity formed in the liver cell is fully occupied by the parasite. As development proceeds the parasite increases in size and the nucleus divides into two. This stage is known as schizogony and the parasite becomes a schizont. Many small, round granules are found in the latter when viewed in the fresh condition. The two nuclei divide and subdivide until from 12 to 20 daughter nuclei are formed. The schizont increases in size and the liver cell becomes a mere shell, the nucleus of which, after undergoing the changes characteristic of degeneration, finally disappears. A delicate cyst wall is formed around the developing schizont, which, at maturity, is ovoid and measures, on the average, 25 by 30 micra. Some, however, are larger and may measure 28 by 35 micra.

The nuclei of the schizont are arranged at the two extremities, as shown in longitudinal sections (pl. III). In fresh cysts studied at this stage the central mass of protoplasm is granular, with a clear area at the ends. At a later stage short rods of clear protoplasm, each containing a nucleus, are seen at the poles. These rods increase in length, the proximal extremities growing toward one another; the central granular mass diminishes in size. Eventually the clear rod-shaped bodies have become fully grown merozoites and occupy the entire cyst except for a small granular "rest" body. When the merozoites are fully grown, the cyst containing them becomes soft and gelatinous; later it ruptures and the merozoites escape, becoming free moving vermicules. The number of merozoites is from 12 to 20; the average 16.

In scrapings made from the cut surface of a liver in which the schizonts and cysts are numerous, their structure and the ease with which they are ruptured is readily determined. In sections from tissue, fixed and embedded in the usual method, the contents of the cyst shrink to a greater degree than the liver tissue and artificial spaces are formed around the schizonts (pl. III, fig. 1).

In heavily infected livers the number of cysts is very large. Their presence is associated with extensive fatty changes in the liver parenchyma. When a small piece of fresh liver tissue is spread out thinly between slide and cover slip and examined with the microscope on a warm stage, the contents of the ripe cysts are seen to be in motion. The merozoites move slowly about, gliding over one another, like a mass of worms. Often such a cyst is seen to rupture and the merozoites escape. In this event they move about as free vermicules, bending and twisting and gliding about exactly as will be described later in the case of vermicules formed from sporozoites, from which they can not be distinguished. Some of these vermicules enter the liver capillaries and later the circulating blood; others enter fresh liver cells and repeat the process of multiplication.

In stained smears made from the liver the various stages in the transformation of the free vermicule into the encysted trophozoite are readily traced (pl. II). In the former the nucleus is composed of large chromatin granules and the parasite is slender and elongated. It then becomes contracted in length and oval in shape; still later the nucleus is disposed in the characteristic parallel skein arrangement, and finally a cyst develops, the parasite being engulfed by a large mononuclear lymphocyte. No evidence of sexual dimorphism of the schizonts has been noted. The merozoites, when released from the cysts, are fully developed and do not subsequently increase in size in the blood. The large mononuclear leucocytes are found in in unusual numbers in the liver, in the sinuses of the lymphatic glands, the thymus, spleen, and bone marrow.

There seems to be little doubt that the vermicules, which are taken in by leucocytes in the liver and elsewhere and become encapsulated trophozoites, undergo no further development in the rat. Those vermicules which escape the phagocytic action of these cells and enter other liver cells repeat the process of schizogony. The majority perhaps are carried into the peripheral circulation in the leucocytes.

The liver cysts and merozoites are almost exactly duplicated in size and appearance by the sporocysts and sporozoites in the body of the intermediate host, to be described later. Encysted trophozoites are especially abundant in the spleen, lungs, kidneys, and brain, in the blood vessels, and lymph spaces.

CLINICAL SYMPTOMS IN WHITE RATS.

When the infection is slight, the rat often presents no evidence of disease. For a month or so the blood may show only a few parasites encysted in the leucocytes. This type of the disease is more common in old rats.

Frequently the disease begins gradually, with a few parasites in the blood. With the gradual increase in the amount of infection the animal becomes more and more anemic. It may continue in this state for two or three weeks and then gradually recover, or death may occur. Other animals, especially those experimentally infected, may die three or four days after the appearance of parasites in the blood; anemia rapidly becomes marked and the number of leucocytes and parasites is very great. Occasionally many free vermicules are observed shortly before death.

In rats with heavy infection there is marked apathy, little food is taken, and the animal sleeps constantly, with the head tucked under the thorax.

The anemia is most striking. The ears and skin, as well as the mucous membranes, become pearly white. When blood is drawn from the tail it is thin and watery, and it is very difficult to make a smooth smear. When death occurs after a long period there is great emaciation. The temperature may rise as high as 38.6° C.

Shortly before death there is often diarrhea, with an occasional admixture of blood, and the temperature is subnormal. In one animal hemoglobinuria was observed.

Lethality.—Of the 17 rats originally found infected, 4 died. However, 4 which showed severe infection were chloroformed to provide material for inoculation and fresh tissue for sectioning. Of 16 rats infected experimentally, 8 died—a lethality of 50 per cent. Young rats appear to be most susceptible and the majority of the fatal infections were in animals not fully grown. In half-grown rats the disease is almost invariably fatal in a few days after the onset of symptoms.

PATHOLOGICAL ANATOMY.

The gross lesions are most marked in animals which succumb to infection. In chronic infections the animals are much emaciated. The muscles and mucous membranes are extremely pale. The heart's blood is thin and watery. The lungs frequently show minute hemorrhages upon the surface. The spleen is greatly enlarged and dark in color; it may be five or six times the normal size, and frequently reaches from the diaphragm diagonally across to the right side of the brim of the pelvis. The small intestine occasionally contains thin fluid blood. The mesenteric glands are enlarged, sometimes hemorrhagic. The liver is distinctly enlarged, dull yellow in color, sometimes mottled, and the thin borders are rounded. Smears and frozen sections show extensive fatty changes. The kidneys show moderate parenchymatous degeneration. The urine rarely contains hemoglobin; more often bile pigment. The bone marrow is red.

THE ARTHROPODA AS AGENTS IN THE TRANSMISSION OF DISEASE.

The sexual cycle of *Hepatozoon perniciosum* takes place in a parasitic mite (an acarien), *Lelaps echidninus* Berlese. Mr. Nathan Banks, of the United States Department of Agriculture, has kindly made the determination. From a careful search of the literature it appears that this is the first recorded instance in which a member of the family *Gamasidæ* has acted as the intermediate host for a protozoan parasite.

So far as accurate knowledge extends, when a parasitic protozoon requires two hosts for the completion of its life cycle one of these hosts will be found to belong to the phylum Arthropoda. An apparent exception exists in *Hæmogregarina stepanovi* in the turtle (*Emys orbicularis*), the invertebrate host of which is a leech (*Placobdella catenigera*).[a]

Of the five subdivisions or classes into which the phylum is divided, two—the Insecta and the Arachnida—include the species which act as intermediate hosts.

Of the insects which serve as parasitic hosts and agents of transmission for pathogenic protozoa the mosquito is the best known, and the malarial parasite the only form which has been accurately worked out in all stages.

The life cycle of malaria in birds (*Proteosoma grassii*) is almost as well worked out as malaria in man. Quite recently Christophers has described the complete sexual cycle of *Piroplasma canis* in the dog tick, *R. sanguineus*.

Of insects, a number, including biting flies, lice, bedbugs, and fleas, are known to be concerned in the transmission of blood parasites. Some of these have been proved to be the agents of transmission, but whether as true intermediate hosts or merely carriers is as yet undetermined, in many instances, the sexual cycle being unknown.

In the class Arachnida the ticks have constituted, up to the present time, the only group capable of transmitting protozoan disease. The number of known tick-borne diseases is increasing and they are of great importance. In an epoch-marking work upon Texas cattle fever Smith and Kilborne,[b] in 1893, were the first to show the part played by the Arthropoda (in this instance ticks) in conveying disease. Various forms of piroplasmosis, relapsing fever, Rocky Mountain spotted fever, and *Leucocytozoon canis* have been shown to be transmissible by ticks.

Until quite recently it was believed that ticks transmit infection only through their progeny; i. e., only ticks derived from the eggs of infected mothers have power to inoculate susceptible animals. Recently it has been shown that infection with certain kinds of piroplasma is taken during the nymphal stage, but is transmitted only by the adult tick.

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[a] See p. 9, ref. b.

[b] Smith, Th., & Kilborne: Texas fever. Bull. No. 1, Bur. Animal Indus., U. S. Dept. Agric., Wash., 1893.

PARASITIC MITES.

Since but little is generally known of the nature and structure of mites, a brief account of their habits and a detailed description of the anatomy of the mite (*Lelaps echidninus*) which is concerned in the transmission of *H. perniciosum* is given below.

The Acarina, an important group of the Arachnida, comprise the mites and ticks, the former being greatly in the majority as regards number of species. The term "mites" is often applied to the entire group of Acarina. In addition to their arachnid characteristics (the absence of antennæ and the possession of four pairs of legs) the Acarina are distinguished by the absence of a division into thorax and abdomen, and by the arrangement and structure of the mouth parts, which are adapted for puncturing and sucking. The larva resembles the parent except in the possession of three pairs of legs and the absence of sexual organs. After casting the skin two or more times (ecdysis) the adult stage, with four pairs of legs and fully formed sexual organs, is attained.

Canestrini's[a] classification, from a scheme devised by Kraemer, divides the Acarina into six subheads:

Astigmata (Sarcoptic and vermiform mites).
Hydracarina (Water mites).
Prostigmata (Harvest mites).
Cryptostigmata (Orbatid mites).
Metastigmata (Ticks).
Mesostigmata (Gamasid mites).

A number of classifications varying in slight degree have been based upon this original. The following classification of the Acarina is from Neumann:[b]

Nonvermiform Acarina	Legs inserted directly into the integument without epimeræ	Legs with five articles			*Oribatidæ*.
		Legs with six articles	With tracheæ. Cheliceræ	Provided with hooklets	*Ixodidæ*.
				Didactylous or styliform	*Gamasidæ*.
			With no tracheæ (marine forms)		*Halacaridæ*.
	Legs articulated on distinct epimeræ	Natatorial legs (aquatic)			*Hydrachnidæ*.
		Ambulatory legs	Chelicerae styliform; palps free, antenniform		*Bdellidæ*.
			Chelicerae styliform or in claws; palps free, ravishers		*Trombididæ*.
			Chelicerae didactylous; palps cylindrical or conical and partially adherent to the tips		*Sarcoptidæ*.
Vermiform Acarina	Legs with five articles				*Phytoptidæ*.
	Legs with three articles				*Demodecidæ*.

Of these ten families only five are parasitic—the *Ixodidæ* (s. l.), *Gamasidæ*, *Trombididæ*, *Sarcoptidæ*, and *Demodecidæ*. Many of these are commensals, obtaining nutriment from the organic detritus,

[a] Canestrini, S.: Prospetto dell' acarofauna Italiana., Padova, 1885–1897.

[b] Neumann, L. G.: Traite des maladies parasitaires non microbiennes des animaux domestiques. Transl. by Fleming. Ed. by J. McQueen. 2d ed. New York, 1906.

epithelial scales, hair, feathers, etc., of the host. The last two families are chiefly of interest to the veterinarian as producing various forms of mange. Only two families are blood-sucking, the *Ixodidæ* (s. l.) and the *Gamasidæ*. The rôle of the former (ticks) in the transmission of disease has already been mentioned. A few species of the *Trombididæ* are known to attack man; a larval form (*Leptus autumnalis, L. americanus*) is the well-known "red bug" or "chigger," also known as the "harvest mite" in England and the "bête-rouge" in South American countries. In Japan the Tsutsugamushi disease or Kedani disease, frequently fatal, is due to the bite of the "akamushi" or "red mite." This disease has recently attracted considerable attention; the etiology is unknown.

Among the *Gamasidæ* the "chicken mite" or "red spider louse" frequently bites those who visit houses in which fowls are kept. The *Gamasidæ* form a larger and more widely distributed family than the *Ixodidæ* (s. l.), but by reason of their small size and apparent harmlessness they have been but little considered. A number of species live on decomposing vegetable and animal matter, others act as commensals upon beetles, reptiles, birds, and small mammals. *Gamasidæ*, which are true bloodsuckers, are found upon lizards and snakes (*Ophionyssus natricis* Megnin); upon vipers and water adders they attack the cornea, rendering it opaque, the reptiles becoming blind in consequence and dying of starvation. *Dermanyssus gallinæ* is a great pest to poultry and horses and even attacks man, withdrawing blood through the punctures made by its pointed mandibles. A number of species infest sparrows, canary birds, and parrots. A great number of small mammals are affected, including rats, field mice, moles, rabbits, etc. Upon bats a very large species (*Pteroptus vespertilionis*), popularly believed to be a bedbug, is found. The number of species of *Gamasidæ* is legion, and doubtless when more attention is paid to this family many others will be discovered.

THE GAMASIDÆ.

The following brief description of the structure and habits of the *Gamasidæ* is taken in part from Banks.[a]

In general the *Gamasidæ* are broad and flat, with a chitinous integument. Eyes are absent, but numerous sensory "hairs" on various parts of the body make up for this deficiency. Many forms are rapid in movement when disturbed. The mouth parts in many species can be withdrawn into the body. The head or capitellum can also be considerably retracted. The mandibles, two in number, are usually chelate, with toothed fingers. In some mites they are

[a] Banks, N.: A treatise on the acarina or mites. Proc. U. S. Nat. Mus., vol. 28, pp. 1–114, 1904.

styliform. In the male the mandibles usually present specific differences.

Beneath the mandibles is a projection called the hypostome. Sometimes an epistome is present above the mandibles. Projecting from either side of the head or capitellum are the palpi, which are provided with sensory organs.

The inferior surface of the *Gamasidæ* is provided with a varying number of chitinous shields or plastrons, upon which are located the genital apertures. The legs are six jointed, being divided into coxa, trochanter, femur, patella, tibia, and the terminal tarsus, provided with hooks or sucking disks. The female genital opening is usually between the sternal and genital plate. In the male it is in the sternal plate.

The act of coition is very remarkable and has been described in detail by Michael.[a] Briefly, it consists in the deposition by the male of a mass ot spermatozoa (often contained in a delicate cyst) into the spermatheca, a sac connected with the uterus in the female. The spermatozoa upon liberation fertilize, from time to time, the ova as they become ripened. *Gamasidæ* lay comparatively large eggs, from which, with a few notable exceptions, six-legged larvæ are hatched. In the genus *Lelaps* the eggs, when deposited, are fully one-eighth the size of the female. One egg only is deposited at a time. It contains a fully formed embryo with six legs and fully developed nervous and digestive system. Very soon after expulsion from the female the delicate membrane or shell which incloses the embryo is ruptured and a six-legged larva appears. After several moultings of the skin the adult is formed. The immature mites are termed nymphs and, according to the number of moults gone through, protonymphs, deutonymphs, tritonymphs, etc. In the nymphal stage many of the *Gamasidæ* are migratory. Often they attach themselves to beetles, mosquitoes, and other insects, it is believed for the purpose of distribution.

LIFE HISTORY OF *LELAPS ECHIDNINUS*.

The species of *Lelaps* (*L. echidninus* Berlese) found upon white rats is a true parasite and sucks the blood of the animal. Its habits differ greatly from those of ticks in that its depredations are constantly repeated and only a small amount of blood is taken at each feeding. The mite is but slightly distensible, hence its capacity is limited. The following biological observations have been made in connection with the studies on *Hepatozoon perniciosum:*

When rats are kept in a cage of some size, in which there is an abundance of straw, the mites appear to feed almost exclusively at night and during the day they leave the host and live in the damp

[a] Michael, A. D.: British Tyroglyphidæ. Ray. Soc. I, 1901, 291 pp.; II, 1903, 183 pp.

straw where the eggs are deposited. Hence when a number of rats are in the same cage the mites may visit a different animal each night. It is an easy matter to cause the mites to get upon the rats in the daytime by stirring the straw about in the cage and making the rats move about. In small cages, such as the glass jars used in the laboratory for holding single animals, the mites behave differently, especially if sand or sawdust is placed in the bottom. The rat moving about frequently in the narrow cage keeps the mites constantly disturbed, and they remain upon their host all the time and may be found in large numbers upon the back of the animal near the root of the tail. The female mite after feeding leaves the rat to deposit her eggs, which are laid one at a time, in damp straw. Each egg contains a well developed embryo. After hatching, the six-legged larva in a few hours moults and becomes a nymph with eight legs. The nymphs, though active, are seldom found upon rats and do not appear to suck blood. The nymphs after two moultings become adult. The young adult females (at first colorless) seek the host, and draw blood from small wounds made by the maxillæ or mandibles. When fully grown they are larger and brown in color. The males do not suck blood, but feed upon animal or vegetable detritus. They are few in number upon rats. The average length of life of the female mite is about six weeks. During this period a number of feedings of rat's blood are necessary to provide material for the development of the embryos.

EXTERNAL ANATOMY OF *LELAPS ECHIDNINUS*.

TECHNIQUE.—In view of the small size of the mite nearly all the details of structure of the exo-skeleton may be determined by examination with low powers of the microscope after a few minutes immersion of the live mite in alcohol.

For brief study the specimens may be mounted in water on a slide with a cover glass. For examination with higher magnifying powers mites must be macerated in caustic potash to render them more transparent. The details of the mouth parts may often be studied in live specimens to advantage, the capitellum being then more markedly protruded.

GENERAL BODY CONSTRUCTION.—The head region or *capitellum* (pl. IV) is distinctly separated from the thorax and contains the mouth parts and certain appendages. The fused thorax and abdomen are covered dorsally by the *scutum*, a single chitinous plate. On the inferior or ventral surface are three chitinous plates—*sternal*, *genital*, and *anal*. The sternal plate serves as a point of attachment for the legs. The latter are eight in number (four pairs) and are composed of six articles, terminating in two hooklets and a sucking disk.

The capitellum is provided with paired sharply pointed *maxillæ*, and it incases for a portion of their length the paired *mandibles*, which are provided with didactylous pinchers in the female. In the male they are modified to form "clasping" organs. The *maxillary palps* are attached, one on either side, to the lateral aspect of the capitellum. They serve as sense organs, eyes being absent, and are composed of five articles. Two *stigmata* are present, with a prolongation forward of a tubular *peritreme*. The *anus* is located in an anal plate, the anterior margin of which is convex and the posterior prolonged into a large spine. In the female the *sexual aperture* is located in the mid-line of the body in the genital plate, near the posterior border of the sternal plate. In the male the *genital pore* is in the center of the sternal plate. The body of the adult female measures from 1.1 to 0.9 mm. in length by 0.7 to 0.6 mm. in breadth. The male is two-thirds the size of the female.

INTERNAL ANATOMY OF *LELAPS ECHIDNINUS*.

TECHNIQUE.—The difficulty of dissecting so small an object as this mite, especially as it is covered with hard chitinous plates, is very considerable. With the aid of fine needles, teased preparations can be made, a small per cent of which will be fairly successful. In the main, serial paraffin sections must be relied upon. The fixatives used were 5 per cent formalin, 80 per cent alcohol containing 5 per cent of formalin, and alcohol in various strengths. In addition, a fixative composed of 3 parts chloroform, 2 parts glacial acetic acid, 1 part saturated solution of bichloride of mercury, and 6 parts absolute alcohol was employed. In some instances soaking in a solution of 2 per cent celloidin preceded the embedding in paraffin.

Some satisfactory sections parallel to the ventral surface were made without softening the chitin, since none is encountered by the knife. For other sections in which the chitinous plates are to be cut, the mites, previously exposed to the action of fixatives, must be treated with a softening solution. For this purpose Labaraques solution in varying dilutions was employed. The length of time of its action depends upon the strength of the reagent and can only be determined by trial. It was found preferable to merely soften the chitin rather than to completely remove it. In the latter event, air bubbles form in the body cavity, which greatly interfere with subsequent dehydration and embedding.

The sections, affixed to slides with Mayer's albumin fixative, were stained with Weigert's iron hematoxylin.

ANATOMY OF THE HEAD REGION.—The capitellum is prolonged anteriorly to form the mouth parts. The dorsal anterior border forms a serrated projection, the *epistome*. The mandibles, which are double jointed and provided with muscles, terminate in pinchers with serrated jaws in the female. In the capitellum they lie in chitinous sheaths and are prolonged posteriorly, to be attached by tendinous expansions to the under surface of the scutum. Muscles attached to the same point serve to retract or protrude the mandibles.

The ventral aspect of the capitellum terminates in a bifid *hypostome* or maxillæ (pl. VI). The maxillæ are situated, one on either side of the mouth opening and form part of its lateral boundary. They are acutely pointed and serve as piercing organs. Between and dorsad of the maxillæ is a single elongated organ, the *lingula* or tongue (pl. VI), which presents a median groove. Its surface is covered with minute papillæ. The outline of the mouth opening is somewhat triangular, the borders being formed by fringed membranous folds.

THE PHARYNX.—The *pharynx* is situated just posterior to the mouth, in which it terminates. It lies almost entirely in the capitellum, forming a canal in the lower portion. It is T shaped (pl. VII, fig. 2) on cross section and its walls are connected by a series of transverse muscular bands with the chitinous covering of the capitellum. These muscles serve to pull apart the walls of the pharynx and produce suction. Interspersed with the transverse muscles are a series of circular muscular bundles (pl. VII, fig. 1) capable of constricting the pharynx.

THE ESOPHAGUS.—Posteriorly the pharynx terminates in a slender tube, the *esophagus* (pl. VII, fig. 1). This in turn communicates with the *ventriculus* or stomach. Surrounding the esophagus is a large *ganglion* or brain.

The ventriculus is a thin-walled sac consisting of a central and two lateral portions (pl. VIII, fig. 2). The former communicates anteriorly and inferiorly with the esophagus. The diverticula are prolonged anteriorly and posteriorly and communicate with the lateral aspect of the ventriculus. The wide central portion (pl. VII, fig. 1) extends from the brain to the middle third of the thorax, resting upon the inner skeletal plate. Regarding each lateral diverticulum as two projections (anterior and posterior) from the ventriculus, there are in addition two others, viz, the median anterior projection to join the esophagus and a posterior projection terminating in the *colon*, which, in turn, empties into the *excretory bladder*. When the uterus contains a large embryo the ventriculus is somewhat distorted and displaced.

The ventriculus and its lateral prolongations are lined with a single irregular layer of large epithelial cells with faintly staining reticular and vacuolated protoplasm and inconspicuous nuclei. The cells rest upon a delicate basement membrane. Encircling the digestive tract are a series of minute parallel muscular fibers, each one isolated and with a single nucleus. These fibers serve to constrict the ventriculus and its diverticula, and in young live mites with transparent scutum the waves of contraction, beginning anteriorly and sweeping backward, are easily discerned. Adherent to the lining layer in some places, but often free in the lumen of the ventriculus, are large spherical cells, sometimes called "liver" cells. They possess very small faintly staining nuclei, excentrically located, and are phagocytic in nature, often containing cell inclusions of various kinds, vacuoles, and yellow pigment. According to Berlese[a] they secrete a digestive ferment and play an important part in the digestive process. That portion of the body not taken up by the digestive and other organs is occupied by muscles which move the legs and mandibles and by fat cells.

The *colon*, a short, thin-walled tube, terminates in an *excretory bladder*, which communicates with the anus. Opening into the lateral aspect of the excretory bladder are paired tubes—the *malphigian* vessels—which run forward parallel beneath the ventriculus almost to the capitellum, to end blindly. They serve as excretory organs, and often contain small dumb-bell shaped crystals of mineral salt.

The *salivary* glands are paired structures in the body cavity, situated beneath the anterior extremity of the scutum (pl. VII, fig. 1). Each gland is pyramidal shaped and composed of several acini, which are formed of large cells of coarse granular structure, with small, indistinctly defined, deeply staining nuclei. The apex, which is below, terminates in a minute duct which empties into the posterior part of the pharynx. Each acinus contains a central cavity, which varies in size according to the activity of the gland. Small accessory glands are sometimes observed.

SEXUAL ORGANS.—The *ovary* is an irregularly shaped, oval body situated in the posterior portion of the body. It is composed of a reticular framework, in which are many young *ova* and small cells which form the stroma. Projecting from its anterior and lateral aspects are ova in various stages of development. Three or four ova in advanced stages of development, one of which is fully ripened, may usually be observed.

In adult females the *uterus*, a thin-walled sac lying in front of the ovary, usually contains an embryo which may show complete development. The ripe ovum is fertilized in the uterus, which communicates by a narrow vagina with the genital aperture. The latter is covered by finger-like sensory organs (pl. IV, fig. 2). Attached to the top of the ovary are peculiar tubular structures—the *lyrate organs*—to which are connected a pear-shaped sac (*sacculus fœmineus*) contain-

[a] Berlese, A.: Ricerche sugli organi e sulla funzione della digestione negli acari. Riv. di Pat. Veg., Terenzi; vol. 5 (5–8), 1896, pp. 129–195.

ing spermatozoa. The lyrate organs pass on either side of the uterus to terminate upon the inner aspect of the acetabula of the coxæ of the third pair of legs.

Since only the female mite is directly concerned in the transmission of *Hepatozoon perniciosum*, the anatomy of the male sexual organs will be only briefly considered. The *testis* is a single oval body situated posteriorly between the ventriculus and the excretory bladder. The paired *vasa deferentia* pass downward from each lateral aspect, then forward near the ventral surface, to terminate in the single genital opening in the center of the sternal plate. The *spermatoza* are large, round or elongated, granular structures, and are usually found in the sacculus fœmineus in the female. Here they are stored until the ova, becoming ripened one at a time, are fertilized.

TRACHEAL SYSTEM.—The stigmata are placed on the lateral aspect of the body, between the third and the fourth pair of legs. A tubular prolongation forward, the *peritreme* or *stigmal canal* (pl. IV, fig. 2) divides into the main tracheal tubes, which ramify throughout the body, some terminating in the legs. The tracheal system is absent in the larvæ.

The following articles may be consulted for a more detailed account of the structure of the *Gamasidæ:*

Winkler, W.: Anatomie der Gamasiden. Arb. a. d. Zoolog. Inst., Wien, vol. 7, part 3, 1888, pp. 1–38.

Berlese, A.: Recherche sugli organi e sulla funzione della digestione negli acari. Riv. di Pat. Veg., Firenzi, vol. 5 (5–8), 1896, pp. 129–195.

Michael, A. D.: On the variations in the internal anatomy of the *Gamasidæ*, especially in that of the genital organs, and on their mode of coition. Trans. Linnean Soc. of Lond., 1892.

Mégnin: Memoire sur l'organization et la distribution zoologique des acariens de la famille des *Gamasides*. Journ. d. l'Anat. et de la Physiol., 1876.

Kramer, P.: Ueber Gamasiden. Arch. f. Naturg., vol. 1, 1882.

Michael, A. D.: British Tyroglyphidæ. Ray. Soc., I, 1901, 291 pp.; II, 1903, 183 pp.

SEXUAL CYCLE OF *HEPATOZOON PERNICIOSUM* IN THE MITE.

Mites removed from rats (in whose blood parasites were abundant) were found, in the majority of cases, to be infected with *H. perniciosum*. Such mites, having fed upon the rat's blood from time to time, usually showed the parasites in several stages of development. Cross sections of mites pictured in pls. IX and X show this condition. Frequently, when many fully developed parasites were present, young forms also were observed in the stomach, indicating a fresh infection with each feeding of blood. Mites which were heavily infected with fully developed parasites could often be distinguished by the naked eye from normal mites. In the former the lateral and caudal portion of the body is distended and pearly white, whereas in the latter this part is dull brown in color.

In order to determine the length of time required for the various stages of development of the parasite, as well as to study them in detail, a number of mites were artificially infected, or, more properly, the usual manner of infection was controlled. For this purpose it was necessary to have a supply of normal mites. Fortunately these could be readily obtained. The stable in which the infected rats had been kept contained a large room in which were a number of rat cages. In two corners large single isolated cages had been built. One of these contained infected rats; the other had been emptied of rats about two weeks. Upon examination both were found to contain damp straw and grain, upon which were myriads of mites. In the empty cage the mites were found to be free of infection. Six healthy rats, fresh from the dealer, were placed in this cage. In a very short time each rat was infested with a large number of mites.

To remove the mites, which run about with considerable rapidity upon the hair and skin, the rats are seized by the loose skin at the nape of the neck with the tip of a pair of long clamps. The tail of the animal is grasped with one hand and by traction the body is extended along the blades of the forceps. In this position it is easy to remove the mites with a fine-toothed comb, from which they are quickly shaken by a tap of the finger into a large glass jar. The inside of the rim of the jar should be previously smeared with a ring of vaseline, which the mites will not traverse. In this manner it is possible in a short time to collect several hundred.

The mites are kept in the jar several days to starve them and then placed upon infected rats. For this purpose four rats were selected in whose blood the parasites were very numerous. To transfer the mites to a rat it is only necessary to place the latter in the jar for a few minutes. In a short time all the mites will have crawled upon the rat and concealed themselves in the fur.

After six, twelve, eighteen, twenty-four, thirty-six, and forty-eight hours, mites were removed and examined in fresh preparations and stained smears, and a number preserved in fixing solutions. From these paraffin serial sections were made. After forty-eight hours a large number of mites were removed and placed upon healthy rats in order that the parasites then present in the mites might develop without admixtures of younger stages, which would have been the result of continued feeding upon the diseased rats. At intervals of twenty-four hours a number of these mites were removed and examined and sections made. Successive collections of mites were fed upon infected rats in order to obtain a large number of infected mites for later experiments.

In order to study the effect of the digestive juices of the mite upon encysted parasites in the rat's blood, the following experiment was performed a number of times. The various stages of the process could be conveniently followed with the microscope for an hour or more.

Blood from a rat containing numerous parasites encysted in lymphocytes was mixed with the expressed body juices of mites, placed upon a slide, and examined with the microscope. In the course of ten to thirty minutes the protoplasm of the lymphocyte was partly dissolved and the parasites were set free.

The encysted parasites, after becoming free from the leucocytes, are slender, elongated bodies, rounded at the extremities. They show no movement or change of form. After thirty minutes or longer the delicate cyst wall surrounding them is ruptured and the parasite becomes an actively motile vermicule. The remains of the cyst, as a transparent shadow, often continues to be attached to the vermicule for some time.

The same changes may be observed when a mite that has just fed upon blood is crushed and the expressed fluid examined.

When mites starved for forty-eight hours or longer are examined six hours after being placed upon heavily infected rats, numerous free vermicules are found in the stomach and its diverticula. When these mites are crushed under a cover slip in a drop of 0.3 per cent salt solution, the vermicules and a considerable number of polymorphonuclear leucocytes are observed. Other kinds of leucocytes appear to be quickly destroyed. Not infrequently the remains of the delicate cyst which inclosed the parasite is seen attached to some portion of the vermicule. In form and appearance the vermicules do not differ from those developed from the sporozoites. The movements, however, although fairly active at first, become quite sluggish, and in mites examined eighteen hours after feeding, motile forms are seldom seen.

The red blood cells disappear very quickly after the ingestion of the blood, but hematin crystals, polymorphonuclear leucocytes, and free parasites are abundant. The polymorphonuclears are especially resistant and appear to remain unchanged after a number of hours in the digestive tract of the acariens. The parasites become detached from the lymphocytes when these are digested. In mites examined twenty-four hours after feeding, the digestive fluid contains single and paired vermicules in which movement has ceased.

The form and arrangement of the paired vermicules is peculiar and characteristic. In the earliest stages they are somewhat spindle shaped and two extremities are in apposition (pl. XIII, fig. 1). This constitutes the earliest phase of conjugation or sexual union. A little later the vermicules are placed side by side and parallel, the extremities even. In appearance they are exactly similar, and hence without distinguishing sexual characters. In slightly later stages the adjoining sides are quite straight and parallel and separated by a slight interval (pl. XIII, fig. 2), although apparently attached in some manner, since their relative position is unchanged by movement of the fluid containing them. Next the two become more rounded and increase slightly in size. The nuclei are large and vesicular and the dividing line still persists (pl. XIII, fig. 3). Later one vermicule grows longer and larger and begins to partly encircle the smaller, which becomes more rounded (figs. 4–7). They may now be properly regarded as gametes. By analogy the smaller and more finely granular is the microgamete, the larger and more coarsely granular the macrogamete. Eventually the line of demarcation between them disappears. The zygote thus formed by fusion of the male and female elements, is slightly elongated, tapered at one extremity, with granular protoplasm and two large vesicular nuclei. The latter approach one another, touch, and become blended. After the union of two sexual elements in the manner described the zygote becomes more elongated (figs. 11–13) and exhibits very slow movements of flexion and extension and something resembling ameboid motion. The term ookinet is applied to such a zygote.

The ookinet continues to increase in size and becomes more and more markedly granular, especially around the nucleus, which contains a small karyosome. When first formed the ookinet measures about 10 by 25 micra. It does not appear to detach any of its substance nor does it contain pigment. From this point development is rapid. The ookinet becomes an elongated or oval body with granular protoplasm and a vesicular nucleus containing a moderate sized karyosome (fig. 13). The nuclear network is quite distinct in both fresh and stained preparations. In mites forty-eight hours after infection the ookinet has reached full size, which averages about 25 by 50 micra.

In sections of mites made at this period the ookinets are fairly numerous in the lumen of the gut and appear in many instances more or less curled up (pl. XII). A similar form is noted in fresh preparations, as well as short spindle and ovoid shapes. They often appear somewhat flattened and occasionally very long and slender. In mites, three and four days after feeding on infected rats, many of the ookinets have disappeared, having doubtless been passed out of the intestinal tract with the feces. A few, however, are adherent to the epithelium of the gut or embedded in the gut wall between the epithelial cells. The protoplasm is distinctly granular and stains much more deeply with iron hematoxylin than do the cells lining the digestive tract. The karyosome is prominent and deeply stained. Some parasites are spherical and lie directly upon the basement membrane, surrounded by epithelial cells. Others are seen outside the stomach wall in the body tissues of the mite. They are granular and deeply colored by basic stains.

The exact manner in which the basement membrane is penetrated by the parasite is unknown. Probably the contraction of the delicate muscular envelope of the alimentary sac causes the parasite to be forced through the spot, weakened by its encroachment upon the basement membrane. At any rate the ookinets are found free in the body cavity. As shown in the description of the anatomy of the mite the body cavities represent the numerous small spaces between the various organs, and between these and the chitinous body envelope. In any of these spaces, in the investing membrane of the salivary glands, or within the sheaths of the muscles inclosed in the body of the mite, the ookinets may be found.

After thus wandering about in the body tissues the ookinets become stationary and undergo a remarkable change. Coincident with an increase in size of the plasma, in which large round granules begin to appear, the karyosome becomes greatly enlarged, exceeding in size the original nucleus. The nuclear membrane is but slightly larger than this giant karyosome, which may measure 10 micra in diameter. When the parasite has reached a diameter (in the fresh specimen) of about 60 to 75 micra, it becomes oval or spherical and a delicate cyst wall makes its appearance. This stage is observed in mites four and five days after infection. It is evident that the large karyosome is the forerunner of active multiplication of the nucleus of the encysted parasite, which is now termed a sporont (Lühe) and the cyst an oocyst.

In sections of mites showing more advanced stages, the parasite often appears oval or flattened by reason of the restraining action of the various organs or tissues upon its growth. In fresh dissections of mites the sporonts are conspicuous by reason of their size, spherical form, and granular structure (pl. XIV). The cyst wall is often very much larger than the parasite it contains, the latter appearing to float freely within it. The sporonts at this stage measure from 100 to 150 micra in diameter. Upon reaching approximately this size the nucleus undergoes division into two. The division is repeated and the daughter nuclei migrate to a position near the surface of the protoplasm, which forms a perfect sphere (pl. XIV, figs. 1–2). Nuclear division continues until a large number of nuclei are formed, all located in a superficial layer of the protoplasmic mass. The oocyst reaches a maximum diameter of 200 to 250 micra, although usually somewhat smaller.

In mites infected six or seven days previously the surface of the sporont is covered with budlike projections (pl. XIV, figs. 3–4), each of which contains a nucleus. A little later the buds become more prominent and elongated and break off from the central mass. A large number (50 to 100 or more) of oval bodies are thus formed, each of which contain a nucleus. These bodies represent sporoblasts and measure about 10 by 15 micra.

When obtained in the fresh condition, by gently teasing apart mites in 0.3 per cent salt solution the sporonts are perfectly spherical or ovoidal (pl. XIV), with a delicate cyst larger than the sporont. In the large budding forms (pl. XIV) the central granular part becomes softened and gradually liquefied, the stored-up material being utilized in the growth of the sporoblasts. Very slight pressure is sufficient to rupture the sporont, causing the escape of the fluid contents and the collapse of the superficial portion with the attached sporoblasts. In sections this collapsed condition always exists, and the flattened sporonts on cross section show usually two rows of sporoblasts with the attached ends adjoining, suggesting a fern leaf in appearance (pl. XVII, fig. 5; and pl. XI).

The subsequent development of the sporoblasts is traced in various stages in pl. XVIII. The nucleus divides and subdivides, the daughter nuclei arranging themselves at the poles. A little later a cyst wall is formed around each sporoblast.

The nuclei become the nuclei of the sporozoites, the protoplasm of which is split off from the central mass as short rods (pls. XVI and XVIII), which increase in length and gradually envelop the diminishing central "rest-body," like fingers. With increasing growth of the sporozoites at the expense of the "'rest-body," the latter is eventually reduced to a small, round, granular mass. The sporozoites fill the interior of the cyst, which measures on the average 25 by 30 micra. They are arranged in two general groups, occupying the two halves of the cyst, as shown in pl. XVI, fig. 12–14, the adjoining extremities of sporozoites interlocking. This arrangement does not always persist, however, since later they may be mixed up indiscriminately and retain merely a general direction corresponding to the long axis of the cyst. When the cysts are ruptured in salt solution, the individual sporozoites appear as cresent or club-shaped bodies (pl. XVI, fig. 1–2). The nucleus, spherical in form, is located nearer the smaller extremity. The thicker and more rounded end, as will be seen later, is the anterior. The protoplasm is highly refractile. The measurements are 14 micra in average length, by 5 micra in greatest breadth, tapering to 2 micra at the narrow posterior extremity.

The rest body persists as a round granular body about 6 micra in diameter. The fully developed cysts are found in mites from ten to twelve days after feeding. The number of sporozoites is from 12 to 20 in each cyst; 16 is the average; occasionally as many as 24 are encountered. The cysts which contain the larger number are elongated and often distinctly bent in the middle. In addition to these, small spherical cysts containing about 10 sporozoites are sometimes met with. The sporozoites in such cysts do not appear to differ from those in the other cysts mentioned.

When ruptured by pressure, the cysts split in the direction of greatest length, the edges curling up. In sections of mites (pls. IX, X, XIX) showing large oocysts containing sporoblasts or sporocysts the outlines of the large cysts are somewhat irregular and not clearly outlined. Also, the varying effect of the pressure of surrounding organs and the shrinkage and distortion in fixation and embedding cause the large cysts to assume a variety of forms other than spherical.

The invasion of the salivary glands by the cysts is particularly interesting. In some specimens the gland on one or both sides has in part or entirely disappeared and been replaced by the parasites.

The sporozoites have never been found free, however, in such sections nor in fresh preparations, but always inclosed in the sporocysts. The possibility of the transmission of the parasite through the salivary glands and their appendages will be considered later.

In the study of the various stages which have just been described sections from a great number of mites and fresh preparations from hundreds of both normal and infected mites have been utilized. The observations were repeated and verified many times.

The presence of the parasites in moderate numbers does not appear to be injurious to the mites. Mites have frequently been observed, with fresh blood in the stomach and ripe sporocysts in the body tissues. However, those found dead have often been very heavily infected.

EXPERIMENTAL INFECTION IN WHITE RATS.

Although Christophers has described what he regarded as the sexual cycle of *Leucocytozoon canis* in a tick, he states in a recent volume [a] that "the sexual cycle can not yet be said to be definitely known." Certainly the experimental transmission of hæmogregarines by arthropoda or by direct inoculation has not, so far as the literature records, been successfully accomplished.

In the present work it was recognized that the conditions for experimental attempts at transmission of *H. perniciosum* were extremely favorable, the principal host being, so to speak, a domesticated animal, and therefore readily handled. The intermediate host—the mite—could easily be obtained in large numbers and its action was susceptible of complete control. In fact, all the conditions of natural infection could readily be reproduced artificially.

As already stated, a large number of mites (several hundred) were infected by being placed upon heavily infected rats for forty-eight hours or longer and then transferred to healthy rats for ten to

[a] Stephens, J. W. W., & Christophers, S. R.: The practical study of malaria. 3d ed. London, 1908, pp. 1–414.

fourteen days. When examined at the expiration of this period about 50 per cent showed heavy infection with ripe cysts. When an infected mite was crushed between slide and cover slip in a drop of salt solution hundreds of sporocysts containing sporozoites were expressed (pl. XV). Only rarely were the sporocysts still contained in the large cyst (oocyst), the latter being usually ruptured in the process of crushing or dissecting.

With a view of obtaining a clew to the manner of infection of rats in nature, a number of experiments were undertaken to determine the effect of various substances upon the sporocysts and the sporozoites. The observations were made partly upon the warm stage of the microscope, but largely without its use; the weather at the time being extremely warm, additional heat was often found unnecessary.

1. Action of fresh rat's blood.—A drop of a healthy rat's blood was mixed on a slide with ripe sporocysts, covered with a cover glass, and ringed with vaseline. No change in the sporocysts took place nor was any movement of the sporozoites in them observed as long as the sporocysts remained intact. On the contrary, when the latter were ruptured by pressure on the cover glass and the sporozoites set free in the blood, marked changes took place. After about fifteen minutes the sporozoites assumed a more elongated form, the principal change of shape taking place in the broad extremity. This end became more slender and elongated (but still slightly wider than the other) and the tip somewhat pointed. The nucleus was visible as a spherical body about one-third distant from the smaller extremity. The movement earliest observed consisted of a gradual bending until the ends were almost touching, then a sudden spring-like resumption of the original shape (pl. XVI). Other sporozoites, after becoming bent, became slowly straightened, a peristaltic wave of contraction beginning anteriorly and a gradually increasing length of sporozoite becoming straightened. A longitudinal contraction of the entire parasite, which rendered it shorter and broader, was followed by slow contraction in a transverse direction, beginning anteriorly, this portion becoming longer and thinner as the wave passed backward and causing the sporozoite to be projected slowly forward. A bending simultaneous with the longitudinal contraction often occurred, and in the following transverse contraction straightening proceeded from before backward, the anterior slender portion often making a distinct angle with the clubbed posterior part.

With high magnifying powers of the microscope and indirect light from the condenser, minute striæ, some arranged in an oblique transverse and others in an oblique longitudinal direction, can be made out without much difficulty. These striæ are caused presumably by minute fibrils—the myocyte-fibrillæ commonly observed in gregarines, which are capable of contraction.

After the slide has been allowed to remain for ten or fifteen minutes in the incubator and is then examined on the warm stage, the sporozoites are found to have become actively moving bodies called vermicules. By a sort of snakelike or wormlike motion, rapid change of place occurred, the red blood corpuscles being vigorously moved about when the vermicule came in contact with them. The anterior extremity was always the one which had been the broadest and most rounded in the sporozoite. In the moving vermicule this extremity is more slender and pointed. The entry of vermicules into leucocytes was never observed. After two or three hours they became sluggish in movement and finally stationary.

2. Action of 0.5 per cent solution of pancreatic extract in normal salt solution.—When sporocysts are exposed to this solution the cysts are rapidly dissolved and the sporozoites set free. The latter very soon become contracted into rounded bodies and lose all motion.

3. The digestive juices of the mite.—Mites containing sporocysts were compressed between a slide and cover slip. The chitinous envelope was then removed and the cover slip replaced and pressed down with sufficient force to rupture the sporocysts and set free the sporozoites. The latter, after a short time, performed slow movements of flexion and extension, and, in addition, some sporozoites showed at times a slow gliding motion forward, with intervals of rest. When in motion a minute point of protoplasm could be seen projecting from the anterior end. The protoplasm showed no perceptible waves of contraction during this movement. Some of the sporozoites at times exhibited slight contractions. No movement took place inside the unruptured cysts.

4. Action of juice from the duodenum of the rat.—A healthy fasting rat was killed, the abdominal cavity opened, and a drop of the clear fluid contents of the lower part of the duodenum mixed on a slide with ripe sporocysts. Immediately upon examining with the microscope the sporozoites in the unruptured cysts were found to be in active motion, moving about with great vigor in the interior of the cyst and suggesting very strongly a mass of worms squirming over one another. After ten or fifteen minutes many cysts were seen to rupture and the sporozoites were set free as actively moving vermicules. They exhibited all the movements previously described as observed when sporozoites were mixed with rat's blood, except that they were intensified. This observation (repeated a number of times) upon the action of the intestinal juice upon the sporocysts and sporozoites pointed to the intestine as a possible point of entry of the infection, in rats, and led to subsequent experiments in this direction.

The first experimental attempts to produce the disease in rats were conducted with the intention of simulating the natural method of infection. Ten healthy white rats about three-fourths grown and just received from a dealer were selected. Each rat was placed in a separate glass battery jar with wire-gauze top. Careful examination failed to disclose any skin parasites except a few lice. Two blood smears were made each day on two successive days from each rat. In every rat the blood was found to be normal. Mites previously infected and showing in about 50 per cent ripe sporocysts when crushed, were placed upon eight of these rats. From 20 to 50 mites were placed upon each rat. Two rats were kept as controls, no mites being placed upon them. The rims of the jars containing these rats were smeared with vaseline and they were kept in a separate part of the laboratory away from the others. The results were as follows:

Rat No. 1.—On the nineteenth day first appearance of parasites in the blood as encysted forms in lymphocytes; leucocytes increased. Twentieth and twenty-first day: Encysted forms more numerous; animal shows marked anemia. Twenty-second day: Lymphocytes greatly increased; in addition to many encysted parasites, numerous free forms. In fresh preparations some of these are actively moving vermicules. Twenty-third day: Leucocytes 120,000, 82 per cent being large mononuclear leucocytes. In counting 200 leucocytes 381 parasites were counted. Animal died.

Post-mortem.—Muscles and skin extremely pale; blood watery; spleen greatly enlarged and dark, measuring 6 by 2 by .5 cm. Liver, yellow in color; punctate hemorrhages in lungs.

Rat No. 2.—Few encysted parasites on twenty-fifth day, gradually increasing in number until thirty-fifth day. Anemia slight. At that time 38,000 leucocytes, large mononuclears 56 per cent. In counting 200 leucocytes 32 parasites encountered. On fiftieth day infection appears to be about the same.

Rat No. 3.—Moderate number of free and encysted forms on twenty-second day. Infection increased until death on twenty-eighth day. Leucocytes, 132,000, large mononuclears 71 per cent. In counting 200 leucocytes 165 parasites observed; few free forms.

Post-mortem.—Lesions as in rat No. 1, except spleen slightly smaller and in addition there is enlargement of abdominal and mesenteric lymph glands.

Rat No. 4.—Infection first observed on twenty-eighth day; few encysted forms; gradually increasing anemia and number of encysted forms; death on thirty-eighth day. Leucocytes 110,000 on thirty-sixth day; large mononuclears 68 per cent. In counting 200 leucocytes 96 parasites observed; very few free; nucleated red cells abundant.

Post-mortem.—Lesions as in No. 1.

Rat No. 5.—Few encysted parasites on twenty-fifth day, but gradual increase up to fiftieth day. Leucocytes 28,000, 37 per cent large mononuclears. In counting 200 leucocytes 28 encysted forms observed; none free. Animal appears healthy; slight anemia.

Rat No. 6.—Few free and encysted parasites on nineteenth day. On twenty-fifth day greatly increased in number, both free and encysted; moderate anemia. On fiftieth day animal alive, but seems sick and is quite anemic. Leucocytes 98,000, 62 per cent large mononuclears; many nucleated reds. In counting 200 leucocytes 231 parasites observed; 157 free.

Rat No. 7.—Infection appeared twenty-seventh day; death thirty-first day; great anemia. On twenty-sixth day leucocytes 91,000, 59 per cent large mononuclears. In counting 200 leucocytes 101 parasites observed, mostly encysted.

Autopsy.—Spleen 6 by 1 by 0.4 cm. Other lesions as in No. 1, except punctate hemorrhages absent in lungs.

Rat No. 8.—Free vermicules only in small numbers on seventeenth day; encysted forms numerous on twenty-fifth day; death thirty-sixth day. Leucocytes 126,000, large mononuclears 78 per cent on thirty-fifth day. In counting 200 leucocytes 83 parasites, mostly encysted, were seen.

Autopsy.—Lesions as in No. 1, except slightly smaller size of spleen.

Rat No. 9—Control.—Examined every five days up to fiftieth day. No parasites found. Leucocytes 10,600, few nucleated reds.

Rat No. 10—Control.—No parasites up to fiftieth day. Leucocytes 5,800.

Control animals were repeatedly examined and found free of mites.

INFECTION IN WILD RATS.

In attempting to infect wild rats (*Mus rattus*) with *H. perniciosum* an obstacle was encountered, due to the difficulty of handling them and of keeping them healthy in captivity.

In the first experiment five large rats, averaging 250 grams in weight, were used. These rats had just been captured in a wire trap. They were placed in separate glass jars, to which infected mites had been added. Unfortunately all the rats died within a week from self-starvation and the effects of confinement.

For a second experiment six young wild rats, apparently of the same age and averaging about 100 grams in weight, were obtained. These were placed in a large wire cage, which in turn was placed in a large wooden cage containing infected mites.

Results: One rat died on the thirty-third day and another on the thirty-ninth day. Examination of the blood and organs showed severe infection with *H. perniciosum*. At the end of six weeks the remaining rats were all infected with the blood parasite, but in a mild degree. At the end of eight weeks the animals still show the parasite. The age of the rats at the beginning of the experiment is assumed to be about four months.

FEEDING EXPERIMENTS.

Ten rats were selected and placed in isolated glass jars with gauze tops. Each cage, as well as each rat, was sprinkled with "insect powder." All the rats were carefully examined and found free of vermin except a few lice. These disappeared within a few days after the "insect powder" was used.

Blood smears from each rat were examined on two successive days. The blood was normal and showed no parasites. Before the feedings with infected material all food was withdrawn for forty-eight hours. Mites containing ripe sporozoites were crushed singly or in small numbers at a time upon double-width glass slides. Immediately after crushing, examination with the low power of the microscope was frequently resorted to, in order to be certain of the abundance of ripe sporocysts. Pieces of moistened white bread were used to absorb the expressed juices of the crushed mites. After a thin piece of bread about 2 cm. square had received from 15 to 20 or more crushed mites and their juices it was gently compressed into a firm round mass and immediately fed to a rat. In this manner eight of the ten rats were fed. The other two were kept as controls. The eight rats were fed as described only once; afterwards ordinary food (bread and oats) was given.

Twenty-four hours after the infected bread was given, and every day thereafter, stained blood smears, and on occasion, fresh preparations, were examined. In the first twenty-four hour preparations, five rats showed a moderate number of actively moving free vermicules in the blood; they were also observed in stained smears and differed in no way from those previously described. The leucocytes were but little if at all increased in number; no encysted or other variety of parasites were observed. When next examined (third day) no parasites were observed in any of the smears examined. The later findings are given below:

Rat No. 1.—A small number of encysted parasites observed on the eighth day; on twelfth day moderate anemia; parasites greatly increased, also leucocytes; death on fourteenth day.

Post-mortem.—Enlarged spleen, fatty liver.

Rat No. 2.—Moderate numbers of encysted and a few free parasites observed on twelfth day; gradual increase in number up to fortieth day; animal shows moderate anemia and seems sick; encysted parasites numerous.

Rat No. 3.—Moderate number of encysted parasites on eighth day; tenth day marked anemia, many free and encysted parasites. Found dead on morning of twelfth day.

Post-mortem.—Hemorrhagic spots on surface of lungs; blood in small intestine; other lesions as in No. 1.

Rat No. 4.—Few encysted parasites on ninth day; moderate number on twelfth day. On fortieth day an occasional parasite observed; animal appears healthy.

Rat No. 5.—Small number of free and occasional encysted parasites on eighth day; tenth day encysted forms numerous; moderate anemia; death on twentieth day.

Post-mortem.—Spleen greatly enlarged; liver mottled yellow; many parasites in blood.

Rat No. 6.—Encysted parasites in small numbers on twelfth day; moderately numerous on twentieth day; animal sick. On fortieth day a few encysted forms still present; animal appears healthy.

Rat No. 7.—Few free and many encysted parasites on ninth day; more abundant on twelfth day; animal anemic, appears sick; leucocystes greatly increased. Increasing anemia and death on twentieth day.

Post-mortem.—Spleen four times normal size; liver enlarged and fatty; mesenteric lymph glands enlarged and hemorrhagic.

Rat No. 8.—Small number of encysted parasites on eleventh day; on fifteenth day considerably increased; anemia apparent. On fortieth day animal still alive, but quite sick and anemic.

Rats Nos. 9 and 10—Control rats.—Were examined in the same manner as the infected ones. No parasites found in the blood at any time. Animals healthy on the fortieth day.

On none of the rats were mites or other ecto-parasites found. The jars in which the rats were confined were placed at some distance in the laboratory from those containing infected mites and were further protected by a ring of vaseline.

In a later experiment, carried out in the same manner, five rats were used. Four were fed with ripe cysts on wet bread. The earliest appearance of encysted parasites in the blood was on the eighth day in two rats; in the other two on the tenth day: Vermicules alone were observed in three on the second day. One rat died on fifteenth; the other on nineteenth day.

In a still later experiment four young rats (2 months old) were fed as in the previous experiments. Encysted parasites were found in the blood on the tenth, twelfth, thirteenth, and fourteenth day, respectively. All four died within a week after the appearance of infection.

Remarks.—Parasites spoken of as "encysted" refer to those forms inclosed in cysts and usually contained in mononuclear lymphocytes.

In the first experiment the five rats in which free vermicules were observed twenty-four hours after feeding infected mites were those in which encysted parasites were first observed. It is probable that a more prolonged search for parasites in the blood of the remaining three rats would have resulted in the finding of a few at that time.

An attempt was made to infect four rats by feeding them livers from infected rats in which cysts (containing merozoites) were numerous. The result was negative. No free vermicules were detected in the blood during the first twenty-four hours, nor did any encysted parasites appear later.

Defibrinated heart's blood from an infected animal was injected into the peritoneum of two healthy rats, 1 c. c. to each rat. Blood smears previously made from the infected rats showed only encysted parasites. Result negative.

In a later experiment defibrinated blood in which there were a few free vermicules was employed. The results were also negative.

Small pieces of liver containing ripe cysts were gently rubbed up in a mortar with salt solution. The liquid, strained through sterilized gauze, was injected into the peritoneum of two rats. Result negative.

Several mites containing ripe sporocysts were gently crushed and rubbed in a mortar containing 0.3 per cent salt solution. The liquid, when strained and examined with the microscope, was found to contain many free sporozoites. A portion of the fluid was injected into the peritoneal cavity of two rats. Result negative.

Action of citrate solutions upon encysted parasites.—Defibrinated blood from an infected rat was added in varying amounts to small test tubes containing 1 c. c. of 1 per cent solution of sodium citrate in normal salt solution. Two tubes were set aside at room temperature (about 30° C.); two tubes were placed in the incubator at 37° C. for twenty-four hours. The same method of procedure was also followed, using normal salt solution instead of soidum citrate solution. Results: In salt solution kept for three days at 30° C. a few sluggishly motile vermicules were found. In other solutions no change took place in the encysted parasites.

A series of sections were made from the middle and lower part of the small intestine of a fasting rat, fed twelve hours before, on a large number of crushed mites heavily infected with ripe sporocysts, inclosed in moist bread. Numerous vermicules were seen between the epithelial cells and in the capillaries and lymph spaces in the villi.

CLASSIFICATION.

Some zoologists have favored a classification of the gregarine-like blood parasites as a subdivision of the gregarines, while others have placed them in a subdivision of the *Hæmosporidia*, making blood parasitism the principal feature upon which to base the classification.

Just at present the question of their systematic position is in a somewhat confused state.

In the classification of Laveran[a] the *Hæmosporidia* are divided into three genera:

1. *Hæmamœba* (including the malarial type of organism).
2. *Piroplasma*.
3. *Hæmogregarina*.

Neveu-Lemaire[b] believes that these primary subdivisions should be into families instead of genera. He divides the *Hæmosporidia* into four families instead of three, viz:

Hæmogregarinidæ	*Lankesterella*. *Karyolysus*. *Hæmogregarina*.
Hæmamœbidæ	*Plasmodium*. *Laverania*. *Hæmamœba*.
Halterididæ	*Halteridium*. *Polychromophilus*.
Achromaticidæ	*Achromaticus*. *Dactylosoma*.

It has been suggested that *Piroplasma*, an important group which has been left out, be included as an additional family.

Lühe agrees with Neveu-Lemaire that the genus *Hæmogregarina* (Laveran) should be given the strength of a family.

The classification of Minchín[c] attempts to accomplish the same end in a somewhat different manner. It is a modification of one proposed by Labbé.

Hæmosporidia	*Hæmosporea*	*Lankesterella*. *Karyolysus*. *Hæmogregarina*.
	Acystosporea	*Plasmodium*. *Laverania*. *Hæmoproteus*. *Halteridium*. *Piroplasma*.

The description of the suborder *Hæmosporea* is as follows:

Trophozoite, typically a vermiform hæmogregarine, endoglobular in early stages, free when full grown; apparently no alteration of hosts; schizogony and sporogony in the same host, which is always a cold-blooded vertebrate, fish, amphibian, or reptile.

The hæmogregarines of mammals could not be included in this suborder without considerable modification. The classification devised

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[a] Laveran, A.: Les hématozoaires endoglobulaires. C. R. soc. biol., Par., vol. 50, 1899, p. 124–133.

———: Essai de classification des hématozoaires endoglobulaires. C. R. soc. biol., Par., vol. 53, 1901, p. 798.

[b] Neveu-Lemaire: Les hématozoaires du paludisme. Thèse, Paris, 1901.

[c] Minchín: The sporozoa. In "A treatise on zoology," ed. by E. Ray Lankester, part 1, London, 1903.

by Neveu-Lemaire and advocated by Lühe divides the *Hæmogregarinidæ* into three genera, as already mentioned.

In view of the restricted knowledge of many of the parasites of this general group, and especially in view of the fact that the complete life cycle for so few forms is definitely established, considerable difficulty naturally arises in attempting to definitely classify *Hepatozoon perniciosum*, the organism under discussion. As Lühe, who has recently published a revisional discussion of this group, has classified an apparently rather closely allied species, namely, *Hæmogregarina balfouri*, from *Jaculus jaculus* in the family *Hæmogregarinidæ*, it seems best to place this new species for rats at least provisionally in the same family.

In attempting to classily the organism in one of the new genera we are faced by two dangers: In the majority of the species of this group the complete life cycle has not yet been established. If, now, the organism under discussion is classified in one of these new genera the danger is present of giving a total misconception to the life cycle of the other forms. If, on the other hand, a new genus is proposed for this species the danger is present that when the life cycle of the other species of this group shall become known this new genus will be shown to be unnecessary. A choice must therefore be made between running the risk of confusing the biology of species with which the present parasite may have nothing whatever to do and of unnecessarily creating a new systematic name. When these considerations are weighed it would appear that it is much more difficult to correct a biological confusion than it is to suppress a synonym. On this account it is believed that the lesser of the two evils lies in proposing for the species under discussion a new generic name. Dr. Charles Waddell Stiles, chief of the division of zoology, Hygienic Laboratory, Public Health and Marine-Hospital Service has suggested the provisional formation of a new genus. The writer accepts this view, and accordingly proposes *Hepatozoon*, kindly suggested by Doctor Stiles as the new mono-typic genus, with *Hepatozoon perniciosum* as type species.

As only one species is at present established for this genus it is naturally difficult to separate the generic from the specific characters. As diagnosis for *Hepatozoon perniciosum* n. g., n. sp., the following may be given:

In the Rat.

When an infected mite is swallowed by a rat the sporocysts are acted upon by the digestive juices and the sporozoites are liberated. The sporozoites become actively motile striated vermicules measuring 16 by 4 micra. They penetrate the intestinal villi of the rat, enter the blood stream, and are carried to the liver. They penetrate the liver cells and undergo schizogony. The nuclei of the oval schizonts are arranged at the poles and an enveloping cyst is developed. Each nucleus becomes the basis of a merozoite, of which an average of 16 are formed, arranged at the poles. The ripe cyst measures 25 by 30 micra. Later the cyst wall becomes softened and is ruptured. The merozoites are set free as actively moving vermicules, indistinguishable from those developed from the sporozoites. Some of the merozoites enter fresh liver cells and repeat the schizogony. Other merozoites enter the blood streams as vermicules, are taken in by the large mononuclear lymphocytes, and develop a definite enveloping cyst wall. A few vermicules may escape the action of the leucocytes and appear in the blood stream.

In the Mite.

When the blood of an infected rat is swallowed by a mite the encysted trophozoites are set free in the stomach by solution of the cyst as free vermicules. Two similar vermicules become associated and conjugate. One, the macrogamete, grows larger and partly surrounds the other, the microgamete. The protoplasm becomes fused and later the nuclei conjugate and fuse to form a zygote. The zygote becomes a sluggishly motile ookinet, which penetrates the stomach wall of the mite and enters the body tissues and becomes encysted (oocyst). Here a remarkable enlargement of the karyosome takes place. The parasite increases enormously in size. The nucleus of the spherical sporont thus formed undergoes division into many daughter nuclei, which migrate to the surface of the sporont. The surface of the latter becomes mammillated. The projections, each of which contains a nucleus, increase in size and length; later they are broken off and each becomes a sporoblast. The nucleus of the sporoblast undergoes division, the resulting nuclei being arranged at the poles. The sporoblast increases in size and a cyst wall develops. Around each nucleus a sporozoite is formed. In the ripe sporocyst, which measures 25 by 30 micra, the sporozoites, 16 in number (average), are arranged at the poles. The large cyst (oocyst) contains from 50 to 100 of such sporocysts. When the mite is swallowed by a rat the cycle is repeated.

It is recognized that the hæmogregarines *Leucocytozoon canis* and *Leucocytozoon funambuli*, which have already been described, in India are more or less similar to *Hepatozoon perniciosum;* but as the genus *Leucocytozoon* has already been taken by a blood parasite, *L. ziemanni*, of an entirely different character, this generic name can not be properly retained.

The relation or possible identity of *Leucocytozoon ratti*, described by Adie, with *H. perniciosum* can only be cleared up when the life cycle of the former is known. Only the trophozoite has been observed, which, according to the description, is smaller than that of *H. perniciosum*, and is without a cyto-cyst. It may be mentioned that there exists a marked similarity in form of the trophozoites of different species of hæmogregarines in both warm- and cold-blooded animals.

Specimens of the type species of the new genus have been deposited in the museum of the division of zoology, hygienic laboratory, United States Public Health and Marine-Hospital Service.

CONCLUSIONS.

Hepatozoon perniciosum is the only hæmogregarine occurring in mammals which has been observed outside of India and northern Africa, both tropical regions.

No other hæmogregarine has been described which exhibits distinct pathogenic properties or causes the death of the animal host.

Other hæmogregarines have been found only in wild animals, in which they appear to exert no harmful effect. It is well known that domesticated animals may succumb to infection by protozoa or bacteria, practically harmless to closely related wild animals. Such may be the case as regards infection of white rats by *H. perniciosum*.

The results thus far obtained in the experimental infection of wild rats (*Mus rattus*) with this parasite are not conclusive as regards pathogenicity. That the wild rat is susceptible to infection is clearly shown. As is the case with white rats, it is probable that the most pathogenic effect is exerted upon young animals. Whether or no this parasite can be turned to account in the destruction of wild rats is a matter for further investigation. The role assumed by wild rats as agents in the transmission of plague makes it a matter of great importance to exterminate them. To do this by spreading disease among them is generally regarded as the simplest and most desirable method.

The Gamasid mite (*Lelaps echidninus*), which is the intermediate host, has frequently been found both upon white and wild rats and in widely separated localities. A study of the habits of rats taken in conjunction with the experimental infections already described, proves beyond question the mode of entry of *Hepatozoon perniciosum* into the body of the rat. That this is the sole manner in which infection takes place is not proved, but is extremely probable.

The puncture produced by the pointed mouth parts of the mite before sucking the blood of the host apparently causes some irritation. Rats infested with mites may frequently be seen to bite at the skin on various parts of the body and frequently they seem to catch and devour the offending mite. The behavior of dogs when disturbed by fleas is more familiar.

If the mite in question has previously sucked blood from a diseased animal and sufficient time has elapsed for the development of sporocysts, the latter, when dissolved in the intestinal juice of the rat, set free the sporozoites. These as vermicules penetrate the intestinal wall, enter the veins and lymphatics, and reach the liver. Here they penetrate the liver cells and undergo multiplication. Some escape into the general circulation. The merozoites when fully developed are set free in the liver capillaries as vermicules. The majority are speedily taken up by the phagocytic large lymphocytes and become encysted; a few may remain as free vermicules.

The habit of mites to leave the rat during the day and return at night affords ample opportunity for a change of hosts if several animals are in the same cage.

As shown by experiment, in rats fed upon infected mites mixed with bread, free vermicules appear in the blood twenty-four hours later and encysted forms after eight or ten days, whereas rats upon which live infected mites are placed show infection only after seventeen to twenty-eight days. The explanation of this difference doubtless depends upon how soon after being placed on the rat an infected mite is devoured.

As already mentioned in the description of the parasite in the body of the mite, there is no indication, either in preparations of fresh mites or in prepared sections, that the sporozoites become free from the sporocysts in the body of the mite or that they are transmitted by the mouth parts. The sporocysts show considerable tenacity and resistance to rupture. Although sometimes encountered in the salivary glands, the sporocysts are common in any part of the body of the mite.

Infection through the alimentary canal from an intermediary host has not previously been described for a protozoan blood parasite.

The fact that all of those animals, viz, frogs, snakes, lizards, turtles, etc., in which hæmogregarines are so common, are insectivorous suggests a possible mode of infection the same as in white rats. *Leucocytozoon canis*, the sexual cycle of which Christophers has described in the dog tick, may possibly be transmitted in the same manner as *H. perniciosum*.

The limited experiments seem to indicate that infection with the latter by inoculation from one animal to another is impossible.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of etheral salts. By J. H. Kastle.

No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car santitation. By Thomas B. McClintic.

No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

No. 33.—Studies in experimental alcoholism. By Reid Hunt.

No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horse-hair worms (species *Paragordius varius*), with summary of all cases reported to date. By Ch. Wardell Stiles.

*No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By M. J. Rosenau and John F. Anderson.

No. 39.—The antiseptic and germicidal properties of solutions of formaldehyde and their action upon toxines. By John F. Anderson.

No. 40.—Miscellaneous zoological papers. By Ch. Wardell Stiles and Joseph Goldberger.

No. 41.—Milk and its relation to the public health. By various authors.

No. 42.—The thermal death points of pathogenic micro-organisms in milk. By M. J. Rosenau.

No. 43.—The standardization of tetanus antitoxin. An American unit established under authority of the act of July 1, 1902. By M. J. Rosenau and John F. Anderson.

No. 44.—Report No. 2 on the origin and prevalence of typhoid fever in the District of Columbia, 1907. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle.

No. 45.—Further studies upon anaphylaxis. By M. J. Rosenau and John F. Anderson.

No. 46.—*Hepatozoon perniciosum* (n. g., n. sp.); a hæmogregarine pathogenic for white rats; with a description of the sexual cycle in the intermediate host, a mite (*Lelaps echidninus*).—By W. W. Miller.

In citing these bulletins, beginning with No. 8, bibliographers and authors are requested to adopt the following abbreviations: Bull. No. ——, Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv., Wash., pp. ——.

MAILING LIST.

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The editions of the publications marked (*), available for distribution by the Surgeon-General of the Public Health and Marine-Hospital Service, have been exhausted. Copies may, however, be obtained from the Superintendent of Documents, Government Printing Office, Washington, D. C., who sells publications at cost and to whom requests for publications thus marked should be made.

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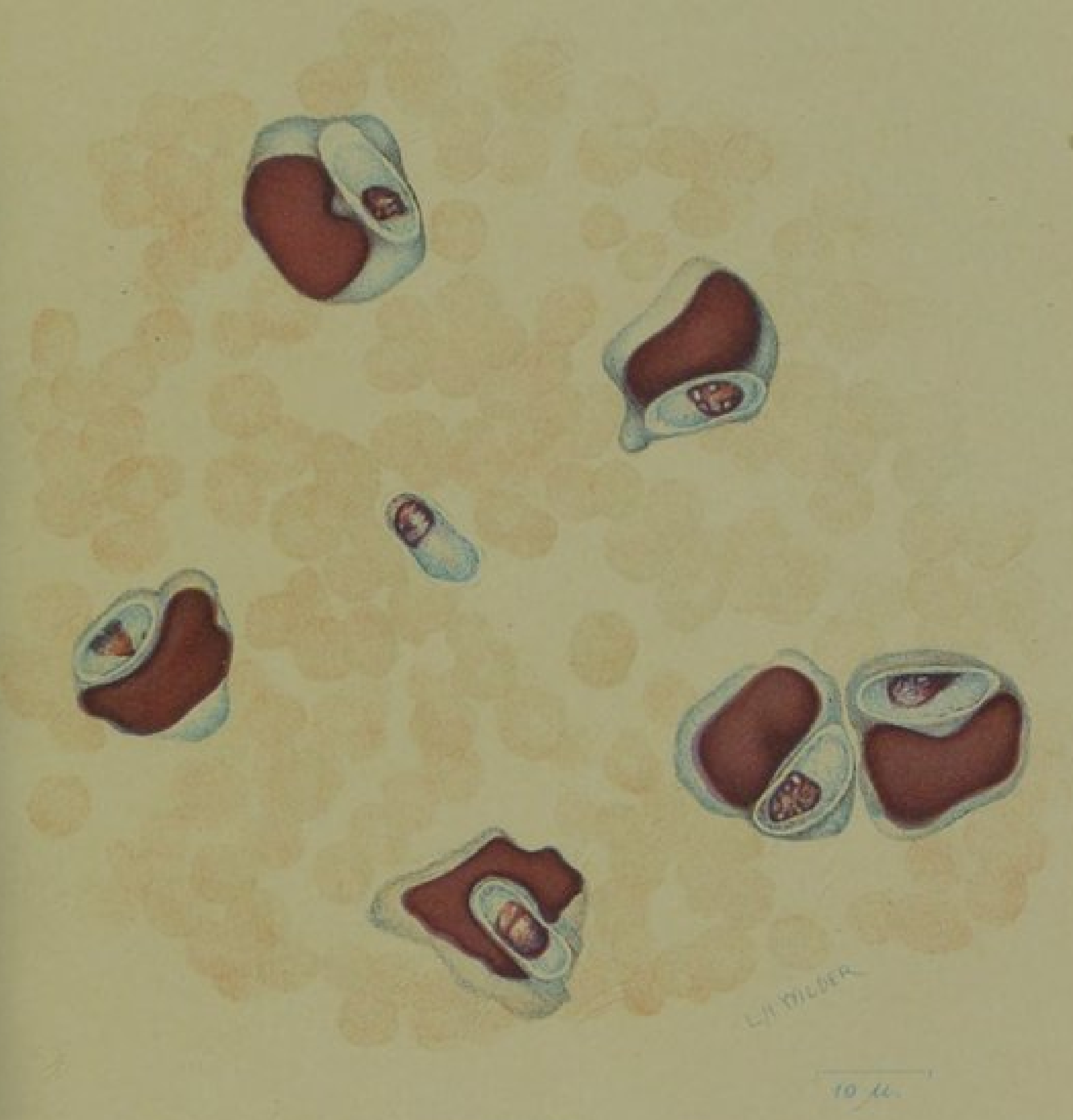


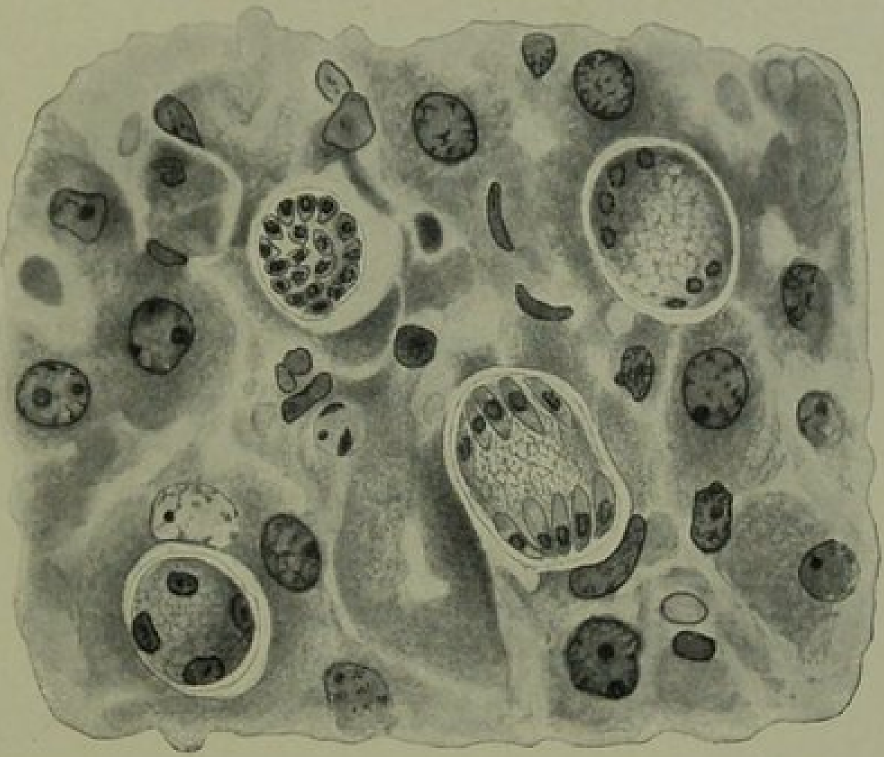
A.Hoen & Co. Baltimore.

Blood smear showing *Hepatozoon perniciosum* encysted in large lymphocytes; also, one free encysted form. Drawn with the aid of the camera lucida from a single field of the microscope. Giemsa stain.

A. Hoen & Co. Baltimore.

Blood smear showing unusual forms of *Hepatozoon perniciosum*. *a.* free vermicules; *b.* motionless, free parasites; *c.* large oval parasite without cyst; *d.* large parasite in transitional leucocyte, unencysted; *e.* ordinary size parasite in transitional leucocyte, without cyst; *f.* large parasite encysted in large mononuclear lymphocyte.

Drawn with camera lucida. Giemsa stain.



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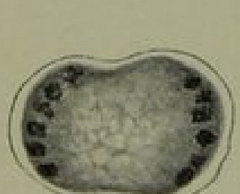

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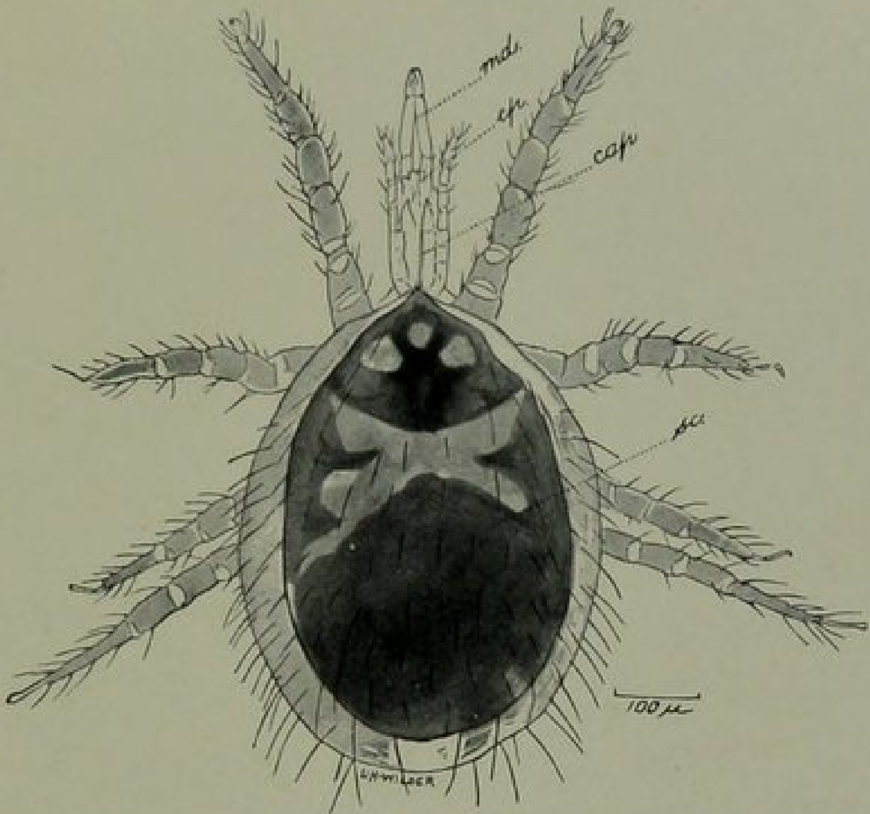
Fig. 1.—Section of liver of infected rat, showing schizonts in various stages and the formation of merozoites. Figs. 2-8.—Detail drawings of schizonts in different stages (from a section of the liver). Drawn with camera lucida. Stain, iron hematoxylin.



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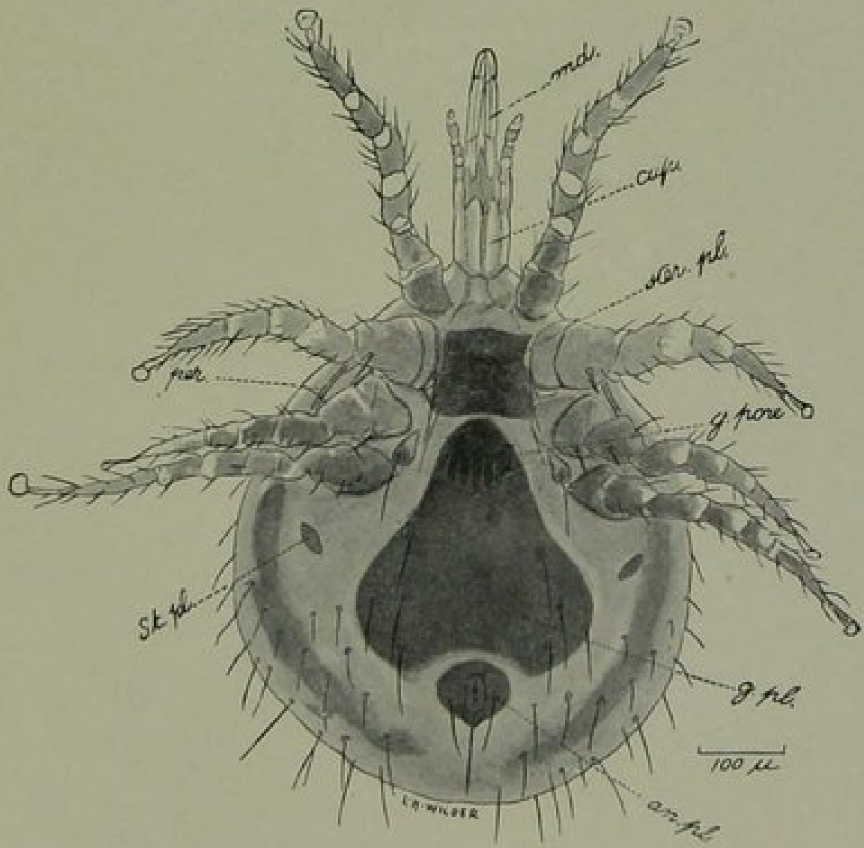

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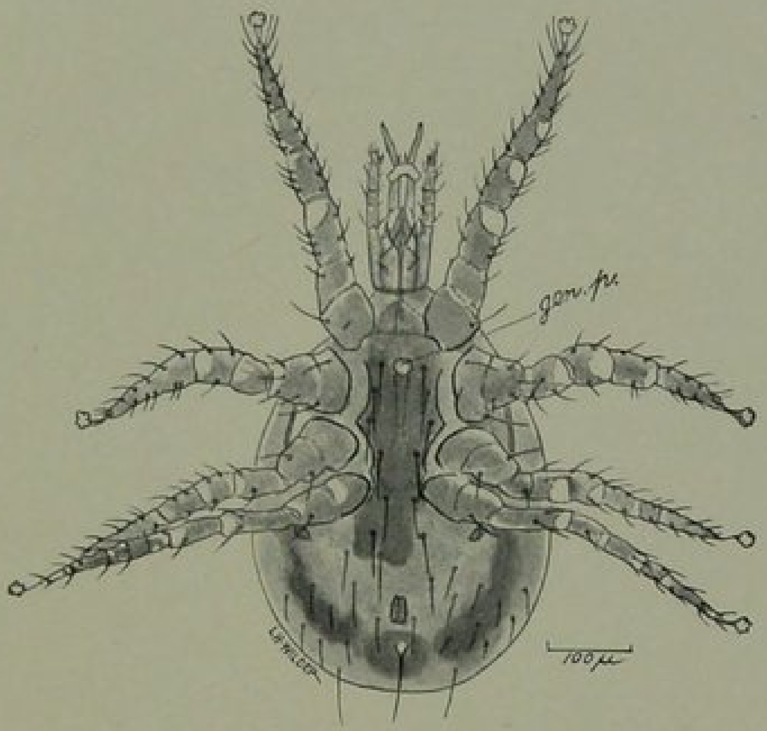
Fig. 1.—Dorsal view of a female mite. Fig. 2.—Ventral view of a female mite. *md.*, mandibles; *ep.*, epistome; *cap.*, capitellum; *sc.*, scutum; *per.*, peritreme; *st. pl.*, stigmal plate; *g. pl.*, genital plate; *an. pl.*, anal plate; *g.*, genital pore; *ster. pl.*, sternal plate. Drawn with camera lucida.



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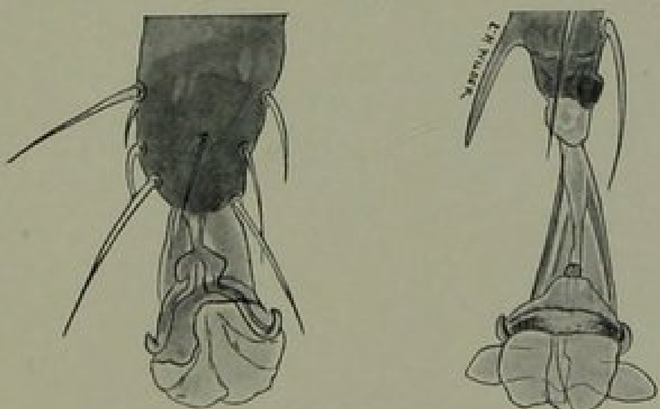

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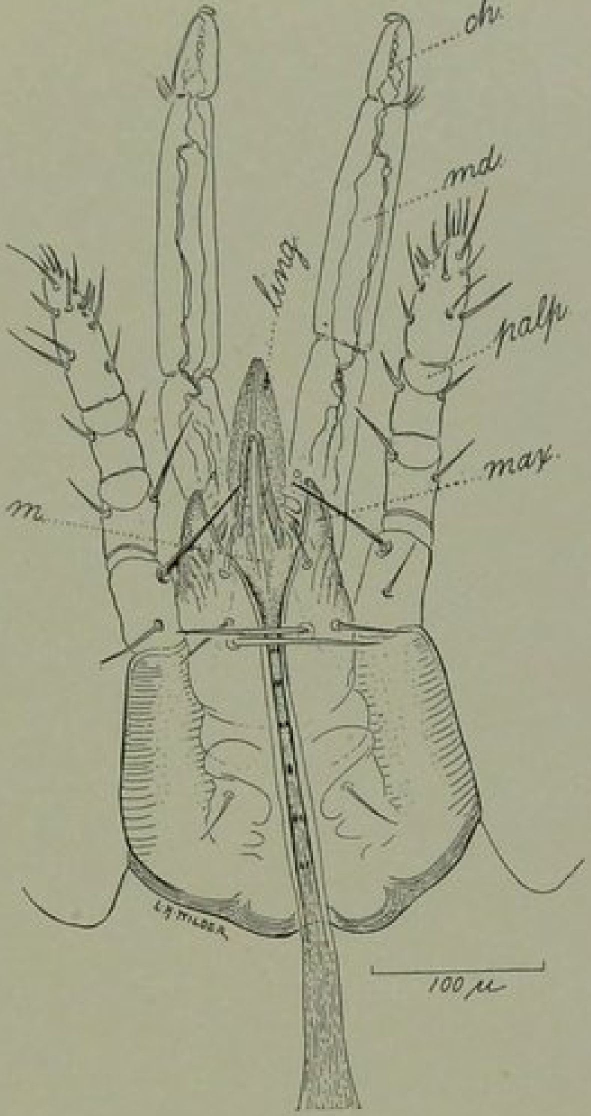
Fig. 1.—Ventral view of male mite. *gen. p.*, genital pore. Drawn with camera lucida. Fig. 2.—Sucking disks and hooklets upon terminal phalanx of first pair of legs. Greatly enlarged.



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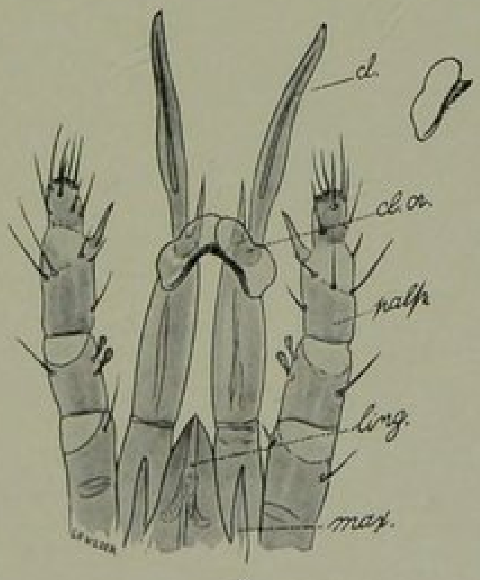

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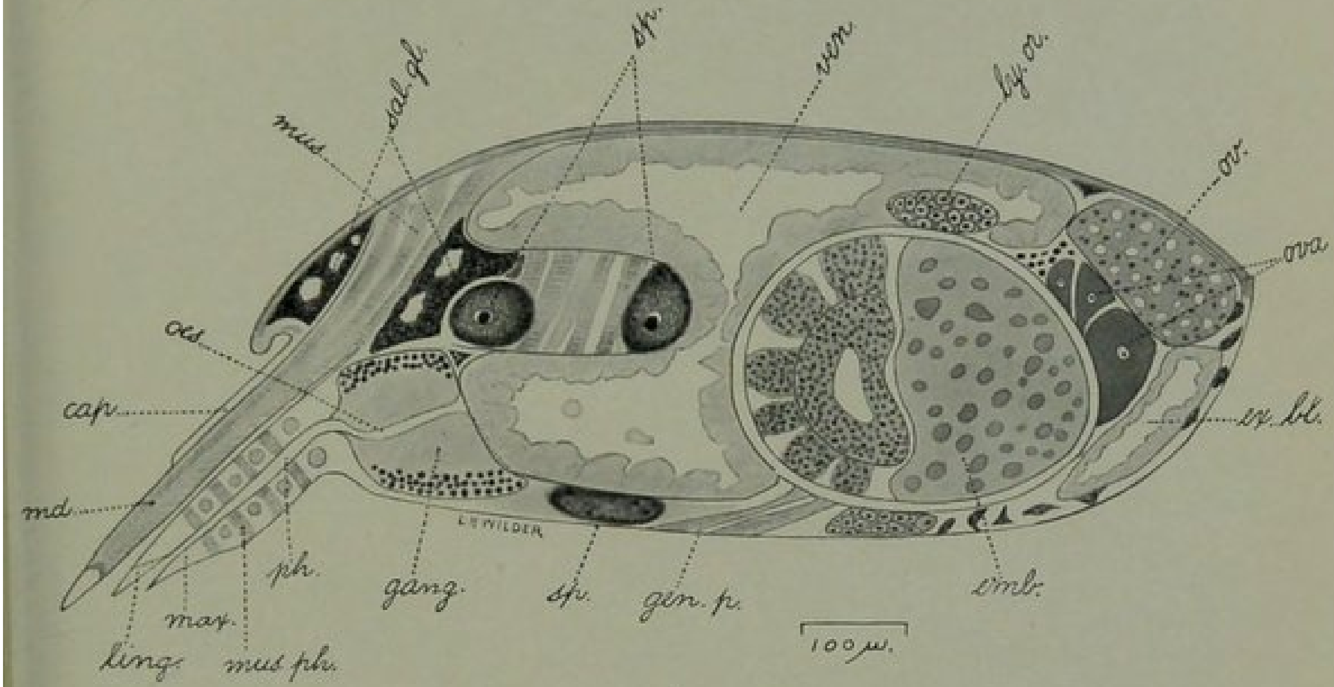
Fig. 1.—Mouth parts of female mite. Fig. 2.—Mouth parts of male mite. *ch.*, chelicerae; *md.*, mandible; *palp*, palp; *max.*, maxilla; *m.*, mouth opening; *cap.*, capitellum; *ling.*, lingula; *cl. or.*, clasping organ. Greatly enlarged. Drawn with camera lucida.



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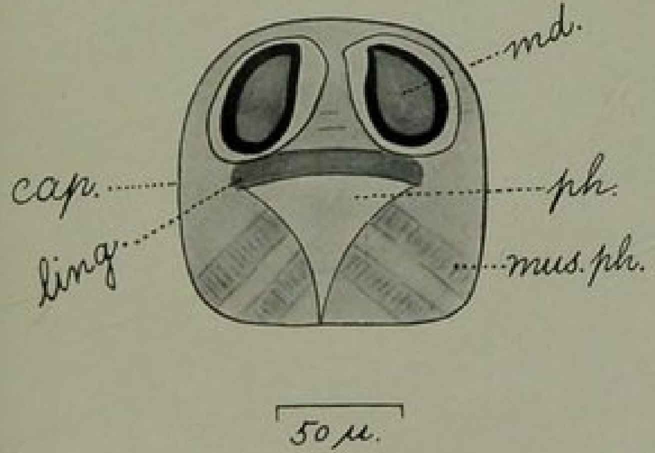

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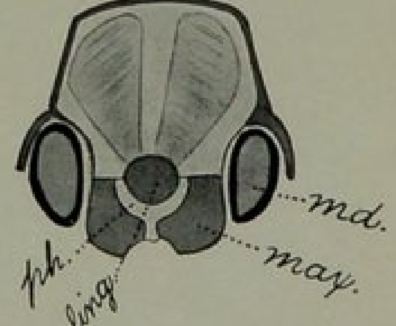

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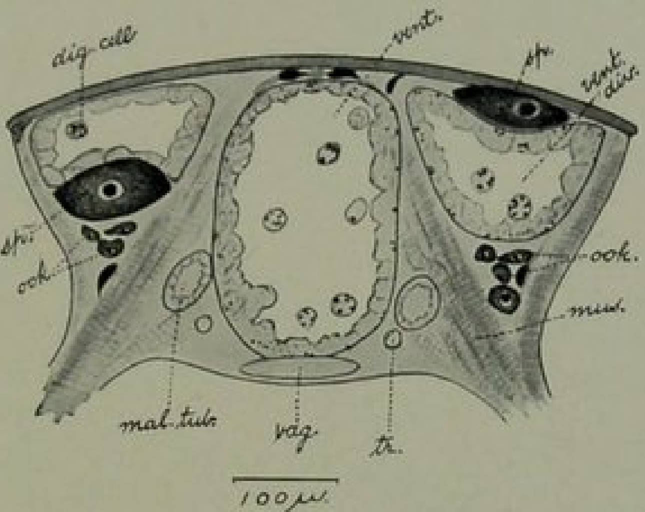
Fig. 1.—Dorsi-ventral section in mid line of female mite. Semidiagrammatic. Fig. 2.—Transverse section of posterior part of capitellum. Fig. 3.—Transverse section of anterior part of capitellum (enlarged). *ven.*, ventriculus; *cap.*, capitellum; *ov.*, ovary; *ova*, ova; *emb.*, embryo; *gang.*, ganglion or brain; *gen. p.*, genital pore; *œs.*, œsophagus; *sal. gl.*, salivary gland; *ly. or.*, lyrate organ; *mus.*, muscle fibers (attached to mandible, and under surface of scutum); *md.*, mandible; *max.*, maxilla; *ex. bl.*, excretory bladder; *ling.*, lingula; *ph.*, pharynx; *mus. ph.*, pharyngeal muscles; *sp.*, sporont (parasites).



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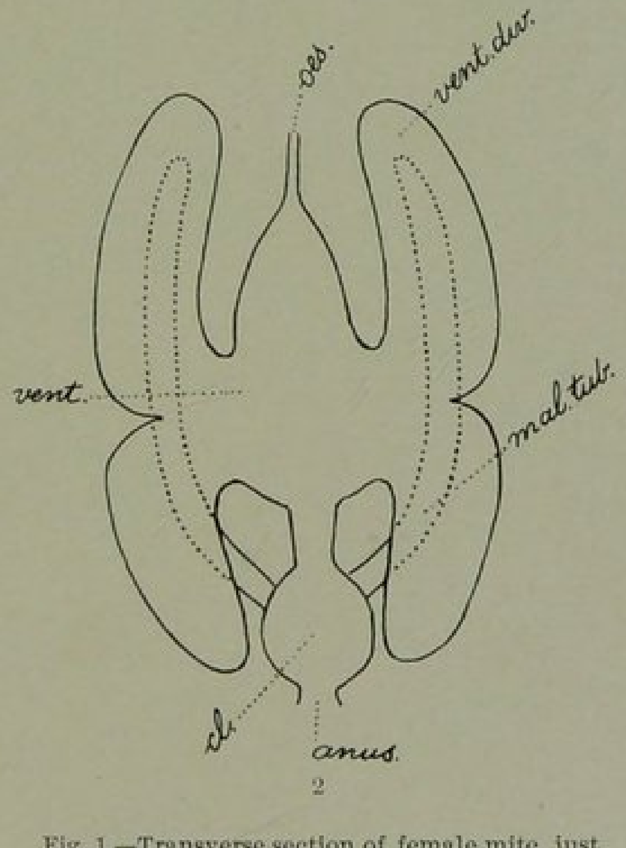

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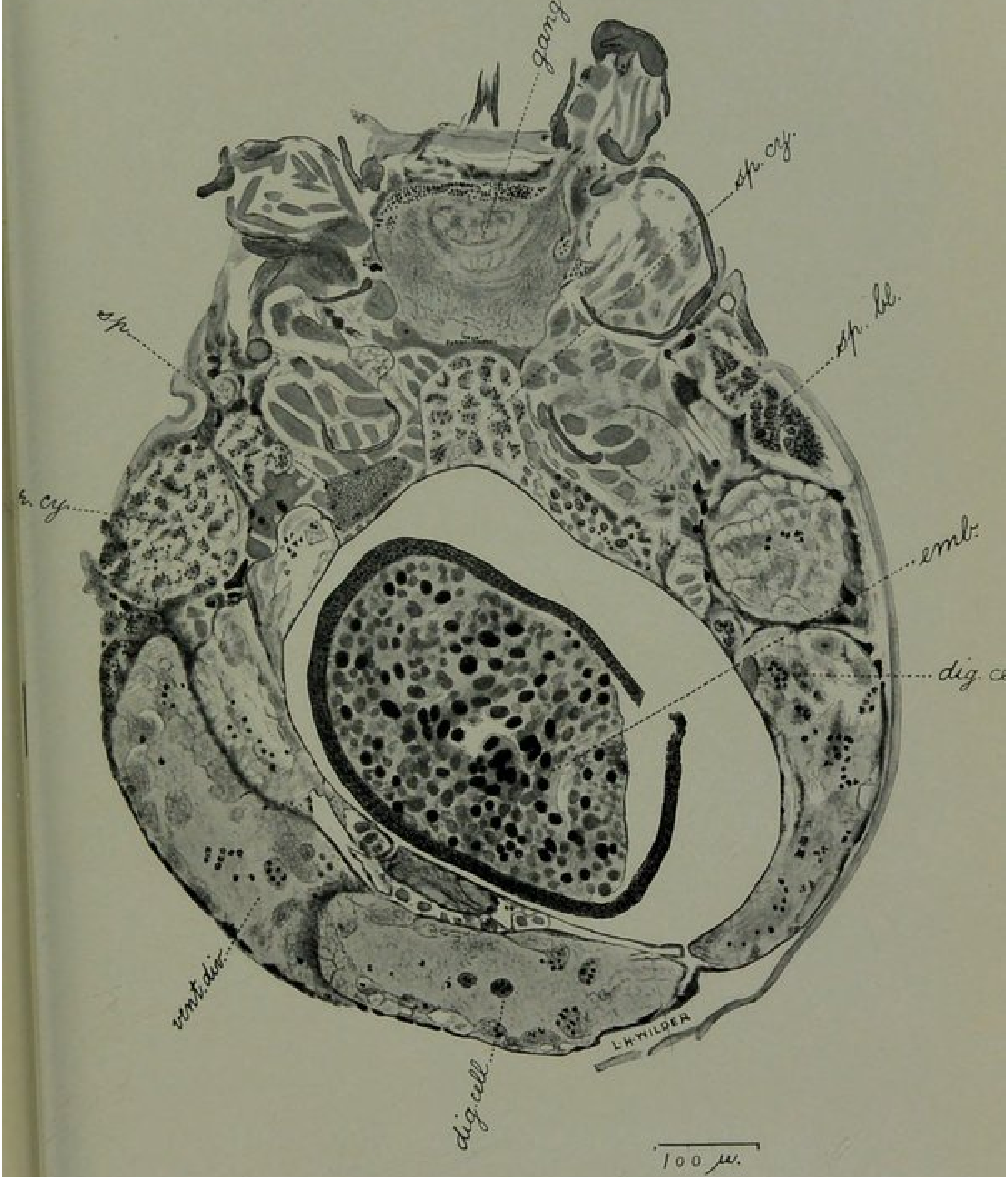
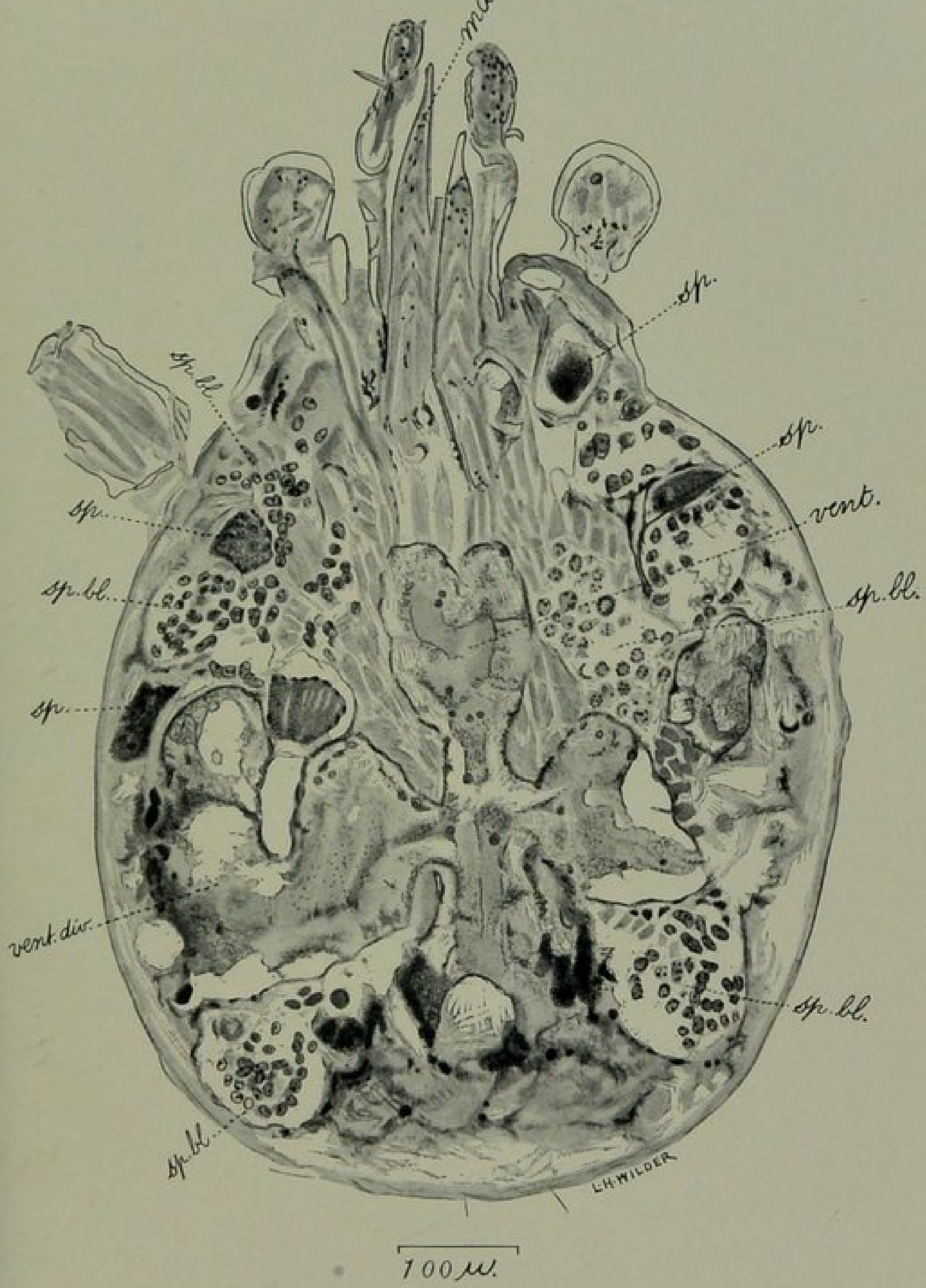
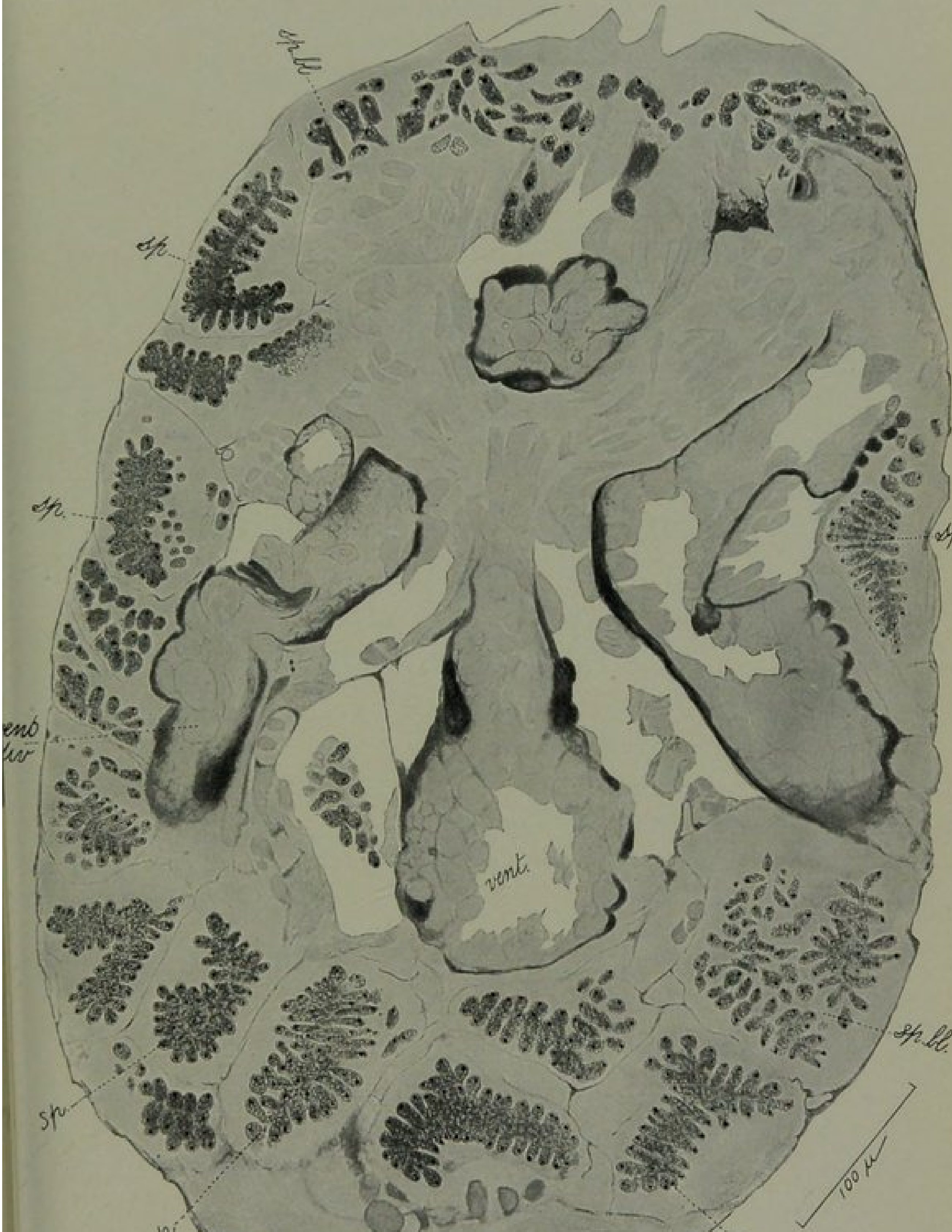
Fig. 1.—Transverse section of female mite, just behind fourth pair of legs. Semidiagrammatic. *dig. cell*, digestive cell; *vag.*, vagina; *tr.*, trachea; *mus.*, muscle attached to legs; *sp.*, sporont; *ook.*, ookinet. Fig. 2.—Diagram of digestive tract and excretory organs. *vent.*, ventriculus; *vent. div.*, ventricular diverticulum; *mal. tub.*, malphigian tubule; *cl.*, excretory bladder; *œs.*, œsophagus.



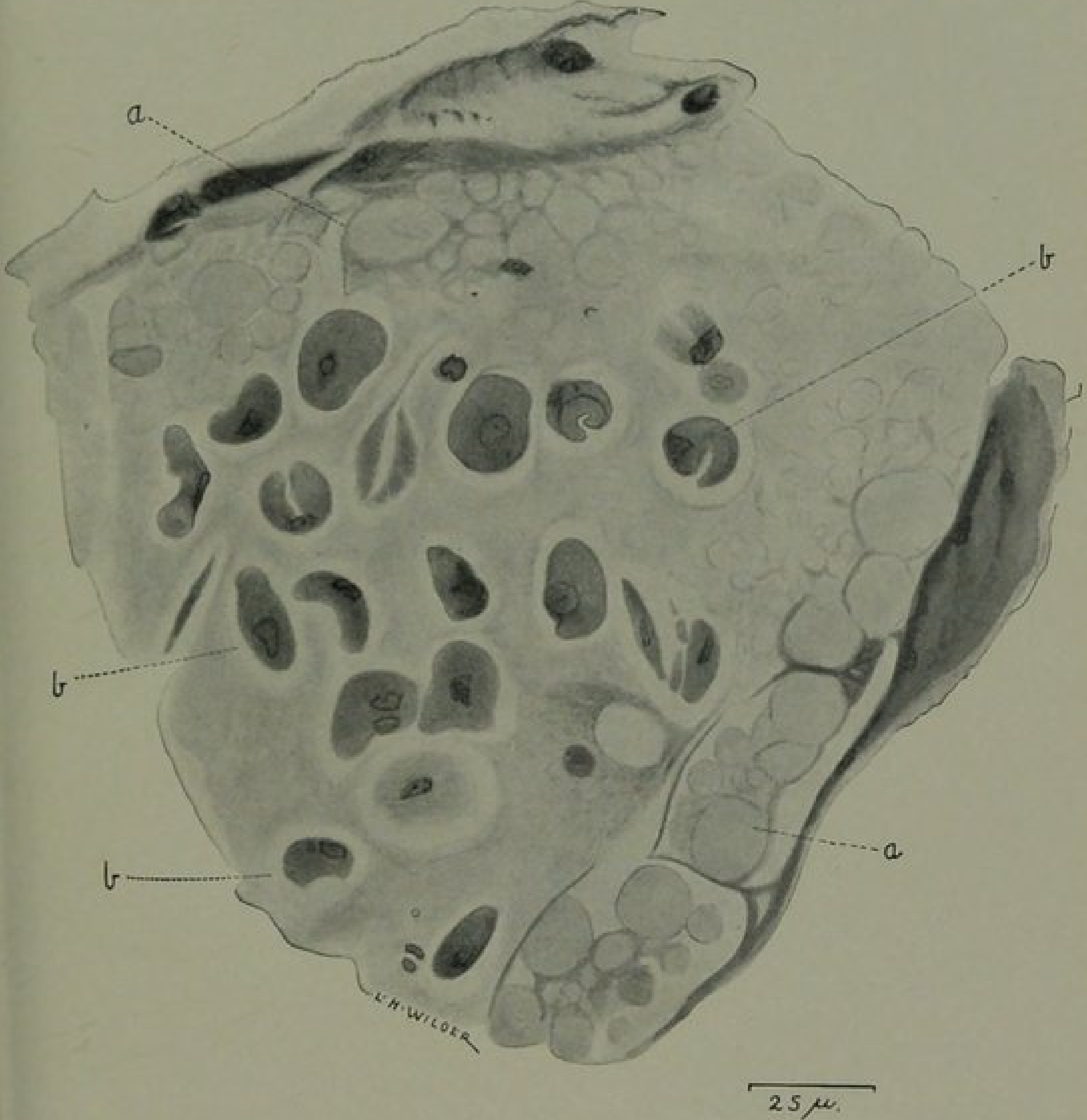
Horizontal section of female mite, removed from an infected rat. *sp. cy.*, large cyst containing many sporocysts with sporozoites—the cyst walls do not show distinctly—the nuclei of the sporozoites are strongly stained; *sp.*, sporont showing peripheral nuclei; *sp. bl.*, large cyst containing sporoblasts; *gang.*, ganglion or brain; *vent. div.*, ventricular diverticulum; *dig. cell*, digestive or liver cell; *emb.*, embryo. Drawn with camera lucida. Stained with iron hematoxylin.



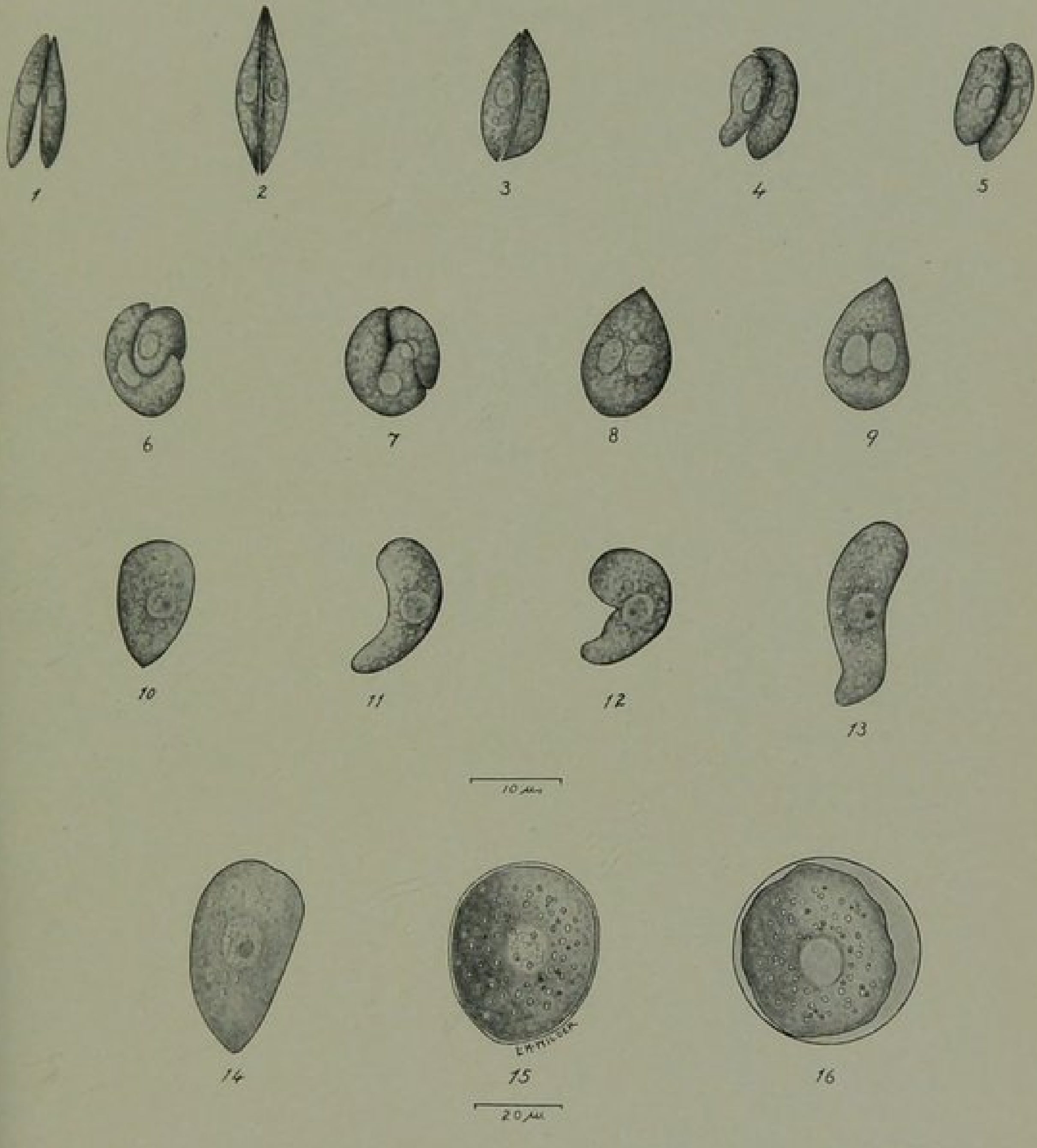
Horizontal section of young female mite from an infected rat, showing parasites. *sp. bl.*, sporoblasts (not yet divided into sporozoites) in large cysts, with indistinct outline; *sp.*, sporont. Lettering as in Pls. V to IX. Drawn with camera lucida. Stained with iron hematoxylin.



Horizontal section of young female mite, from a lot placed upon a heavily infected rat for 24 hours, then removed and placed upon a normal rat; about seventh day; section shows enormous number of segmenting sporonts. The large cyst surrounding each sporont is irregular in outline. Some of the sporonts are shown completely divided up into sporoblasts. Drawn with camera lucida. Stained with iron hematoxylin.

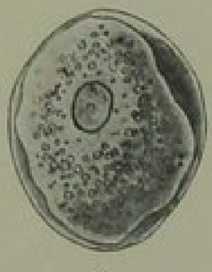


From a horizontal section of a mite 48 hours after feeding upon an infected rat. Figure represents the anterior portion of the left lateral diverticulum of the ventriculus in section. Stained with iron hematoxylin. *a*, Epithelial and muscular layer of ventriculus; *b*, zygotes and ookinets of various forms. Drawn with camera lucida.



SEXUAL PHASE OF HEPATOZOON PERNICIOSUM IN THE STOMACH AND BODY TISSUES OF A FEMALE MITE.

Fig. 1.—Association of vermicules (gametocytes). Figs. 2–5.—Conjugation of gametes. Figs. 6–9.—Copulation of gametes to form a zygote. Figs. 10–14.—Formation and development of ookinet. Figs. 15–16.—Formation of an enveloping cyst and beginning of sporont stage. Figs. 1–12.—In the ventriculus. Figs. 13–16.—In the body tissues. Drawn from fresh preparations.



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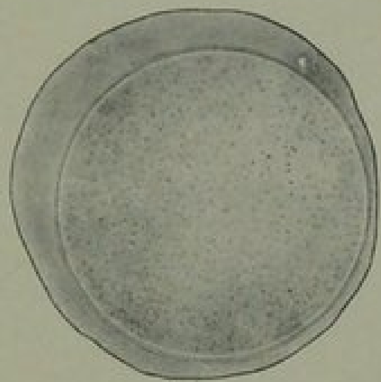

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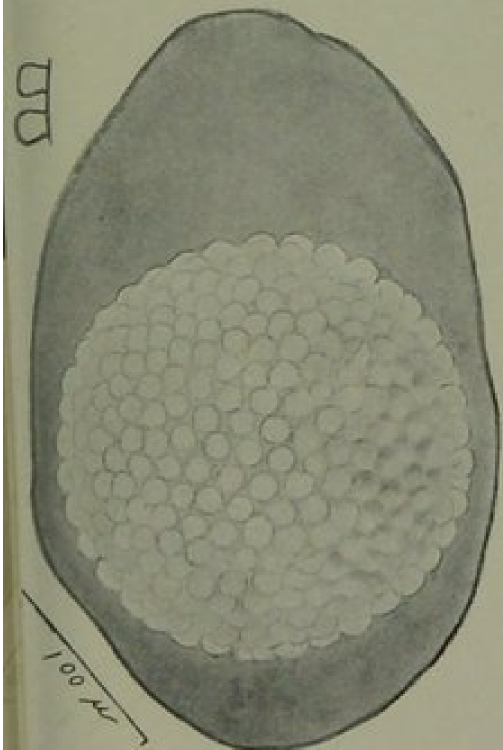

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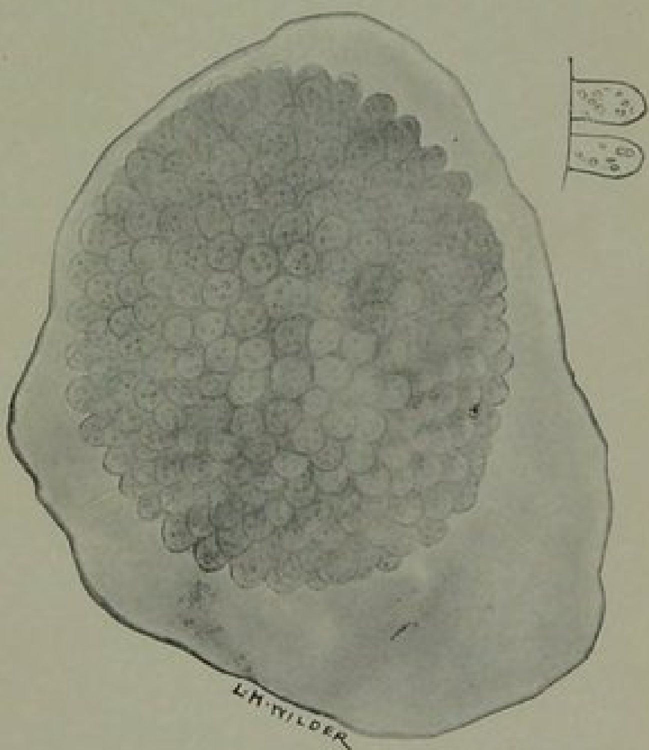
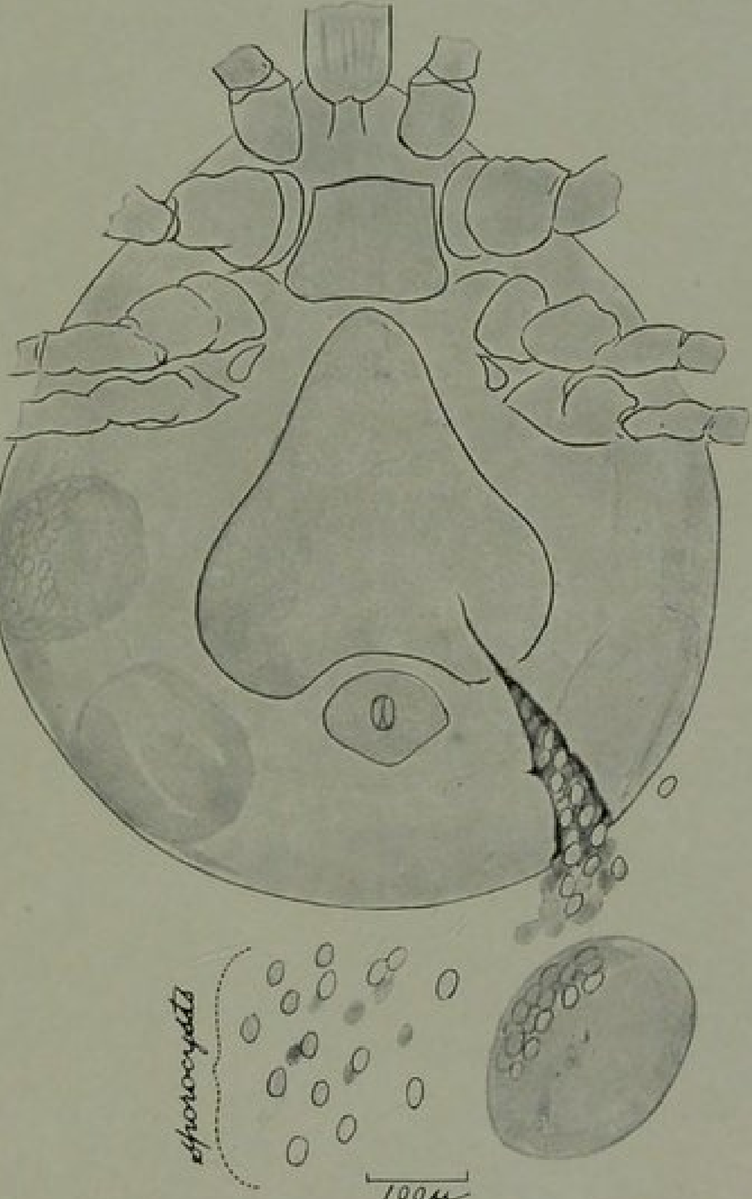


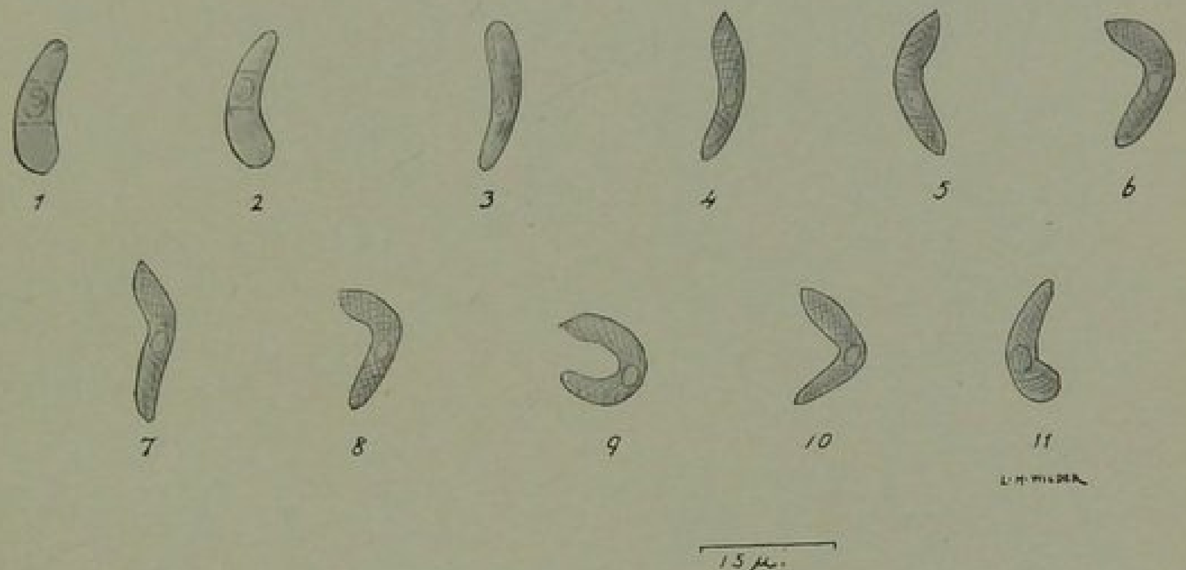
4

SHOWING SPORONTS IN VARIOUS STAGES, IN FRESH PREPARATIONS, MADE BY TEASING APART LIVE MITES FROM 3 TO 6 DAYS AFTER INFECTION, IN 0.3 PER CENT SALT SOLUTION.

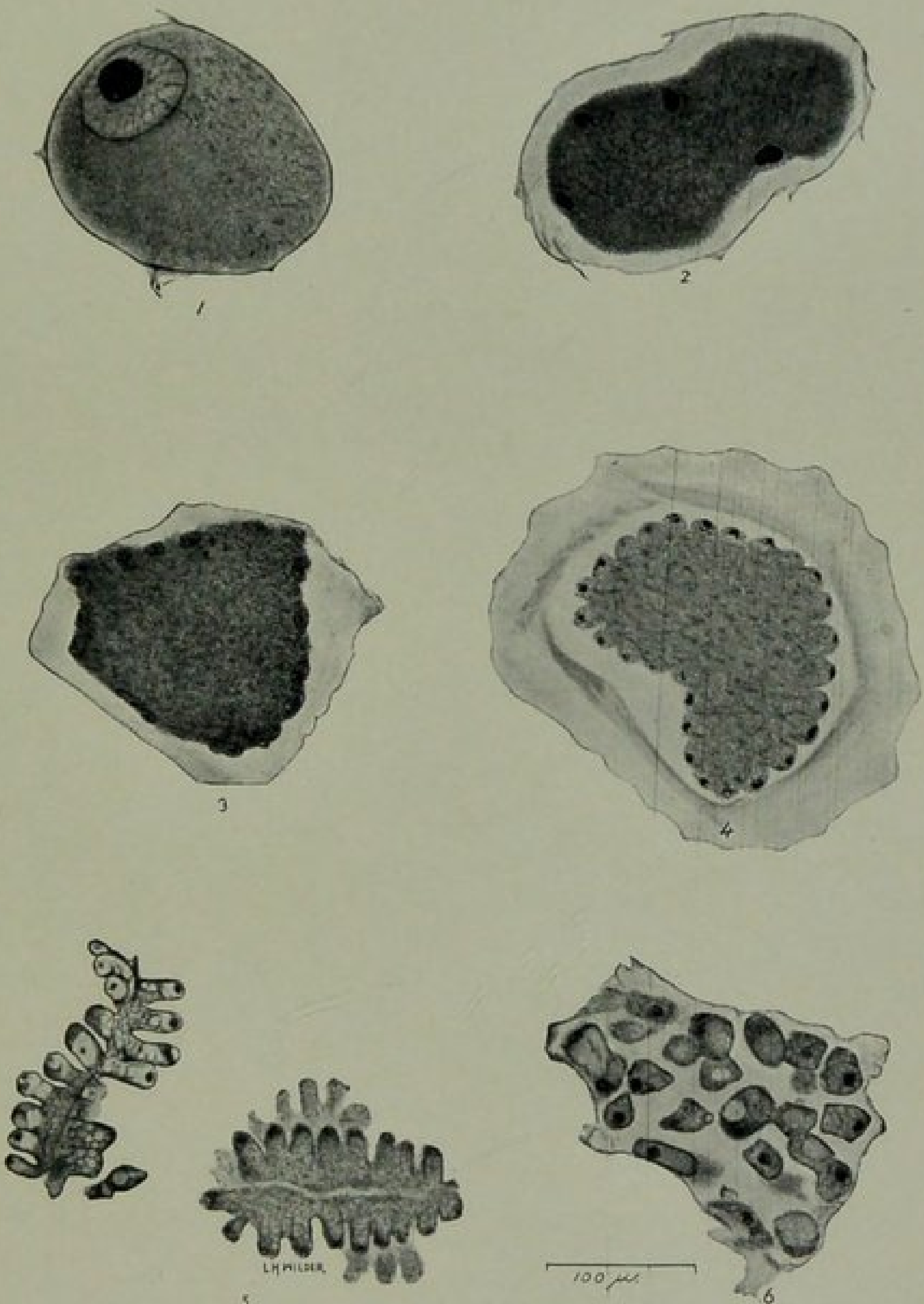
Figs. 1-2.—Unsegmented sporonts with cyst. Figs. 3-4.—Sporonts showing budding from surface to form sporoblasts; with cyst. Drawn with camera lucida.



A MITE CONTAINING 3 LARGE CYSTS, WHICH HAS BEEN GENTLY CRUSHED IN SALT SOLUTION BETWEEN SLIDE AND COVER SLIP. SHOWING EXTRUSION OF A LARGE CYST CONTAINING MANY SPOROCYSTS; AND MANY FREE SPOROCYSTS. FRESH PREPARATION. SEMIDIAGRAMMATIC.



Figs. 1-11.—Free vermicules. Drawn from a fresh preparation, prepared by mixing ripe sporocysts from a crushed mite with duodenal juice obtained from a freshly killed healthy rat. Figs. 1-2.—Sporozoites unchanged. Figs. 3-11.—Sporozoites which have become active vermicules; showing striation (exaggerated) and various shapes assumed. Fig. 12.—Sporocyst from freshly crushed mite; showing central "rest" body and young sporozoites at the extremities. Figs. 13-14.—Lateral and end views of fresh sporocysts, showing completely developed sporozoites and small "rest" body.

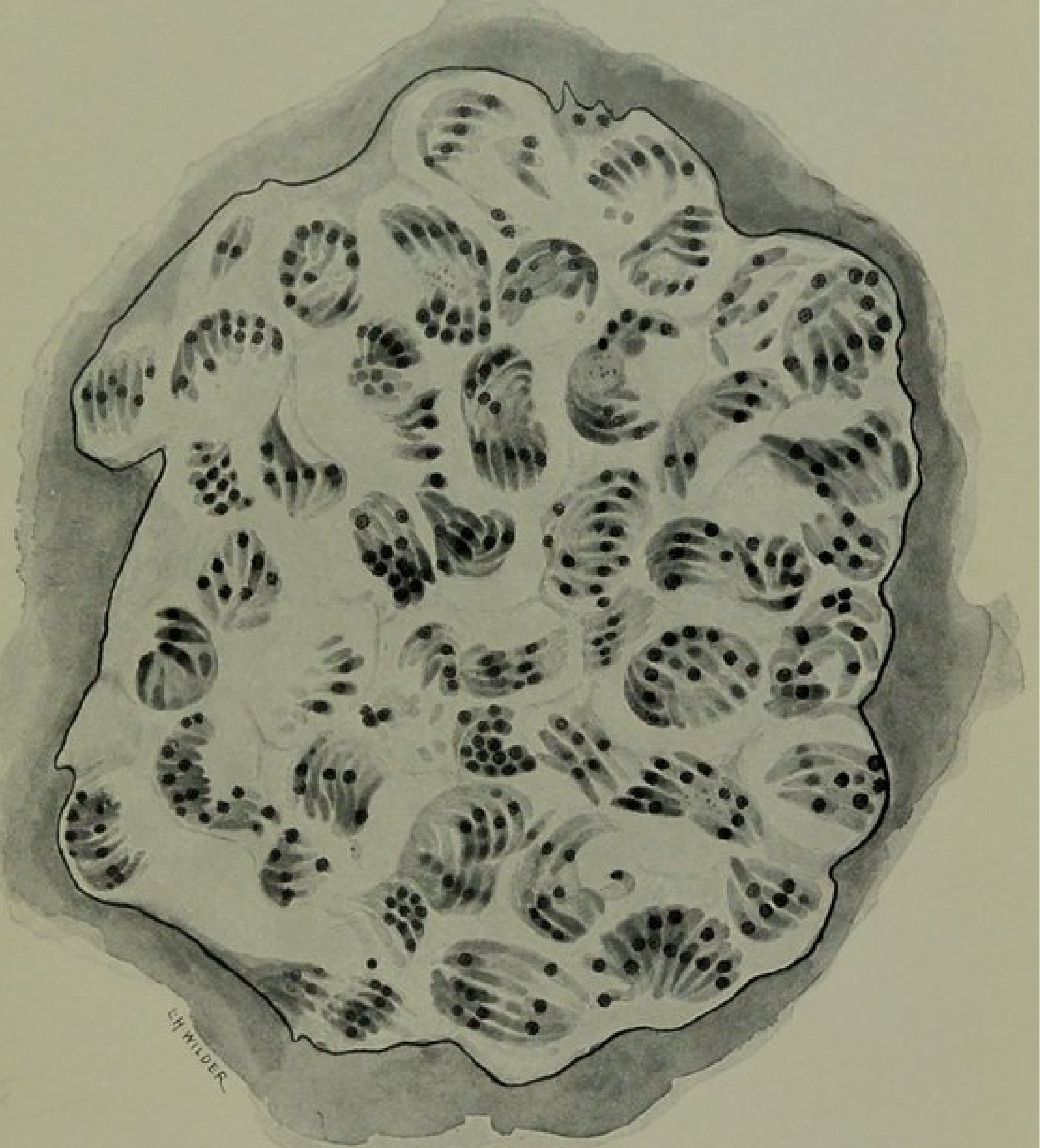


PARASITES DRAWN FROM SECTIONS OF MITES. STAINED WITH IRON HEMATOXYLIN.

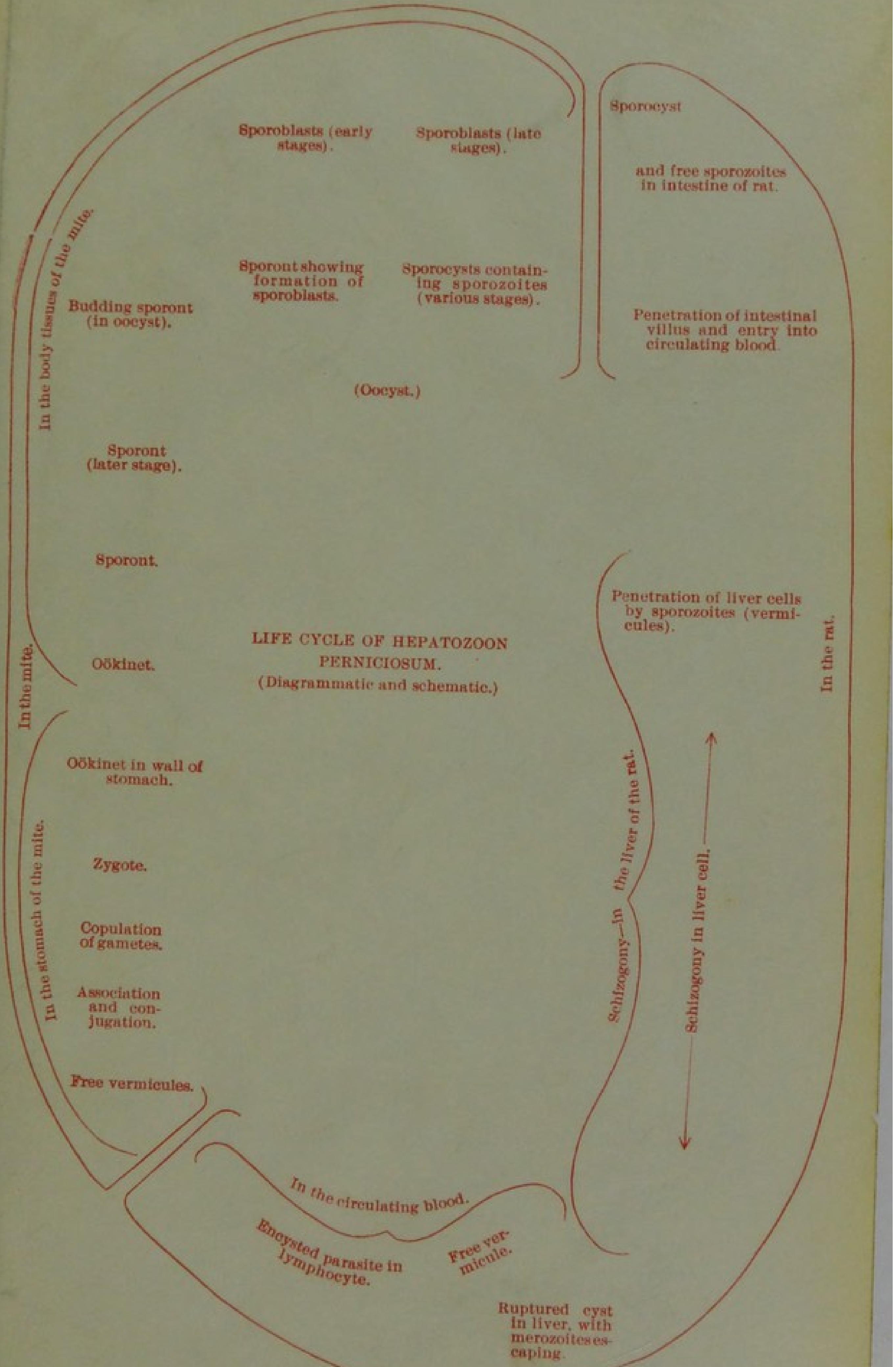
Fig. 1.—Large sporont. Figs. 2-3.—Sporonts with daughter nuclei. Fig. 4.—Early bud formation. Fig. 5.—Segmentation into sporoblasts almost complete. Fig. 6.—Complete segmentation into sporoblasts. Stages shown in figs. 4, 5, and 6 are numerous in Pl. XI. Drawn with camera lucida.

SPOROBLASTS SHOWING VARIOUS STAGES OF NUCLEAR DIVISION AND SEGMENTATION INTO SPOROZOITES; FROM SECTIONS OF INFECTED MITES, 7 TO 10 DAYS AFTER BEING PLACED UPON INFECTED RATS. STAINED WITH IRON HEMATOXYLIN.

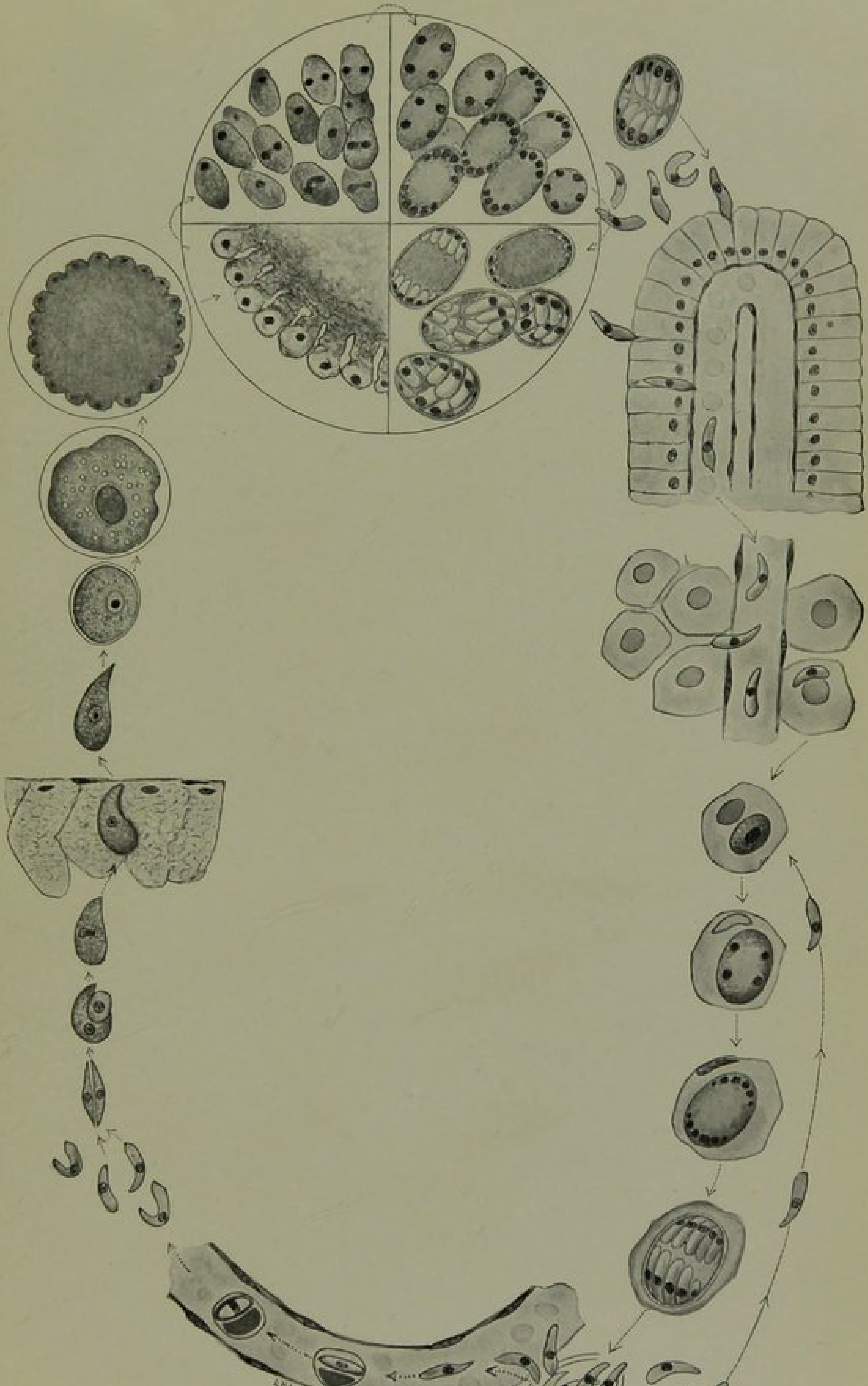
Figs. 8 and 10 show cross sections of sporoblasts near one extremity. Drawn with camera lucida.



CROSS SECTION OF LARGE OOCYST (FROM SECTION OF A MITE) CONTAINING MANY RIPE SPOROCYSTS WITH SPOROZOITES. STAINED WITH IRON HEMATOXYLIN. DRAWN WITH CAMERA LUCIDA.



LIFE CYCLE OF HEPATOZOON PERNICIOSUM.
(Diagrammatic and schematic.)


L.H.WILDER