## TWO NEW SARCOSPORIDIA.

BY HOWARD CRAWLEY.

**Sarcocystis leporum** sp. n.

The material on which the present study is based consists of the arm and shoulder of a very old male rabbit shot at Bowie, Md., on December 13, 1913. The presence of *Sarcosporidia* in rabbits has been recorded from time to time in the literature, and there are four specimens of this parasite in the collection of the Zoological Division of the Bureau of Animal Industry, the localities of which were Maryland, Pennsylvania, New York, and Illinois. No description of the organism has ever been published, nor has it received a name. With regard to this latter point certain authors have of late assumed that the *Sarcosporidia*, like other parasites, are in the case of each species capable of dwelling in any one of several hosts, and there is a certain amount of experimental evidence that this is true. Nevertheless, in the event of a duplication of names, it is very easy to relegate one of them to synonymy, whereas it is extremely awkward to be obliged to refer to a parasite as the sarcosporidian found by a certain author in a certain animal from a certain locality on a certain date. Hence it seems best to make a new species, and I propose to call this parasite *Sarcocystis leporum*.

In the fresh tissue the parasitic cysts were visible as short, delicate threads or rods lying in the muscles. They were about two millimeters long, and the diameter measured in paraffin sections was from 200 to 250 microns. It may incidentally be mentioned, however, that the size of a sarcosporidian cyst is of no diagnostic value, since it is wholly a function of the age, and the cysts of this particular specimen were probably much under the possible maximum size.

Compared with the infestations seen in rats, mice, and ducks, that of the rabbit here under consideration was very slight, and a casual glance at the flesh would probably not have revealed anything amiss. Data as to how heavily rabbits may become infested are, however, wholly lacking.

The cysts, in paraffin sections, showed nothing noteworthy. The cyst wall was from 5 to 6 microns thick, and seen under low powers presented the typical striated appearance. Under high magnifica-

tion, however (500 to 1000), it was easy to see that the wall was composed of a great number of papilliform processes, standing closely packed together upon a sort of basement membrane and with their outer ends wholly free. That is, the cyst wall, at least in this case, does not consist of a membrane pierced by pores, nor of a congeries of rods bounded both without and within by a membrane, but of rods or papillæ projecting freely from a basement membrane. Furthermore, in this case, there was nothing to show that any part of the cyst wall was derived from the surrounding host tissue.

As already stated, the papillæ rested upon a basement membrane with which they were apparently continuous. Within there was to be seen the structure usual for sarcosporidian cysts; that is, a division of the central space into compartments, the walls of which were a continuation of the membrane inclosing the cyst. Finally, in the central portions of the cysts there was a small area free of spores, and here the coarsely alveolar structure of the frame work could readily be seen. This is in itself an indication that these cysts were young rather than old, since it is a matter of common observation that in old cysts there is always present a central space of considerable extent in which there are no spores.

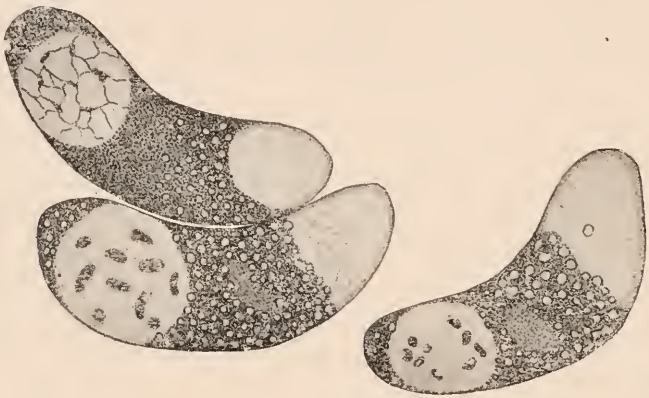
As is usual, the cysts were closely packed with spores which showed a certain disposition to be arranged in files, radiating from the centre to the periphery.

A study of the spores themselves revealed data of considerably greater interest. Several mounts were prepared by smearing out the contents of the cysts on slides, drying, fixing in absolute alcohol and staining in Giemsa. As thus prepared, the spores measured about 13$\mu$ long by 5$\mu$ wide, the precise figures for the average of 20 specimens measured being 13.14$\mu$ long by 5.16$\mu$ broad. The longest spore measured was 16$\mu$ long, the broadest 6$\mu$ wide. The figure given for the length, however, is a trifle too small, since the measurement was taken in a straight line, no allowance being made for the curvature. Furthermore, it is not unlikely that the fixed spores are too wide, since they are quite large enough to be flattened in the process of fixation.

The spores, although possessing the typical banana shape, are not quite symmetrical, it being generally possible to distinguish between a narrower, more pointed and a broader, more rounded end. This narrower end, which may be regarded as anterior, is occupied by a very solid mass of homogeneous cytoplasm, which has but little affinity for the stain and contrasts very sharply with the deeply

staining cytoplasm of the remainder of the spore. Thus, when viewed with powers of only 200 to 300, the spores show two very clear-cut oval areas, the nucleus in the posterior half and the differentiated area in front, and this latter is sometimes so faintly stained that the complete outline of the spore cannot be followed.

The clear region is sometimes oval, sometimes truncated behind, as shown in the figure. It may be spoken of as the rostrum of the spore. Behind it, the cytoplasm abruptly assumes the character which it possesses in the remaining portion of the spore where it is densely staining and conspicuously alveolar. It is to be noted, how-



Spores of *Sarcocystis leporum*. × 3500.

ever, that the cytoplasm nearest the rostrum shows the coarsest alveoli, while backward the alveoli become smaller and smaller, so that in many cases the cytoplasm in the posterior half of the spore becomes very dense, on account of the excessive minuteness of the alveoli. In other cases, however, the alveoli are distinctly visible throughout the entire extent of the dense spongioplasm of the cell. At times, also, the spongioplasm encroaches somewhat upon the homogeneous cytoplasm of the rostrum, there being here visible one or more alveoli or one or more strands of spongioplasm. There is, finally, often to be seen one or two clearer regions in the cytoplasm between the rostrum and nucleus, but it is not believed that these represent morphological entities.

Following the rule for the spores of *Sarcosporidia*, there is no membrane, the spores being naked masses of protoplasm.

The nucleus may occupy nearly any position in the cell, but it is usually placed near the posterior end. Although, as already stated,

it stands out very clearly when the spores are viewed with rather low powers, it seems for the most part to be no more than a clear space in the cytoplasm, and it is only occasionally that a definitive nuclear membrane can be demonstrated. Within it is normally provided with a number of chromatin granules. These granules vary a good deal in size. At times they are quite minute and occur in clusters and chains. More frequently, however, they are quite large, round, or elongated, and appear to be wholly free in the nuclear sap. At times, also, there is a more or less typical chromatin net. Whereas these differences may have some significance, it is perhaps best to regard them merely as variants of some fundamental plan.

The spores of certain species of the *Sarcosporidia* which attack mammals are described and figured as being liberally provided with rather densely staining granules, concerning which there has been a good deal of theoretical discussion. Frequently, also, such spores have been described as showing a differentiated area at one end, and attempts have been made to correlate this area with the polar capsules of the spores of the *Myxosporidia*. In the case in hand, the spores of *Sarcocystis leporum*, there is at least no question about the structure, which is remarkably clear cut and perfectly obvious. And the rostrum of this spore, whatever may be its homologies, is clearly the analogue of the rostra of the sporozoites and merozoites of *Coccidia*. Moreover, it seems an entirely safe assumption that its function is to enable the spore to drill its way into the intestinal epithelium of its host, without prejudice as to whether this host be an invertebrate, another rabbit, or some carnivorous mammal or bird which preys upon the rabbit.

**Sarcocystis setophagæ** sp. n.

Stiles (1895 c)[1] notes that Dr. Hassall, of the Bureau of Animal Industry, discovered a sarcosporidian in the muscles of a redstart (*Setophaga ruticilla*), and a description of this parasite was promised at the time. This description, however, was never published, and the material, consisting of two cysts embedded in paraffin, was recently given to me by Dr. Hassall.

Based upon the number of sections yielded by the cysts, their length was about 2.5 mm., while the largest cross sections measured about 1 mm. in diameter. Hence the cysts are thick in proportion to their length, which seems characteristic for *Sarcosporidia* of birds. The cysts were divided into the usual compartments.

[1] Stiles. 1895 c.—New American finds of Sarcosporidia (Notes on parasites. 28.) *Vet. Mag.*, Phila., v. 1 (11) (Nov., 1894), Jan. 17, pp. 728, 729.

Unfortunately, the material was not in good condition for cytological study, and very little could be made out as to the structure of the spores. As well, however, as could be determined, the form was that shown by the spore of *Sarcocystis rileyi*, that is, one end was rounded, the other pointed. The spores were sometimes straight, sometimes curved, but in the latter case the curvature was slight, which also seems characteristic for avian *Sarcosporidia*. Within, in a few of the better preserved specimens, it was possible to make out a vacuole in which was a chromatin granule. This structure is probably to be interpreted as a vesicular nucleus.

The spores were small, measuring from 4 to 5 microns long by .75 to 1.00 micron broad.