EXPERIMENTAL SARCOSPORIDIOSIS IN THE GUINEA-PIG AND ITS RELATION TO A CASE OF SARCOSPORIDIOSIS IN MAN.¹

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PLATES I AND II.

Notwithstanding the fact that infections by representatives of the genus Sarcocystis are very common among some of the domestic animals, little is known about these interesting sporozoa, and very few experiments have been conducted for the purpose of clearing away our ignorance of the mode of infection and the life history of the parasites in their various hosts. There are at least two reasons for this: first, sarcosporidiosis gives rise to few, if any, symptoms; and secondly, the infection is very rarely communicated to man.

Sarcosporidia have been found in the tissues of mammals, birds and reptiles, by different observers in various parts of the world, more than twenty-eight species of hosts being represented. Some of the domestic animals are infected to a very high degree; 98 per cent. of sheep in Europe are said to be infected with sarcosporidia, and 98.5 per cent. of swine. Theobald Smith (1) found that 6 2/3 per cent. of the gray mice in his laboratory at Boston were naturally infected. The writer (2), from an examination of rats caught in or near the City of Panama in 1908, found the following number of naturally infected animals:

<table>
<thead>
<tr>
<th>Number of adult rats examined</th>
<th>Number of immature rats examined</th>
<th>Number of adult rats, Mus rattus, examined</th>
<th>Number of adult rats, Mus decumanus, examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>131</td>
<td>89</td>
<td>28</td>
<td>65</td>
</tr>
</tbody>
</table>

1 Received for publication September 7, 1909.
Number of rats examined, both species, all ages ......................... 324

Number of these found to be infected upon microscopic examination. Per cent. infected upon microscopic examination.

Number of rats examined that were not infected to gross examination... 26 2 7.6

From this table it appears that adult rats alone show gross evidences of infection, and that some of the rats negative to gross examination may be found to be infected upon microscopic examination; that is, the sarcosporidia are young, not fully developed, and are obscured by being enclosed within a muscle fiber (Fig. 1).

The larger proportion of infected specimens of *Mus decumanus* is in keeping with our knowledge of the habits of this species. The following is an illustration of this:

On October 20, 1908, 15 gray rats (*Mus decumanus*), and 15 black rats (*Mus rattus*) were placed in a roomy, well ventilated cage about 3 by 4 by 4 feet, to be fed with the carcasses of rats infected with *Sarcocystis muris*. Within a few days all the black rats had been killed and eaten by the gray rats, although they had been regularly supplied with food (bread and killed infected rats). It is not uncommon, also, to see gray rats kill and eat individuals of their own species.

Sarcosporidiosis in man, as Theobald Smith (1) has pointed out, has been rarely observed. This is because the infection is extraordinarily rare and also, no doubt, for the reason that the "muscular system in man is not subjected to that scrutiny which the viscera undergo in pathological enquiries, and that sarcosporidia may be present and yet not be recognized."

There are three assured instances of this infection in man. Kartulis (3), who reported the first case in 1893, found Miescher's cylinders of various sizes in the liver (?) and muscular system of a Sudanese who had succumbed to multiple abscesses of the liver and abdominal muscles. Döflein (4) regarded this case as doubtful. Braun (5), however, after examining Kartulis's preparations agreed with the latter's diagnosis of sarcosporidiosis.

The second case was that reported by Baraban and St. Remy (6) in 1894. These observers found sarcosporidia in the laryngeal muscles of a man who had been executed by hanging. The sporozoan distended the muscle fibers to four times their normal thickness,
and were 1.6 millimeters long and 0.17 millimeter wide, with a thin capsule. The sporozoites were banana-shaped, and were 8 to 9 microns long in sections. All individuals were in the same stage of development and the sporoblasts developed in room systems (Kammerung).

The third positive case was found by the writer (2) and was reported in April, 1909. The parasites were found in tissue taken from the right and left biceps (Figs. 2 and 3). They were microscopic in size and disappeared within a period of four months after the first examination. This case is of special interest because the parasite was discovered in the tissues of its living host, who was under observation for a period of one year, during which time tissue for examination was removed on three occasions. The close resemblance between the sarcosporidia in this case and those in guinea-pigs experimentally infected with Sarcocystis muris from rats was very striking.

In the writer's description of the case of sarcosporidiosis in man, he stated that the infection was probably a chance one by sarcosporidia whose customary habitat was some one of the domestic animals, or still lower order of life, and that the sporozoan described was probably abortive. This opinion has since become more firmly fixed by the results of Negri's (7) experiments, and by the results of the experiment described here in confirmation of Negri's in which a number of guinea-pigs were fed with the muscle of rats infected with Sarcocystis muris. In this experiment it was demonstrated that after an interval of five months from the last feeding the guinea-pigs had become infected, and parasites were found in sections of the thigh muscles. A most interesting and instructive fact developed from the experiment was that the sarcosporidium of the rat became profoundly modified in the muscles of the guinea-pig; that it became greatly reduced in size; that its mode of development in room systems was probably altered; and that, on the whole, it was strikingly like the sarcosporidium found in man (writer's case).

Theobald Smith (1), in a series of carefully conducted feeding experiments, demonstrated that mice could be infected by feeding them with infected muscle of mice.

Nègre (8) and Koch have also infected mice by feeding them
with infected muscle of other mice. Negri (7) has recently suc-
cceeded in infecting guinea-pigs with sarcosporidia by feeding them
with muscle of rats infected by Sarcocystis muris. His experiments
are of considerable importance in showing that guinea-pigs may
become infected with Sarcocystis muris in this way, and also that
the sarcosporidium undergoes an alteration in morphology in the
muscle of the guinea-pig. This last observation, as Negri says,
deserves consideration from a systematic standpoint, for inasmuch
as the sarcosporidia attain special characteristics in the new host,
they might be mistaken for a new species.

Negri (7) selected guinea-pigs in his experiments for the reason
that these animals were not naturally infected so far as is known.
His guinea-pigs were fed with infected rat's muscle, chopped and
mixed thoroughly with bread or bran moistened with saline solution.
The guinea-pigs were killed at intervals after the last feeding and
sections of their pectoral muscles chiefly examined microscopically
for sarcosporidia. Nine out of eleven animals fed became infected
fifty days after the first feeding.

These sarcosporidia from guinea-pigs, described by Negri, so
closely resembled those found by the writer in man that the follow-
ing experiment was carried out to determine whether guinea-pigs
could be infected in this tropical region, which is more nearly within
the climatic limits of their natural habitat (Brazil) than in Pavia,
Italy, where Negri's experiments were carried out, and also to deter-
mine whether there was any likeness between the guinea-pig sar-
cosporidia and those found in man (writer's case).

Six adult guinea-pigs, male and female, were placed in a clean pen October
19, 1908. The animals had been bred at the laboratory since 1905 from a lot ob-
tained in the United States. They had always been fed with coarse fresh grass
from the hill near by, which they received morning and evening. In this experi-
ment the infected muscle was fed in the following way: on the morning of a
feeding their regular supply of grass was withheld, and a much smaller amount
was cut up into pieces, 1 to 3 cm. in length; this was placed in a zinc dish to
prevent waste and was sprinkled over with a saline suspension of teased-out
infected muscle or of teased-out ripe parasites from richly infected muscle.

October 19, 1908. Fed with teased chopped emulsified infected rat's muscle
mixed with chopped grass blades and stalks.
October 26. Fed as on October 19.
October 31. On this date the method of feeding was changed by first
teasing out the ripe sarcosporidia in saline solution from heavily infected rat's
muscle. The capsules of the sarcosporidia were torn so as to liberate the sporozoites and the suspension was then sprinkled over chopped grass blades and stalks.

November 2. Fed as on October 31.
November 17. Fed as on October 31.
November 27. Fed as on October 31.

The most favorable feeding was, probably, that of October 31, when a very large number of parasites were fed. The suspension, when examined microscopically, was found to contain innumerable adult mobile sporozoites. The guinea-pigs remained well and vigorous and, save for the first feeding, when only one guinea-pig was seen to eat of the infected grass, all the guinea-pigs were seen to eat a portion of the infected grass at each feeding. The guinea-pigs were sacrificed one at a time on the following dates.

<table>
<thead>
<tr>
<th>No. of guinea-pig</th>
<th>Sacrificed</th>
<th>Days after 1st feeding</th>
<th>Days after most favorable feeding, October 31</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>November 29</td>
<td>40</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>January 4</td>
<td>76</td>
<td>64</td>
</tr>
<tr>
<td>3</td>
<td>January 25</td>
<td>97</td>
<td>85</td>
</tr>
<tr>
<td>4</td>
<td>February 23</td>
<td>126</td>
<td>114</td>
</tr>
<tr>
<td>5</td>
<td>April 2</td>
<td>164</td>
<td>152 (Became infected)</td>
</tr>
<tr>
<td>6</td>
<td>April 21</td>
<td>183</td>
<td>171 (Became infected)</td>
</tr>
</tbody>
</table>

Blocks of muscle were taken from the right and left thigh, right and left foreleg, one pectoral, abdominal muscle, diaphragm and psoas. The esophagus was also sectioned and the liver of three of the animals. The muscle was fixed in alcohol 95 per cent. and paraffine sections stained with eosin and hematoxylin were used. Two to ten sections from each block of tissue were examined carefully first with the low power and then with the high power and a mechanical stage, but nothing was found until sections of the thigh of No. 5 were examined. These sections contained one sarcosporidium resembling the one figured and described by Negri (7), and very much like the one found by the writer in man.

In No. 6, sacrificed 19 days later, another sarcosporidium exactly like the one found in No. 5 was found in sections of muscle from the thigh. When the animals were sacrificed, their muscles were carefully examined in gross for evidences of sarcosporidia resembling *Sarcocystis muris*, but nothing was ever noted at all suggestive of the latter or, indeed, of any species, although the writer was quite
familiar with the gross appearance of this parasite from his previous examinations of many infected rats. It should be remarked that it is strange that instances of rats infected with Sarcocystis muris have not been reported oftener when one considers the hundreds of thousands of rats examined at plague laboratories. The explanation, no doubt, is that plague rats are examined for enlarged groin glands and for the purpose of making bacteriological examination of the spleen; in this way, the thigh muscles, which are most commonly invaded, would be overlooked.

DESCRIPTION OF SARCOSPORIDIA IN INFECTED GUINEA-PIGS.

Guinea-pig No. 5 (Fig. 4).—There is an obliquely cut parasite .111 millimeter in length, and 0.024 millimeter in width, in the interior of a muscle fiber, the diameter of which is 0.033 millimeter. The parasite is oval, and in a few sections at one end of the body of the parasite a very thin unstriated eosin staining capsule, or envelop enclosing the sporozoites can be seen. The sarcosporidium contains hundreds of minute sporozoites, which are slightly curved or banana-shaped, each one of which has a single nucleus, staining deeply with hematoxylin. The nucleus is round, oval, or oat-shaped, and occupies about one-third the space of the eosin staining sporozoite; it is placed at one end of the sporozoite. The opposite end of the sporozoite is generally acuminate. The sporozoite is 3.75 microns long and 1.0 micron wide. In most of the sections, the sporozoites are closely packed together without any arrangement, but in one section they were matted together in little groups of twenty or more (in section) suggestive of clusters in room-chambers (Kammerung), but no reticulum enclosing and separating the groups such as is seen in Sarcocystis muris could be detected. Another block of muscle from this guinea-pig contains many muscle fibers that have undergone a peculiar basophilic degeneration. The fibers are fibrillated and the fibrillar blocks are dissociated and separated one from another. They stain blue with hematoxylin. There is no associated leucocytic or cellular infiltration, and no evidences of myositis. No sarcosporidia could be found in sections of this block. This basophilic degeneration may have been caused by a sarcotoxin formed by the sarcosporidia such as has been studied by Laveran (9).
Guinea-pig No. 6.—A block taken from one of the thighs contains, in section, a sarcosporidium just beneath the fascia but enclosed partly within a muscle fiber. This apparently shows one of the ends of the sarcosporidium. The capsule is wrinkled, slightly shrunk, and encloses a number of sporozoites which are separated sufficiently from one another so that their morphology can be made out. No room-chambers could be detected.

Occasionally, in sections of Guinea-pigs Nos. 2, 3 and 4, a few atypical large nuclei in unusual locations were seen, which were suggestive of resting spores (sporont).

Guinea-pigs Nos. 1, 2, 3, and 4, while presenting no direct evidences of sarcosporidia, would occasionally show a few small foci of cellular infiltration within a single muscle fiber. These foci of infiltration may have replaced sarcosporidia that had died.

The results of this experiment confirm the one previously conducted by Negri (7) with guinea-pigs infected with *Sarcocystis muris*. The greater length of time required before parasites could be demonstrated in the guinea-pigs muscle may be due in some way to the fact that the guinea-pigs were more nearly in their normal environment—the tropics—and may have been slightly more resistant to infection.

It must be said that parasites in Guinea-pigs Nos. 5 and 6 were extremely sparse, and if a much greater number of sections from Guinea-pigs Nos. 2, 3, and 4 had been examined, it is possible that parasites might have been found. It was not thought necessary to do this, inasmuch as the important fact of infection had been demonstrated.

The results of the experiment then are:

The infection of guinea-pigs with a sarcosporidium derived from *Sarcocystis muris* of rats, the parasite becoming evident in the muscular tissue after a period of five months, and appearing to be undeveloped, abortive, and being microscopic in size.

A comparative examination of the sarcosporidium obtained from guinea-pigs, with the one found by the writer in man, brings out several points of similarity.

Both forms are microscopic; they apparently develop without the formation of room-chambers. Their contour, size and general ap-
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pearance is about the same, and their sporozoites are practically the same size.

When the guinea-pig sarcosporidium is compared with the one from which it is derived, some remarkable alterations in morphology in the guinea-pig parasite are noted.

When tabulated, they appear as follows:

<table>
<thead>
<tr>
<th></th>
<th>From rat Sarcocystis muris (mm.)</th>
<th>From guinea-pig (rat) Writer's experiment (mm.)</th>
<th>From guinea-pig (rat) Negri's experiment (mm.)</th>
<th>From man Writer's case (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>13.000²</td>
<td>0.111²</td>
<td>0.1³</td>
<td>0.084³</td>
</tr>
<tr>
<td>Width</td>
<td>0.208²</td>
<td>0.024²</td>
<td>0.03³</td>
<td>0.037³</td>
</tr>
<tr>
<td>Sporozoite</td>
<td>0.012⁴</td>
<td>0.00375³</td>
<td>0.003 to 0.005³</td>
<td>0.00425³</td>
</tr>
<tr>
<td>Width</td>
<td>0.005⁴</td>
<td>0.001³</td>
<td>?</td>
<td>0.00175³</td>
</tr>
</tbody>
</table>

Beside the great reduction in size of the ripe parasite and its sporozoites in the guinea-pig sarcosporidium, there are finer changes in the sporozoite, such as staining qualities and the location of the nucleus. In the guinea-pig parasite, and in that of man, the nucleus is at one end of the sporozoite, while in Sarcocystis muris, studied from smears, the nucleus is nearer the middle and has a well defined nuclear membrane. However, an exact comparison cannot be made because the finer structures of the sporozoites can be seen only in stained smears and the guinea-pig parasite has been detected only in sections of tissue which are too coarse to show anything more than the size, form, and location of the nucleus.

The sarcosporidium found by the writer in man is so small, and resembles the guinea-pig parasite so closely, that one cannot escape the conviction that each is an example of an aberrant form—a sarcosporidium developing in the muscular tissues of an unusual host.

Naturally, it remains to be seen whether the guinea-pig sarcosporidium can be made to develop back into Sarcocystis muris and regain their normal morphology by feeding infected guinea-pigs to rats or mice. This would be an extremely difficult experiment to carry out on account of the sparseness of sarcosporidia in the guinea-pigs, and because of the many naturally infected rats.

One of the most striking things noted in this experiment was the

² Fresh specimen.
³ Sections fixed in alcohol, etc.
⁴ Smears stained with Hastings's stain.
Samuel T. Darling.

extreme sparseness of the parasites in the guinea-pig, notwithstanding the enormous number of sporozoites which must have been ingested.

The mode of infection in man, in the writer’s case, might be explained by his having eaten some uncooked, or insufficiently cooked, infected meat; the pig-tail, or fowl, which entered into his diet, may have been infected. So far as we know, the mode of infection is always by way of the gastro-intestinal tract, and it is not unlikely that the man’s infection was by *Sarcocystis miescheriana*, or by some one of the sarcosporidia of fowl, or possibly a parasite in some lower form of life as its host.

CONCLUSIONS.

1. Guinea-pigs, naturally uninfected by sarcosporidia, were infected by feeding them with rat’s muscle that was naturally infected by *Sarcocystis muris*, and by ripe mobile sporozoites from the same source. The infection was not visible grossly, but was detected upon very careful search through many sections of muscle.

2. Sarcosporidia were not found in the guinea-pigs until after an interval of 164 days from the first feeding, or 152 days after the most favorable feeding, when many teased-out mobile sporozoites were fed.

3. The prolonged period of incubation or latency, and the greater time required for infection of guinea-pigs here over that required by Negri (7) in Pavia, may be related to the fact that the experiment here was conducted in the tropics where the guinea-pig is a native.

4. Sarcosporidia of this apparently abortive type in unusual hosts cannot be specifically identified until their derivation and host relationships have been determined.

5. Morphologically, the guinea-pig sarcosporidia derived from *Sarcocystis muris* are identical with those found by the writer in the biceps of a Barbadian negro, and both probably represent abortive or aberrant forms.

BIBLIOGRAPHY.

Experimental Sarcosporidiosis in the Guinea-Pig.


EXPLANATION OF PLATES I AND II.

Fig. 1. Rat muscle (Mus rattus) showing cross-section of parasite—Sarcocystis muris.
Fig. 2. Sarcocystis hominis (writer's case) section of biceps showing cross-section of parasite.
Fig. 3. Sarcocystis hominis (writer's case) section of biceps showing long section of parasite.
Fig. 4. Guinea-pig No. 5. Sarcosporidium derived from Sarcocystis muris.
Fig. 1.

Fig. 2.