Bulletin No. 259 - Life History of Sarcosporidia, with Particular Reference to Sarcocystis tenella

University of Wyoming Agricultural Experiment Station

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Life History of Sarcosporidia, with Particular Reference to Sarcocystis tenella
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Life History of Sarcosporidia, with Particular Reference to Sarcocystis tenella

By John W. Scott

INTRODUCTION

The solution of any difficult major problem in parasitology involves many scattered observations, the drawing of tentative, frequently erroneous, conclusions from incomplete data or inaccurate observations, and the critical analysis of all the pertinent facts available. This must be done before one can obtain an accurate conception of the biological significance of the problem. The study of the biology of the Sarcosporidia presents no exception to the rule, as will appear later. In a previous bulletin (Wyoming Experiment Station Bulletin 124, 1920) there was given briefly the general biological relations of the muscle parasite of sheep, Sarcocystis tenella, a summary of the experimental evidence relating to infection, and methods suggested for control. In this bulletin we will report on our observations and experiments with particular reference to the life history of S. tenella, mention the results bearing on the life history obtained by other workers on Sarcosporidia, and attempt to interpret the facts and observations available pertaining to the life cycle of this group of animals.

After an extensive series of experiments and observations, extending over a period of more than 25 years, and a careful study of the reports of other workers, my conclusions in regard to the life history of S. tenella may be expressed briefly as follows:

1. A sarcocyst of S. tenella begins as a one-celled amoeboid parasite within a striated muscle cell.

2. By growth and repeated cell division a young sarcocyst develops, consisting of a number of round cells or sporoblasts, each with a nucleus surrounded by cytoplasm. Rarely, it appears that the sporoblasts at this stage may break up, wander out, and start new infections in other muscle cells.

3. As development progresses the sporoblasts continue to divide and all become enclosed in a cyst wall derived primarily from the muscle and connective tissues of the host.

4. The sporoblasts are transformed into ellipsoid and then into banana-shaped spores.

5. In old, large sarcocysts the centrally located spores
degenerate and disappear. The cyst walls of many, perhaps all, mature sarcocysts rupture and the spores find their way into the blood stream. (6) The next stage is somewhat uncertain. I have found the spores in nasal secretions, and in the feces, and though I have not seen them, in urine, it has been conclusively proved that the feces of infected sheep contain an infective stage which by contaminating food, transmits the parasite to a new sheep host. (7) It has been experimentally proved that no intermediate host is necessary. (8) How, in what form, or by what path, the parasite gets from the alimentary canal to the striated muscle cell is a blank, so far as our observations go. This requires an approximately six weeks period. The work of others, on this point, is so inconclusive, uncertain, unconvincing, and so lacking in genetic continuity that any opinion would be based on unsatisfactory evidence.

The biological position of the Sarcosporidia is briefly as follows. One branch of the animal kingdom, the Protozoa, consists in general of one-celled animals, and among these one-celled animals are found a large proportion of the minute animal parasites. One class of the Protozoa, the Sporozoa, consists entirely of parasitic forms. The Sporozoa are characterized by the fact that at one time in the cycle of development each individual produces by growth and division a large number of other forms called spores. This method of reproduction usually takes place within a cyst (or within a host cell), and when the cyst wall, or cell, breaks down spores are set free. The spores are frequently widely spread and because of their vast numbers, some of them are likely to reach another host. The order Sarcosporidia to which S. tenella belongs includes those Sporozoa that are parasites in the muscles of land-living vertebrates, principally mammals and birds. They are especially abundant in herbivorous animals and in some aquatic birds.

Babudieri (1932) made an extensive and critical synopsis of the Sarcosporidia and considers this group as an independent sub-class of Sporozoa closer to Coccidia than to Cnidosporidia. He states that Globidia are not intestinal stages of Sarcocystis and outlines the classification as follows:
Barretto (1940) reviewed the literature dealing with the systematic position of Sarcosporidia, and concluded that most authors think they should be definitely separated from Cnidosporidia Doflein, 1901, and from Telosporidia Schaudinn, 1900, or Sporozoa s. str. Lueckart, 1879 emend. Wenyon, 1926. This author believes that the Sarcosporidia should constitute a class of Plasmodroma Doflein, 1901, between Sporozoa s. str. Leuckart, 1879 emend. Wenyon, 1926, and Cnidosporidia Doflein, 1901.

There is still considerable uncertainty with regard to the classification of this group. The life history, when completely known, will aid in placing them in their true biological position.

Miescher (1843) first observed Sarcosporidia in mice, and these were called "Miescher's bodies." Rainey (1857) made the mistake of describing the sarcocysts found in the muscles of the pig as young stages of a tapeworm. Knoch (1860) mentions that the cysts were found in cattle, common in swine, and even more plentiful in sheep. Leiserung (1865) found "Rainey's corpuscles" repeatedly in sheep, and the next year he and Winkler found whitish knots on the oesophagus of sheep the size of peas to hazel nuts. Sticker (1886) found sarcocysts in the heart of a sheep. Zurn (1872) found cysts in many muscles of the body and two large cysts on the brain covering of the sheep. Barrows (1883) found sarcocysts in the muscles of a bird in Uruguay. Railliet (1886) gave the name Sarcocystis tenella to the muscle parasite of the sheep. He found large cysts (Balbiania) on the oesophagus of an ewe and other sarcocysts present in muscles of the tongue, larynx, pharynx, jaws, lips, neck, shoulders, fleshy portion of the diaphragm, and in thoracic and abdominal muscles, and even in
the skin. Space will not permit here a complete historical account of the Sarcosporidia. Other workers gradually supplied additional information about the group, and the work of these will be mentioned later in so far as it pertains to the life history of these parasites.

As we shall see later, the Sarcosporidia may occur in enormous numbers and produce serious pathological effects in the muscles of domestic animals and birds, and are therefore of important economic interest to man. Besides the economic interest, man is also directly concerned. Lindemann (1863) reported that he found "Psorospermien" in the semi-lunar valves of the heart of a cadaver. Rosenberg (1892) found cysts in the heart muscle of a 40-year-old woman which he regarded as Sarco sporidia. Baraban and St. Remy (1894) who regarded the two previous cases as doubtful, reported what is perhaps the first authentic case of the occurrence of Sarco sporidia in man. Since that date a considerable number of human cases have been reported. For example, Vuillemin (1902) described two cases from man at Nancy, and by study of these, and a comparison with *S. tenella*, states that they correspond to this species as diagnosed by Railliet. Darling (1909) discovered cysts in the bicep muscles of the living host, a 20-year-old Negro, ill with typhoid fever, and removed these on two occasions. His figures, however, give the impression that he was not dealing with Sarco sporidia, or else with an abortive form. Darling (1919) described another case from an East Indian in which the sporoblasts were much smaller than in *S. tenella*. Manifold (1924) reported a new case from heart muscle. Naidu (1928) reported a case in an East Indian that may have been a *Globidium*. Lambert (1927) reviewed the human cases of sarcosporidiosis previously reported and added two new cases from human heart muscle. Vasudevan (1927) described a sarcosporidian from man which he believes is an undescribed species. Hertig (1934) reported a sarcocystis infection from the myocardium of a 26-day-old premature infant. This case is of special significance as it indicates a transmission through the placenta. Gilmore, Kean and Posey (1942) reported invasion of the myocardium of an eleven-year-old Panamanian child with Sarco sporidia.
Human infection with sarcocysts is rare, probably accidental, and apparently without any relation to the normal life history. The spores of human sarcocysts are usually described as similar to *S. tenella* but presenting an abortive appearance in size and form.

**PITFALLS IN THE STUDY OF SARCOSPORIDIA**

It will be observed that in the following pages the observations and findings of some workers have been rejected. There are many reasons why errors creep into scientific work, besides the intellectual honesty of the worker and inaccuracy of his observations. One of the most important pitfalls is interpreting behavior, function or causal relation on the basis of morphology or anatomy. Regressive or degeneration changes may be interpreted as growth and development. Form and structure are like circumstantial evidence; if the chain of circumstances is incomplete, the evidence is regarded as more or less worthless. On the other hand an integrated series of forms or structures may be the best of evidence. Erdmann described a protozoan, found in the epithelium and lumen of the intestine six days after a feeding experiment, as representing stages in the development of *S. muris*. Actually there was no relation either in structure or sequence to the spores fed. Crawley and Marullaz made similar interpretations, and Crawley described the development of male and female gametes on the basis of erroneous observations. The work of Nègre (1910) appears more authentic, but this also needs to be carefully repeated under controlled conditions. The writer found (1927) an amoebula apparently leaving one end of a spore of *S. tenella*, after feeding Balbiania of this parasite to young rats. Other evidence proved that the small amoeba was only closely applied to one end of a degenerating spore. Numerous examples of similar errors could be cited in the literature.

Crude technique has provided another source of error. McGowan (1914), using crude methods, observed that spores burst on a slide, setting free granules. On the basis of this observation he infers that the spores burst in the blood stream and the granules provide a means for congenital infection, *in utero*, or infection through the milk. Mrowka (1925) combined both faulty
technique and erroneous interpretation. He found that under certain conditions the spores break up into granules and infers that infection and reproduction are similar to a filterable virus.

Association does not prove causal relation. Darling (1915) observed that the Neosporidia are very common in insects, and that the feces of insects are widely scattered on grass. To account for infection in herbivorous animals, he advanced the theory that Sarcosporidia are merely aberrant Neosporidia of insects, a hypothesis that our experiments disproved. Sergent (1920) put forward the theory of insect transmission on the basis of a single observation, the fact that he found sarcosporidian spores in a droplet of blood after puncturing through the skin on the jaw of a calf. The chances are that he ruptured a sarcocyst in the muscles of the jaw rather than that he picked up the spores in the circulating blood. To account for the wide spread infection of herbivora, Minchin (1903) assumed that an intermediate host was necessary, presumably a carnivore, to digest infected muscle and set the spores free to be eaten by grass feeding animals. Missiroli (1928) found a parasite in large numbers in the thoracic muscles of Anopheles maculipennis which on account of its general shape he regarded as a sarcosporidian. This led him to favor the theory of Sergent. However, the falciform bodies he observed were smaller than the spores of sarcosporidia, and his figures indicate that they have a very different internal structure.

These instances represent some of the more flagrant errors made in the study of Sarcosporidia and illustrate how easy it is to make mistakes in various ways. Many similar minor errors in drawing conclusions, in making observations, and in methods of technique could be cited. Some of these have been elucidated in the following pages.

ACKNOWLEDGMENTS

In undertaking a work with a scope of this kind one becomes indebted in many ways to many individuals, too numerous to mention. Of outstanding importance in giving advice and encouragement in the early years of this work were the late J. Allen of the Office of Experiment Stations and the late Henry G. Knight,
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LIFE HISTORY

The growth and development of the sarcocysts in muscle tissue have been studied more than any other portion of the life history. This stage of the parasite is comparatively well known and for that reason this report will start with an account of the phases of the life cycle found in muscle tissue.

Some general observations are of interest. Scott (1918b), employing rigid methods of examination, found at that time that apparently 100 per cent of all range sheep on the Laramie Plains were infected. Infection occurred under all different pasturing conditions used, but the percentage of lambs infested and degree of infection were decreased by feeding lambs in a dry lot. Scott and O’Roke (1920) named the counties in which *S. tenella* had been found and called attention to the fact that this parasite probably has a distribution as wide as the sheep industry itself. The conditions for completing the life history must be well nigh independent of latitude, altitude and most other climatic conditions.

In the century since their discovery much has been written about Sarcosporidia, and still our knowledge of their life history is somewhat fragmentary and incomplete. The work at this experiment station has aided materially in rectifying erroneous observations, refuting fallacious theories, and adding to our knowledge numerous facts pertaining to the life history of *Sarcocystis tenella*. The *Sarcosporidia* infest the striated muscle tissue and occasionally the connective tissue of mammals, birds and reptiles. They have been found chiefly in mammals with herbivorous or omnivorous habits, in a good many birds, and in a few reptiles. They may be found in great numbers in the sheep, pig, ox, horse, and in some birds, and apparently have a worldwide distribution.
in several of these animals. Their chief economic interest is for the veterinarian and users of meat rather than for the physician.

Location of the parasites. In general S. tenella is widely distributed in the voluntary muscles, the tongue, masseter, diaphragm, throat, neck, body and limb muscles. It is also found in the heart muscle, and both large and small sarcocysts are found in muscles of the oesophagus but are entirely absent in muscles of the stomach wall only a few centimeters removed, and absent in the muscles of the intestine. Upon several occasions we have found sarcocysts in Purkinje fibers of the heart, as well as in numerous other locations cited. Siedagrotzky (1872) found the cysts in horses were most constant, in greatest numbers and largest in size, in the striated muscles of the oesophagus. Pfeiffer (1891) found sarcocysts in the eye muscles. We have found the large cysts, Balbiania, most frequently in the oesophagus, pharynx, and tongue muscles of the sheep, or enclosed in loose, adjacent connective tissue. Bertram (1892) and others have found the large cysts only in the larynx, pharynx, oesophagus, tongue and palatal muscles. Schlegel (1918) found S. blanchardi in the neck ligament of an ox. The parasite, in early stages at least, is intra-cellular (Laveran and Mesnil, 1899) or inter-fibrillar in location. This was first observed by Laulanie (1884). Railliet (1886) found two cysts in one muscle fiber, in swine. In general the parasites are located in voluntary and heart muscles or occasionally in connective tissue closely associated with voluntary muscles.

Early stages in the muscle. Bertram (1892) observed a sarcocyst consisting of numerous cells only 40 micra in length and 6 micra in width. While such small cysts are not frequently seen, much younger stages have been observed. Alexieff (1913a) states that in early stages the parasite is represented by round uninucleated cells (pansporoblasts) which multiply by divisions and transform into spores. Each sarcocyst consists of a large number of uninucleate parasites enclosed by an envelope and according to his view the septa are derived from the host. Hartmann (1915) described a young stage within a muscle cell which had two nuclei and consisted of two apparently naked ameboid cells. These cells
appeared to increase by cleavage and later develop into a sarcocyst which contained a number of rounded cells enclosed by a cyst wall built from the muscular tissue. In older cysts the contents were divided into chambers by secondary walls of host tissue. The "prosporoblasts" (rounded cells) transformed into ellipsoid and then into sickle-form spores.

Crawley (1916) found a single partly divided "sporoblast" of *S. tenella* in a minute cavity within a cell of the heart. The next stage he found consisted of eight sporoblasts. In 1933 I found a young sarcocyst of *S. tenella* consisting of only two rounded cells. Erdmann (1910b) also observed a single cell with two nuclei. Nakanishi (1929) reported that the contents of fresh young cysts always consisted of rounded, uninucleated, cytoplasmic bodies from 4 to 8 micra in diameter. In these lymphocyte-like cells, granules and nuclei soon become distinct and under favorable conditions proliferate by division or sometimes by budding; then each cell is transformed into a sickle form. Nowak (1930) published some good photomicrographs of *S. tenella*. Several authors, Manz (1867), Fiebiger (1910), Tiechmann (1911a), Fantham and Porter (1914), Hartmann (1915), and others report that the spores may increase by longitudinal division. According to Fantham and Porter (1914) each spore of *S. tenella* contains an amoebula which finds its way into the muscle, grows, and the nucleus divides giving rise to a multi-nucleated mass; the protoplasm segregates around each nucleus and the young pansporoblasts are formed, which may wander out and start new infections. According to my observation this migration of pansporoblasts to other cells seldom occurs in *S. tenella*. In one lamb I once observed a great "swarm" of parasites of about the same age in the heart muscle which from staining reactions and appearance were thought to be young stages of *S. tenella*. In later stages of growth septa form between the pansporoblasts, which continue to divide and give rise to several or to numerous spores within each compartment.

*Later stages in the muscle.* A number of authors have described in detail the structure, growth and development of the older sarcocysts, including the finer structure of the spores. It is not within the scope of this bulletin to relate this detailed
anatomy. In our study of *S. tenella* the development proceeds as follows: The sporoblasts grow and rapidly multiply by division, and growth of the entire cyst takes place. Within the cyst the more actively dividing sporoblasts are found near the periphery. As the cells increase in numbers they push inward toward the center of the cyst, the sporoblasts take on an oval shape and become transformed into "sickle-shaped," or better banana-shaped spores. These young spores may divide by longitudinal division, first shown by Negri (1908). As the cyst becomes larger, partitions extend into the interior from the inner surface of the cyst covering, dividing the interior into chambers. In cross-sections of a fully-developed sarcocyst one may find in the chambers next to the cyst wall sporoblast cells in various stages of growth; nearer the center there is a zone where the sporoblast cells are transforming into spores. In large and aging sarcocysts the most central part is filled with the detritus of disintegrating spores and partitions. In very large, and presumably still older cysts, the preceding zonal structures may be observed, but the central area appears entirely empty.

The size of the cyst depends upon certain external factors such as compactness or looseness of the enclosing tissue and the nutrition available. The shape of the sarcocyst depends upon its location, its age, and the nature of the enclosing tissue. Mature cysts in the diaphragm are much elongated and spindle-shaped. In the heart muscle, the young cysts are usually circular in cross-section and elliptical in longitudinal section; in early stages the length is frequently several times the diameter, while later the diameter increases proportionately faster than the length. Some cysts are irregular in outline, probably due to unequal resistance to growth pressure from within. Such cysts may be on the point of disintegration. Very large cysts, Balbiania, located chiefly in the muscle and connective tissue of the oesophagus, tongue, pharynx, and palatal muscles, are usually oval-shaped and have been reported as large as peas to hazel nuts by Leiserung and Bertram, up to the size of a peanut by Morot and up to 17 mm. in the horse by Siedamgrotzky. Braun (1915) states that Balbiania of *S. tenella* may reach 20 mm. in diameter. We have
found none more than 8 or 10 mm. in length. Alvarez (1926) pointed out that in giant cysts the sporozoites appear densely packed together in the peripheral zone, and loose and scattered in the central part.

The cyst wall. Ferret (1903b) was of the opinion that the cyst wall was derived from the parasite at an early stage when the elements grouped in the muscle cell did not yet have the character of typical cells. Most authors disagree with this view. Our studies indicate that he was partially correct. Fiebiger (1910, 1923) regarded the wall of the cyst as transformed muscle tissue. After extensive study of *S. tenella*, Alexieff (1913a) concluded that the envelope of the cyst consists of two layers, both elaborated entirely by the host, as well as the partitions which detach themselves from the inner layer. The inner layer, according to this author, consists of three zones. Braun (1915) states the sarcocyst is covered by two membranes, an outer, thicker, striated one and an inner, thinner, homogeneous layer from which are derived outgrowths forming the septa within the cyst. Chatton and Avel (1923), working on a sarcosporidian found in the Gecko, concluded that the envelope belongs to the parasite and is not derived from the host. Alcobe y Noguer (1928) concluded the cyst consisted of three concentric layers: (1) a layer of muscle fibers contiguous with the cyst wall, (2) a middle layer of typical collagenous connective tissue and (3) a third layer composed of connective tissue, in which three secondary layers may be distinguished, probably arising as a pathological modification of connective tissues, altered by the pressure of the parasite in growth. These differences in opinion are probably due to the study of different species of *Sarcocystis* in different hosts. Babudieri (1932) after a monographic study of the Sarcosporidia, stated that the cyst wall may be produced entirely by the parasite as in *S. muris* or in part by the host as in *S. tenella*. Barretto (1940) states that his study of the cyst wall of *S. darlingi* Brumpt shows that the Sarcosporidia have highly differentiated and nucleated membranes, which due to their great complexity and staining reactions, are of parasitic origin. In our study of *S. tenella*, the outer layer of the cyst wall is undoubtedly derived from the host;
the appearance, development, and staining reactions indicate that the inner layer including the septa is derived from the parasite.

*Balbiania.* Railliet (1886), on the basis of the thin envelope and location in connective tissue, placed the large cysts found in sheep in a new genus, *Balbiania,* and species, *gigantea.* Later investigators generally regard these structures as all belonging to *Sarcocystis tenella,* that the difference is due largely to a matter of location and external environment. The spores from both kinds of cysts have the same size, structure and appearance. *Balbiania* are most frequently found in the muscles of the oesophagus, palatal and pharyngeal muscles, and tongue, but they do not seem to be common among Wyoming sheep; in fact, are not often found. No satisfactory explanation has been given why they appear in one individual and not in another of the same flock. They are present only in older sheep.

**Fate of the sarcocysts.** Railliet (1886b) observed *Balbiania* on the oesophagus of a goat in the process of calcification. Schlegel (1920) found some cysts of *S. tenella* in a six-year-old goat in the form of caseous, chalky knots. I have infrequently, seen this taking place in sheep. Mason (1910) expressed the belief that new infections occur in the camel by bursting of the cyst, and that spores are carried in the blood stream. Scott (1918b) in Wyoming, found that in old ewes, larger cysts are not so abundant as smaller ones. In ewe 717, which was killed December 28 at the age of 3 years and 9 months, and therefore exposed to four seasons of infection, 100 sarcocysts were measured. By comparison with the size of sarcocysts in lambs and other sheep of known age, four age groups of sarcocysts could be distinguished in ewe 717 as follows: Belonging to the first infective season, 6; to the second season, 15; to the third, 34; and to the fourth or last preceding, 45. While the current seasonal infection of a two-year-old sheep was apparently the same as for lambs of the same season, the number of parasites remaining from the first infective season in two-year-olds was considerably less than for the current season. The average age of sarcocysts in any one animal was two years or less. Either the sarcocysts disappear as they grow older, some never grow to a large size, or
they break up into smaller cysts as they increase in age. There is no evidence for the last hypothesis. While there is some evidence for believing that some sarcocysts never grow large, this view, except for age, is inconsistent with the fact that large and medium-sized cysts are frequently found side by side in the same tissue where nutrition is presumably the same for both. All evidence indicates the older sarcocysts break up and disappear, though occasionally a sarcocyst evidently reaches considerable age. While we are not fully informed as to the conditions under which dissolution of the cyst takes place, the thin wall in some old cysts suggest that not only does the cyst wall break down but that sudden mechanical pressure or strong muscular action by the host may be the cause. The presence of spores found in the blood stream of ewes is in agreement with the indirect evidence. Alvarez (1926) found indications that the cyst ruptures in the pig. Leese (1928), in a treatise on the one-humped camel, asserts the cysts develop, reach maturity, and burst, setting free the spores which are carried into the blood stream and then, if arrested in the red muscle, develop into daughter cysts. The presence of mature spores in the blood stream is additional proof that the cysts are ruptured. We have mentioned above the view, expressed by Fantham and Porter, that the cyst may break up in the early sporoblast stage, and each sporoblast wander out and invade a new muscle cell.

The mature spores. The mature spores have been studied in both fixed and fresh preparations. Plasmolysis of spores does not occur when placed in a 4 M, or a saturated solution of sodium chloride. Apparently the membrane is impermeable. The morphology of the spores has been studied by Fiebiger (1912), Alexieff (1913), Erdmann (1914), Wenyon (1926) and many others. Cleavage by longitudinal fission has been observed by Negri (1908), Betegh (1909), Fiebiger (1910), Teichmann (1911a), Fantham and Porter (1914) and many others. Koch (1902) macerated cysts in physiological salt solution, heated the preparation somewhat above blood temperature in a thermostat on the stage of a microscope, and observed movement of spores of S. musis from freshly killed mice. This movement was described as a backward, lively rotation of a spore about its long axis.
Betegh (1909) worked on fresh sarcosporidia partly with dark field illumination, and on fixed, stained preparations. Cysts of *S. tenella* were freed from the muscle fibers, cut open with a sharp razor, the contents transferred to a slide with a platinum needle, and studied in lymph from the cyst. At first sight fine cilia appeared to be present. However, locomotion was not demonstrated, though there was a noteworthy longitudinal contraction of that part of the spore that has no chromatin. Alvarez (1928) found the structure of the spores was complex, including a nucleus, granulations, filamentous structures, and vacuoles. Alcobé y Noguer (1928), working on goats, found the spores were non-motile, and have no trace of polar capsules or filaments as had been maintained by some earlier writers. By dark field illumination refringent granules were found in the anterior portion of the spore; these were homogeneous by transmitted light. In most spores the anterior portion takes eosin, but some do not, these variations depending upon the age of the cell and intensity of the stain. The ovoid nucleus, found in the posterior third of the spore, contains chromatin granules uniformly distributed. In the median third are numerous siderophile granules. He suggests that the eosinophile substance of the anterior part of the spore may possibly be the toxic substance called “sarcocystin.” Breindl (1927), and Breindl and Komarek (1928) used moist fixation of smears fixed in osmium and found that no striations were present, first shown to be an artificial product by Perrier, 1907; that there is no epimerite lengthening of the anterior end (vs. Alexieff); that there is no flagellum or polar thread (vs. v. Ratz, van Eecke, Pfeiffer); that there is no polar capsule (vs. van Eecke, Watson, Erdmann); and that the nucleus is a group of sharply differentiated granules in the half of the spore near the blunt end (vs. Hartmann, Erdmann, Wasielewsky).

In this laboratory, the writer (1930) has used the following method for the examination of fresh spores: The cyst is cut open with a sharp razor, the contents spread loosely on a cover glass, and the cover glass immediately inverted on a hollow ground slide and sealed around the edges. By this method the fresh spores may be observed with the high powers of the microscope, in their
natural medium, without disturbing either form or structure. By a similar method and fixing and staining on the cover glass, one may get excellent preparations, not subject to the production of artefacts such as are found by the use of some other methods or treatments. As having a bearing on the life history, a further reference to the nature of spores will be found under culturing experiments. My own observations agree closely with those of Breindl and Komarek.

*Sarcosporidia in the blood stream.* If our hypothesis is correct that the old, fully matured sarcocysts break down and set free the mature spores, as indicated above, then we should, occasionally at least, find the spores in the blood stream. After long research we first succeeded in finding the spores of *S. tenella* in the blood of old ewes of different ages, but not in lambs, in 1929. Again in 1930 spores were found in smears prepared from the heart blood of ewe No. 288, and in blood smears, taken from the ear, of both ewe 288 and ewe 205. The number of spores found was small, but assuming equal distribution of the spores there must have been thousands of the spores present in the circulating blood at that time. These spores were apparently unchanged in form and structure from that found in hanging drop preparations. Since a drop of blood contains millions of blood cells, and since the spores are still smaller in size, it is not surprising that spores in the blood are not always found, and never in large numbers.

In spite of the fact that the spores are difficult to find in the blood, abundant evidence has accumulated to show that this is probably a significant stage in the life history. Mettam (1905), writing of *S. tenella*, states, “I have seen the spore in the blood.” Chatterjee (1907) found spores resembling *S. tenella* in a blood smear from the heart of a cow in Calcutta. Spores from cysts in the same animal were identical with those in the smears; Dodd (1909) states that occasionally the spores have been demonstrated free in the blood of cattle. Probst (1910) found sarcocyst spores in a blood smear from the heart blood of a calf, and in blood smears from Texas cattle. Castellani and Chalmers (1919) reported *S. tenella bubuli* in the blood of *Bos indicus*. Croveri (1920) also observed sarcosporidia in the circulating blood of a
sick calf. Fanthan (1920) claimed that he found the spores of *S. tenella* in the reticulum and caecum as well as in blood smears from the heart, lymphatic glands and spleen. It is possible that he was here dealing with a *Globidium*, a closely related genus. In scrapings and smears of heart muscle, the sarcocysts were not usually seen in smears, but free spores occurred. Sergent (1921) obtained spores in a droplet of blood secured by piercing the skin of the jaw of a calf, but these may have come from a ruptured sarcocyst. He was unable to repeat the experiment. Wroblewski (1923) found sarcosporidia in the blood of a sheep that was sick and dying of an unknown “epizootic.” Arai (1925) found spores of *S. muris* in the blood of all mice in which ripe Sarcosporidia were found in the muscles. According to Chiwy and Colback (1926), one may find the spores in circulating blood of oxen.

Considering all these facts, the presence of spores in the blood does not appear to be accidental. If so, their presence is probably related to disintegration of the sarcocysts and represents a definite step in the life cycle.

**Fate of the mature spores.** Very little is known of what becomes of the spores when they leave the blood stream. Do they again penetrate muscle cells and start new sarcocysts? If so, the young parasite found in a muscle cell is not recognizable as having been derived from a mature spore. If this were an ordinary method of re-infection, it would be difficult to reconcile such a method of reproduction with the established facts of seasonal infection (Scott, 1918b). Opposed to this idea is the fact that Nègre (1907) failed to infect mice by inoculating spores of *S. muris* under the skin or in the peritoneum. Do the mature spores leave the host and escape to the outside? There is some evidence, both direct and indirect, to indicate that this is true. The exact path of exit from the blood stream is not known. Alexieff (1912) states that many times he has found the banana-shaped spores in the fourth stomach (rennet-bag) and other parts of the stomach of the sheep. However, he was probably dealing with a related form, *Globidium girruthi*, which we have found in the sheep in a similar location. Nègre (1907, 1918) proved the existence of an infective stage in the feces of mice which themselves had been
fed with sarcocysts. The infective stage appeared in the feces fifteen days after the mice ingested infected muscle, and disappeared about the seventy-fifth day. Whether this infective stage was derived from sarcocysts of the infested host is not known. Crawley (1916) obtained similar results with *S. muris*. Babudieri (1932) states that secondary auto-infection probably does not occur.

It is certain that the spores must escape from one host before they can infect another. Our actual knowledge of the life history is not clear at this point, but such evidence as we have suggests that the path of exit from the blood is by way of the alimentary canal. With this in mind numerous fecal smears were prepared taken at different levels in the intestine and in the four stomach divisions. All urine examinations were negative. So were all fecal samples examined with one exception. In smears prepared from nasal secretions one spore was found. This suggests that if our search had continued further we might have found other spores in the faeces. That the spores can penetrate the tissues was shown by the following experiments: (1) Spores were washed in normal saline and injected into the peritoneal cavity of a young rat. The result showed that the spores were able to penetrate normal tissue and contained an endotoxin. (2) Again using a young white rat, spores were injected into loops of the intestine which had been ligatured. After eight hours toxic effects were evident, but no spores were found within the loops. Considering all the evidence there is reason to believe that the spores escape from the blood through the walls of the alimentary canal. Additional circumstantial evidence will be found later in this report.

**Culturing the spores.** Since the mature spores are the end product of a developing sarcocyst, numerous attempts have been made to culture the spores or observe any behavior that indicated the next step in the life history. Opening the cysts at room temperature, one very rarely sees any movement whatever. Eecke, (1892), in describing the sporozoites in cattle, states that they clearly at times have continuous movements, in part progressive, in part rotating, proceeding from the pointed to the blunt end. Negri (1907) showed that the spores could withstand drying or
heating for 5 minutes to 65 degrees Centigrade, but were killed by heating for 5 minutes to 85-90 degrees Centigrade. When Betegh (1909) opened the cysts of *S. tenella* with a sharp razor, transferred the spores with a platinum needle to a slide and studied them directly in lymph from the cyst, he did not observe any locomotion, but there was a notable longitudinal contraction in that part of the spore without chromatin.

Numerous other attempts have been made to culture the spores. As early as 1872, Siedamgrotzky failed to culture the spores in water, salt, sugar and albumin solutions. Pfeiffer (1888) transferred the contents of a fresh cyst, immediately after removal, to aqueous humor of the sheep and observed movement of the young spores of *S. tenella* in a hanging drop, on a warm stage, at 30 degrees Centigrade. Such movements were seldom seen in like preparations, but may last for hours with gradual cessation of activity. Stiles (1891) saw movements, as described by Pfeiffer, but believed these movements were certainly caused by outside influences. He was unable to find movement in spores, when placed in bouillon, normal saline, water, or aqueous humor from sheep's eyes, either warmed or at room temperature. Pfeiffer (1892) reported that spores from swine in filtered human saliva on a warm stage soon transform into moving amoeboid forms and later into round cell forms. One suspects that retrogressive changes were involved. According to Galli-Valerio, Piana (1896) thought he was able to culture the spores of *S. tenella* on sterilized, moistened filter paper, on moist earth, and on gelatine, at a temperature of 18 to 25 degrees Centigrade. After 25 to 60 days, bodies with a nucleus developed amoeboid movements and encysted. Galli-Valerio (1913) repeated Piana's experiments with *S. muris*. After 8 days he found amoeboid bodies showing slow movement at 20 to 37 degrees Centigrade, which later encysted. Inoculation into the white rat and black mouse gave negative results and Galli-Valerio was not sure that the amoeboid forms were derived from the spores. These results are therefore of very doubtful significance. Behla (1897) tried to culture spores in hay infusion, straw infusion, sterilized dung infusion, dung decoction, muscle juice, etc., with negative results. Koch (1902) observed, figured
and described a backward, lively rotation movement of a spore about its long axis. The spores were from freshly killed mice and were obtained by macerating the cyst in physiological salt solution and heating somewhat above blood temperature in a thermostat on the stage of a microscope.

Willey, Chalmers and Philip (1904), working on the carcasses of Indian buffaloes, found both intra-cellular, microscopic and macroscopic forms. They, like many other workers, found the spores were motionless in physiological salt solution, but when the temperature was raised the spores showed two different movements; "a gliding movement about the center of their curvature" and "a spiral rotation of the body giving the effect of an act of boring." The latter movement was characteristic and the more important. The blunter end of the spore was generally directed forward. On October 29 cysts were placed in white of egg in a covered glass cell. On October 31 the cysts were still intact and the spores moved when heated. November 2 the spores appeared normal and moved vigorously when heated, continuing indefinitely. November 5 the cysts appeared unchanged. Using milk gave similar results. The spores did not resist heat, putrefaction, dessication, or live in running water. This species was named S. tenella bubuli, or S. bubuli. Janin (1908) saw rotating movements in some spores, but was not sure this was a vital phenomenon.

Nakanishi (1929) made an extensive study of sarcosporidia in Korean cattle. For the study of fresh material he employed saline solution, glucose (1-5%), Roger's solution, Ringer's solution, cattle serum, saliva, bile and digestive juice. Young cysts, 16 to 26 micra, sometimes 10 to 15 micra, in width were often found. The contents of these cysts when fresh always consisted of rounded uninucleated, cytoplasmic bodies. Under favorable conditions and media, granules and nuclei soon became distinct in these lymphocyte-like cells and the cells began to proliferate by simple division, or sometimes by budding or chain formation; then each body changed into a sickle form. Active movements of the sickle forms took place in guinea pig bile. Fresh cysts were kept for one hour in one part of inactivated calf serum mixed
with 19 parts of 0.4% saline solution at 25-27 degrees Centigrade; then treated with fresh guinea pig bile and observed at room temperature, or under a warmed stage of a microscope, or with a dark field apparatus. Two sorts of movements were seen: The one was a circular or pushing movement, the sharp end of the sickle being ahead and the whole body being more or less elongated in its curved position; from one to four motions were needed to finish one revolution, but sometimes one motion made two or three revolutions. The second kind of motion was very active, the sickle pushing forwards, or spirally swimming as the halibut does in water, but not as a trypanosome. Active movements continued for two or three hours at 26 degrees Centigrade, but after 18 hours they were less active. He states that Sato (1921) observed similar movements in *S. blanchardi* when treated with pancreatic juice, but the movements were less active.

From time to time I have tried numerous experiments designed to culture the spores, and made numerous observations on living spores under different conditions. In 1929, Piana's experiment was repeated with a negative result. I believe the amoeboid organisms which he found had no genetic connection with the spores of *S. tenella* with which he started. The most important of our experiments are briefly described and the results given below. The method for removing the spores from the cyst has previously been given.

**Experiment 1.** The cultures consisted of sarcocyst fluid mixed with normal saline, (a) at room temperature and (b) at 37½ degrees Centigrade. Result: No activation observed.

**Experiment 2.** Used serum from sheep's blood, (a) at room temperature and (b) at 37½ degrees Centigrade. Result: In one instance the spores showed remarkable movement unlike anything we had previously observed.

**Experiment 3.** Used cyst fluid as a culture medium (a) at room temperature and (b) at 37½ degrees Centigrade.

**Experiment 4.** Used normal saline (a) at room temperature and (b) at 37½ degrees Centigrade.
Experiment 5. Used a weak urea solution (a) at room temperature and (b) at 37½ degrees Centigrade. Results of experiments 3, 4, and 5 were all negative or showed only slight indications of any change.

Experiment 6. A large cyst placed for one hour in a mixture of sterile inactivated sheep serum and sterile saline solution. The cyst was then opened in sterile sheep bile and hanging drop cultures prepared. These cultures kept (a) at 37½ degrees Centigrade and (b) at room temperature. Results: (a) Spores were only slightly active; (b) the spores showed rapid movement.

Experiment 7. Two small cysts were opened in sheep bile; hanging drop cultures were made and kept at 37½ degrees Centigrade. Result: Many spores showed movement and locomotion in a helical path. Contraction and expansion of the spore occurred, accompanied by a flowing of the endoplasm.

Experiment 8. A cyst opened in inactivated sheep serum, and cultured (a) at room temperature and (b) at 37½ degrees Centigrade. Results were negative.

Experiment 9. Cysts were opened in human saliva and cultures kept (a) at room temperature and (b) at 37½ degrees Centigrade. Results were negative.

Experiment 10. Cysts opened in Ringer's solution, and cultures kept under similar conditions. Results, negative.

Experiment 11. A cyst was kept in sheep bile for 17 hours and then opened in sterile salt solution. Results not promising.

Experiment 12. A cyst was placed in inactivated sheep serum and kept over night at 37½ degrees Centigrade. It was then opened in a sterile saline solution. Result: Somewhat later, movement was observed in some of the spores and the method was considered fairly satisfactory.

Experiment 13. An attempt was made to culture spores in (a) a bacto-nutrient broth (one part) and sheep serum (one part), pH 7.2, (b) In a bacto-nutrient broth (one part) and sheep bile (one part) pH 6.7. The results were negative.
Experiment 14. Spores were washed in 0.85% NaCl solution, centrifuged at 600 r. p. m. for five minutes; the supernatant fluid was removed, 10 cc. of fresh 0.85% NaCl added, and the process repeated. After allowing to stand for 30 minutes, the spores were shaken up and placed in a counting chamber, where they were allowed to settle for 10 minutes. Results: The spores showed movements similar to those in bile solutions, but there was no reaction in 0.85% NaCl without other treatment.

Experiment 15. Spores treated with sterile sheep bile at 37½ degrees Centigrade, or at room temperature, gave a specific reaction. This consisted of twisting, boring and darting movements, during which the spore may twist on its long axis and change its radius of curvature. Locomotion also took place in the form of a continuous uniform motion, the cause of which was not ascertained. Such free swimming spores moved in a spiral path, but no means of locomotion was to be seen. The greatest velocity attained was about 9.5 micra per second, and the path of the spore was calculated by Mr. R. F. Honess, Research Assistant, as taking the form of a helix.

Aside from the experiments listed the writer has observed movements of the spores of S. tenella on several occasions and in one instance (1930) described a pronounced locomotion as follows: "The tongue of an old ewe was kept for a week at a temperature that ranged from 15 to 20 degrees Centigrade. On the seventh day a large cyst was removed from the deep muscles of the tongue, cut in halves, and one-half was placed on a slide moistened with sterile normal salt solution. After mixing the contents with the liquid, the mixture was transferred by means of a pipette to a cover glass for a hanging drop preparation. Both bacteria and spores were present; the bacteria exhibited Brownian movement and the Sarcosporidia showed slight traces of motility. The slide was kept at room temperature. The next day the bacteria were much more numerous, the rod-shaped forms were motile and the round forms showed Brownian movement. The Sarcosporidia were nearly all motile, would turn over, straighten out and move one-third the way across the field in a short time; they would twist and turn and change position almost constantly. The hyaline
end of a spore would lengthen out, causing the spore as a whole to assume a long spiral shape, then as it contracted and resumed the normal or typical banana shape, the spore moved forward a distance sometimes almost equal to its full length. Twisting and turning movements, resulting in moderate changes of position, were more characteristic than progressive locomotion.” Experienced co-workers, a botanist and a bacteriologist, both agreed that we had a true case of movement and locomotion unlike anything we had previously seen. On the third day the spores were inactive and apparently dead. Whatever the significance, it appears that the spores under certain conditions are capable of locomotion and twisting movements, such as would probably be required to pass from the blood stream to the alimentary tract.

It is difficult to interpret the significance of the preceding results. In general, bile is the most successful agent in producing active movements and locomotion of mature spores; reactions occur both at body and room temperatures. Nakanishi, using bile as a culture medium, also found that by taking young sarcocysts, he could produce, cell division of the young rounded cells (sporoblasts), growth and transformation into the sickle-formed spores. Pancreatic juice is said to have a similar effect. Mechanical centrifuging affords a sufficient stimulus in some cases, and our result in obtaining very active spores from fermenting flesh suggests that a chemical stimulus was responsible for the activity. Comparatively mild but definite responses are sometimes produced in sheep serum, and in aqueous humor. Heat, likewise, may bring about activity of the mature spores. Both physical and chemical agents are capable of producing activity of the spores.

From the preceding account it is noted that authors differ as to details in describing the movement of the spores. Our own observations agree rather closely with those of Nakanishi. It is evident that under certain physical conditions, and in certain chemical media, the spores are capable of a definite type of behavior which is probably related to a significant phase of the life history, namely the exit of spores from the blood stream through tissues into the alimentary canal. We have recovered the spores from nasal secretions and faeces of the sheep. Alexieff reported
the spores in the lumen of the fourth stomach and in other parts of the stomach of the sheep, and Fantham reported spores in the reticulum and caecum, but these may have belonged to the genus *Globidium*.

*Experiments with spores and infected muscle.* A large number of investigators have fed infected muscle to different species of animals known to have a variety of feeding habits.

*Feeding S. tenella to sheep.* Scott (1915b) reported negative results after feeding several lambs with infective heart muscle. Scott also reported negative results for each of the following experiments. (1) September 30, 1920. A lamb fed muscle known to contain numerous sarcocysts. (2) September 30, 1926. Two lambs fed *Balbiania* for the purpose of obtaining intestinal stages. It was found that development of the spores, after they were freed by digestion, proceeded no further than they did in the white rat, that is, failed to produce an infection. (3) On August 5, 1926, a lamb was fed several *Balbiania* (many millions of spores) for the purpose of obtaining muscle stages. When killed March 31, 1927, some of the control lambs showed an equally heavy infection. The result therefore could not be attributed to feeding *Balbiania*. Feeding a young dog (1915a) liberally with infected muscle, and permitting the feces from this dog to contaminate grass in an enclosure, and then grazing lambs on the contaminated grass, also gave a negative result. Behla (1897) failed to infect sheep by feeding infected muscle.

*Feeding sarcocysts to white rats.* A long series of experiments were tried with young white rats. (1) On August 29, 1925, sarcocysts from an old ewe were fed to a young white rat. When this rat was killed some 18 hours later, a few intra-cellular parasites were found in epithelial cells bordering the glandular pits in various regions of the intestine, but these parasites bore no resemblance to the spores of *S. tenella*. (2) On August 29 and 30 three young rats were fed parts of diaphragm, neck, heart, and leg muscles of an old ewe, No. 279. These rats were killed 53, 65 and 86 days after feeding, but no sarcocysts could be found in the muscle. (3) On February 24, 1926, two rats were fed sheep
muscle (heart, oesophagus, diaphragm, skeletal). When killed from six to twenty weeks later no sarcocysts could be found in the muscles. (4) On February 24, young white rats one-half to two-thirds grown, after fasting for 15-20 hours, were fed two or more Balbiania (Scott, 1927). Some of these rats were killed at intervals from one and one-fourth up to 28 hours after feeding. Amoebulae were found in the intestine and some had penetrated the epithelium. If numerous, they destroyed a large part of the villus and were found along the injured surface or within the partially destroyed villi. Many spores found in the lower part of the intestine were empty and it was thought, at the time, that these amoebula had escaped from the spores. However, this was probably incorrect, for blood smears taken from rats killed 3 to 6 hours after feeding disclosed free, unchanged spores in the bloodstream. The amoebulae apparently had no relation to the sarcocyst spores. (5) On July 31, 1929, an old ewe, No. 288, was killed and within one hour Balbiania were fed to young rats which were killed 0, 1, 1½, 2, 2½, 3, 4½ and 6 hours after feeding. Spores unchanged in form were found in the blood of these rats at 3, 4½ and 6 hours after feeding. (6) On March 31, 1930, Balbiania from ewe No. 295 were fed to eight young rats which were killed from one to ten hours after feeding. Results: Spores of S. tenella were recovered in the blood 1½ and 5 hours after feeding. Intestinal smears showed that spores had reached various levels depending chiefly upon time elapsed. Sections of the intestine showed two spores in the act of penetrating the intestinal epithelium. Sections from livers failed to show, with one possible exception, any spores in this region. (7) On December 6, 1930, ten fresh Balbiania were fed to six young rats. Previously we had shown that when fed fresh, some of the spores pass into the blood within a few hours. The object of this experiment was to see if sarcocysts became established in the muscles. The rats were killed at intervals from one to fourteen weeks, but no Sarcocysts were found in the muscles.

Vanni (1932a) states that the spore of S. tenella is disintegrated in the rat. From these experiments it appears that S. tenella is unable to complete its life cycle in the white rat. The
distinctive, pathological effect of feeding the Balbiania will be described later.

Feeding sarcocysts to mice. Smith (1901) was the first to determine the intestinal origin of sarcosporidial infection. He infected mice by feeding infected mouse muscle, and (1905) states that he maintained the infection for nearly seven years by feeding infected muscle. He also, in the earlier paper, states that spontaneous infection went on at the same time. The parasites did not appear in the muscle until 45 days or more after feeding. Koch (1904) apparently got positive results from ingestion of muscle. He removed parts of leg muscle from both experimental and non-experimental mice with the invariable result that almost all mice killed after 45 days were infected. He suggested that in nature mice may become infected by eating the bodies of dead mice, but there must be a different mode of infection among herbivorous animals. Nègre (1907) confirmed Smith's results; the parasites did not appear in the muscle before 45 days and were mature in from 65 to 90 days; at the end of this development the sarcocyst showed the maximum power to infect. Young mice were more easily infected than adults. Sarcocysts were found in different stages in the same and different muscles and sarcocysts of the same age varied in size in different mice. More mice were infected by eating muscle if confined together than if isolated. Inoculating spores under the skin failed to infest. Negri (1908b) obtained similar results by feeding S. muris to Mus decumanus.

Erdmann (1910) transmitted S. tenella to mice by feeding sheep sarcocysts mixed with wheat bran. The same year, 6 days after feeding sarcocysts, she found and described small amoeboid forms in the faeces and lumen of the gut, and later in the lymph spaces of the gut musculature; five weeks after feeding she found these forms in lymph spaces of muscles. She did not observe these amoeboid forms escape from the spore, and in 1914, hesitates to say that they were derived from or connected with the sarcosporidian spores which were fed. Inspection of her illustrations suggests that she was dealing with an intestinal protozoan. Crawley (1916) repeated Nègre's experiments with similar results. He also figures male and female forms, but we have pointed out
(1930) that he was in error. Marulluz (1920) failed to find the fertilization processes described by Crawley, but claimed he found the spores in mitosis in the epithelial cells and later in the lymph spaces. These could be taken for cleavage stages of lymphocytes and their identity with *S. muris* is in no way probable.

Arai Kei (1925) found that infecting mice with *S. tenella* by feeding experiments is not as easy as previous results had indicated. The material must be quite fresh after slaughter. He killed mice 2, 4, 6, 10 and 20 hours after feeding and examined both smears and section preparations, from the stomach, different parts of the small intestine, the large intestine and the caecum. Two hours after feeding, unchanged spores were found penetrating the epithelium between the cells and into sub-epithelial spaces, most numerous in the upper part of the small intestine. Four hours after feeding, no spores were found in the gut tissue but were abundant in the lower, small intestine where they were undergoing degenerative changes. In one mouse he found unchanged spores in blood taken from the tail of a mouse 5 hours after feeding, but not at an earlier or later period. In another mouse he found spores in the heart blood six hours after feeding. The spores that do not succeed in entering the epithelium pass down and undergo digestion. The young parasites appeared in muscle fibers in 35-50 days after feeding. It is pointed out that these results are inconsistent with those of Erdmann, Crawley and Marullaz, and Arai Kei concludes that the entrance of the spores into the gut wall and their transfer to the blood is of an accidental nature. This view is supported by our results in feeding the white rat. Koegel (1926), in experiments transmitting *S. tenella* to the mouse, demonstrated the parasites in the gut epithelium, and concludes that wandering to the lymph or blood vessels can be accepted as a fact.

(1) On August 29, 1925, sarcocysts from the oesophagus of an old ewe were fed to nine mice. The mice were killed at intervals from two to eighteen hours after feeding. The results were similar to those previously described for rats. (2) On the same date 15 mice were fed parts of diaphragm from the same ewe, which they ate greedily. The next day they were fed heart
muscle, and on August 31 all were fed leg muscle from the same source. When killed several weeks later, *S. muris* was discovered in the controls as well as in this group. There was no evidence that they became infected with *S. tenella*. (3) In an attempt to obtain intestinal stages Scott (1926) fed large sarcocysts (Balbiania) to two white mice. The results were similar to those obtained by feeding white rats, mentioned above.

It is well established that the mature spores may penetrate various body tissues, but in view of conflicting experimental results the experiments of Nègre and Arai Kei should be repeated under rigidly controlled conditions. There is no proof that the passage of the spores from the intestine into the blood represents a step in the life history.

*Feeding infected muscle to other animals.* Numerous feeding experiments on a great variety of hosts have been undertaken. Probably some of the experiments were not under adequate control, but it is interesting to summarize the results. Cobbold (1866) considered sarcocysts were harmless. At two meals he swallowed not less than 18,000 cysts from sheep and bullock hearts, with no after effect. Moule (1888) and his colleague ate several bits of raw beef containing sarcocysts, but experienced nothing to indicate development. Leuckart (1866) reports he infected a swine by feeding it infected swine flesh. Virchow, the same year, fed Miescher’s cysts to dogs and rabbits but observed no trace of illness or later infestation. Pfeiffer (1891) pointed out that butcher’s dogs remain free from sarcocysts though fed oesophagus of sheep containing millions of spores. Later (1892) he showed that injections of cyst contents into rabbits was fatal, though feeding to rabbits, swine and sheep was without apparent results. Behla (1897) reported he was unable to infect rabbits, swine, guinea pigs, rats and white mice, by feeding *S. miescheriana*, the muscle parasite of swine. Willey, Chalmers and Philip (1904) were unable to infect a dog with *S. tenella bubuli*. Negri (1908) first successfully transmitted *S. muris* by feeding infected muscle to a foreign host, to the guinea pig, and to the rat. In the guinea pig the cysts and individual spores were smaller than in the mouse,
thus demonstrating that changing hosts produces morphological changes. Darling (1910a) reported that he had infected guinea pigs by feeding rat muscle infected with S. muris. However, the sarcosporidia were not found until 164 days from first feeding and 152 days after most favorable feeding, and were apparently an abortive type. The same year he injected two cysts from an opossum into the muscles of two guinea pigs; one killed at 60 days showed a few sarcocysts, the other killed after 146 days showed none.

Betegh and Dorcich (1912) infected a hen and two ducks with S. tenella by feeding sarcocysts from the sheep. Development was slow and the sarcocysts small in size. Since S. tenella can develop also in the mouse and guinea pig, they are of the opinion that the same species of Sarcosporidia may exist in different animals, a difference in environment producing a difference in form of the parasite. Galli-Valerio (1913) reported that guinea pigs which ate fragments of muscle containing S. muris, did not develop sarcosporidiosis. Scott (1915) found that a dog fed repeatedly on heart muscle from the sheep failed to become infected. On March 26, 1929, he fed 8 Balbiania to a guinea pig with negative results. No sarcocysts were found in sections of oesophagus, heart, diaphragm, and skeletal muscles when killed on August 9 of the same year. Krause and Goranoff (1933) succeeded in infecting the fowl with a strain of S. blanchardi from the buffalo. Chiwy and Colback (1926) failed to infect two young goats by injecting spores in a jugular vein, in a subcutaneous vein, or by feeding the spores from cattle. Patton and Hindle (1926) fed 12 hamsters with a suspension of spores from a freshly killed animal. Results were negative except in one case which they thought had probably acquired the infection before the feeding. Vanni (1932b) fed spores of S. tenella to the blow fly and to the flesh fly. The spores left the intestine and invaded all tissues. Structures of changed form, which Vanni thought were modified spores, were found in adult flies that were raised from larvae fed upon S. tenella. Similar staining reactions were apparently the only proof of such a belief.
Results and conclusions. (1) Transmitting *S. muris* by feeding muscle has been most successful in the mouse, but also in the rat and guinea pig. However, Smith (1901) explains that “spontaneous infection went on at the same time,” and this raises a reasonable scepticism in regard to other experiments of this nature. In his later experiments, Smith found that the sarcocysts developed more slowly and were smaller in mice after feeding muscle than in natural infections. Similar results were obtained by Darling in the guinea pig.

(2) Transmission of *S. tenella* to the mouse, hen and duck has apparently been successful, but it is not an easy matter, and attempts to transmit this species to the sheep and other hosts have been negative.

(3) The reported transmission of a sarcocyst from the opossum to the guinea pig by inoculation in the muscle is of doubtful significance so far as the life cycle is concerned, especially since the organisms produced were of an abortive type. The transmission of *S. blanchardi* from the buffalo to the fowl appears of little significance.

(4) In general, it is difficult, and frequently impossible to transmit sarcosporidia by ingestion to a foreign host, or even to a host of the same species. Natural transmission by ingestion of spores from the muscle is impossible in the case of herbivorous animals. The failure to transmit sarcosporidia by ingestion to carnivorous animals and the results of feeding to all other animals strongly indicate that this is not the usual method of transmission even in the mouse, which is known to eat its own kind.

Experiments with faeces. The writer (1915) failed to infect lambs by allowing them to graze on grass that had been contaminated with the faeces from a dog that had been fed on heart muscle from infected sheep. The conclusion appears evident that the theory mentioned by Minchim (1903), and Creech (1922), namely that a large carnivore, as the dog, acts as an intermediate host for the sarcosporidia of sheep and swine, must be regarded as untenable. Besides, not all infected flocks are exposed to the faeces of carnivores. The writer (1918) showed that natural in-
Infections occurred in lambs under various environmental conditions as follows: On the range, in a wet pasture, in a dry lot with no green feed, in a dry lot where the lambs were allowed to graze twice weekly in a dry grassy pen, in a dry lot where lambs were watered twice weekly in a small pond, and in a dry lot where each lamb twice weekly was fed a different kind of insect in large numbers. In all cases ewes, which were later found infected, were allowed to run with the lambs. Again (1920) the writer found additional proof that natural infection occurs under widely varying, controlled conditions and that a second or intermediate host is necessary for the development of *S. tenella*. A factor common to all the experiments described was the fact that fecal contamination of food was possible. In general, lambs grazed in a wet pasture were more heavily infected than lambs fed dry feed in a dry lot; grazing or feeding lambs in restricted areas, whether in a screened cage or in small lots outside, favored heavy infection.

In 1918, lambs 1, 2, 3, 4, 5, and 6 were raised in a screened cage. Lambs 4 and 5 were given identical treatment with the other four except that twice per week each was fed grass from a pen that had been repeatedly contaminated with feces from infected sheep. Of the six lambs kept in the screened cage, lambs 4 and 5 showed a considerably heavier infection than the other four (an average of 432 sarcocysts per cubic centimeter of flesh as compared with 272 per cubic centimeter). Three other lambs, kept in a dry lot and fed contaminated grass from the same pen, were more heavily infected than four controls kept with them in the same dry lot.

A test was also made of the effect of raising lambs apart from their ewes and far from sheep of any kind. Four early spring lambs, Nos. 16, 27, 59, and 91, within four to seven days after birth were removed to a distance of about two miles from other sheep and raised by hand. Until removed these lambs had not been outside of the sheep barn and it is not likely that they had ingested spores of *S. tenella* before removal. These lambs were given cows milk and grazed together on vacant lots, confined in a small movable pen sixteen feet square. One of the lambs, No. 27, was fed grass 21 times by hand from a far distant
stationary pen where the grass had been contaminated with feces. There was no chance for lambs 16, 59 and 91 to get any of this contaminated grass. When killed, this lamb showed an infection many times heavier than the lambs raised the same year under other conditions, 2060 sarcocysts per cubic centimeter of flesh compared with an average of 266 for 13 lambs raised under other conditions. Unexpected results were obtained with the other three lambs of this group. Lamb 16 showed 2650 sarcocysts per cubic centimeter of muscle tissue; lamb 59 showed 2950 sarcocysts per cubic centimeter, and lamb 91 showed 634 which, though comparatively light, was heavier than in any lamb raised the same year with different treatment. The results in this group of four lambs are of particular interest in furnishing evidence for the faeces-food contamination theory of transmission.

First, these four lambs had not reached an age for taking any other food than milk, before they were removed from their dams, and there was very little chance of any infection before isolation. A study of a great many other lambs had shown that seasonal infection with sarcocysts in Wyoming certainly does not ordinarily begin until later in the spring. Seldom have we obtained an equally heavy infection, and then only when conditions were very favorable for fecal contamination of the grass eaten. There is no reason for suspecting that cow’s milk has any connection with *S. tenella*, and we had previously found considerable evidence that confinement within narrow limits, as in a screened cage or restricted grazing, is an important factor in producing heavy infection. In this respect the conditions were ideal. These lambs were compelled to do close restricted grazing. Since no enclosed pasture was available which had not been used for sheep, a small movable pen, sixteen feet square, was built and set up on some vacant city lots. This pen was moved early in the morning, at noon, at about four in the afternoon, and again about eight in the evening. When first moved in the morning, the lambs grazed eagerly; they moved across and around the enclosure and, as is the habit of sheep after lying down at night, they scattered pellets of feces as they moved about. A considerable part of the grass was quickly contaminated; by noon the rather sparse grass was
practically all grazed down to the ground. Later in the day the grass in the different squares was frequently not so closely cropped. In three or four weeks, after the grass grew up again, the lambs were grazed over the same areas. In this experiment it was clear that lamb 27 had become infected by means of the contaminated grass, agreeing with the results of other experiments, but this does not explain the heavy infection that resulted in the other three lambs. The result can be best explained on the theory that, after spores of *S. tenella* reach the intestine on contaminated grass, there develops in the intestine resistant, infective spores, similar to those that are said to exist in the feces of young mice after they are infected with *S. muris*. The heavy degree of infection of three out of four of these lambs and the medium heavy infection of the other indicates they were probably reinfected from their own feces. *All of the results obtained, after several years of experiment, correlate with the idea that infection with S. tenella is derived from sheep without the necessity of any other host,* and it appears hardly possible to explain the infections in lambs 16, 59, 91, and 27 without assuming an infective stage in the feces of at least some of these lambs. If this assumption is correct, there appears to be no reasonable doubt about accepting this hypothesis as a satisfactory explanation of a part of the life cycle of *S. tenella*.

The following experiment furnishes additional proof that infection with *S. tenella* can occur without any sort of intermediate host being present. Two lambs, Nos. 97 and 98, born in mid-winter, January 23 and 24 respectively, were placed in a screened cage with their ewes, early in the spring, long before any insects appeared. These lambs were killed on July 1, and both were infected with *S. tenella*. There was no possibility of an intermediate host, for the screened cage was free from anything of this sort (mice, mosquitoes, etc.). There was of course abundant opportunity for contamination of food from faeces of the mother ewes.

It is known that mature spores occur in the blood stream, and these spores have been found in the nasal secretions and according to some authors in parts of the alimentary canal. Further, it has been demonstrated that an infective stage of the parasite
exists in the feces, and we have a satisfactory explanation of how the sarcosporidia of herbivorous animals pass from one host to another. We are not fully aware of the exact nature of development in the intestinal stage. The work of Erdmann, Crawley, Galli-Valerio and Marulluz, dealing with a so-called reproductive cycle in the intestine, is not well founded and probably entirely erroneous. While such a reproductive cycle may exist in or outside of the digestive tract it has not been satisfactorily demonstrated.

Experiments on mice. Nègre (1907) demonstrated experimentally an infective stage of *S. muris* in the intestine of mice. He found that the faeces of mice which are infected by eating muscle have power to infect healthy mice from 15 to 60 days after the ingestion of muscle. In mice infected by means of faeces the sarcocysts appeared in the muscles in 45 days. In 1910, he found a protozoan cyst in the digestive tube of a mouse whose faeces had, by ingestion, infected with sarcosporidiosis all of a lot of well mice. This mouse was killed on the 22nd day after ingestion. These cysts, measuring 25 by 30 micra, and containing 9 or 10 protoplasmic masses similar to cells of the sporoblast stage in the muscles, were found in sections of the duodenum included in the intestinal mucosa. A cyst on the point of falling into the intestinal cavity was similar to other free cysts in the intestine. Crawley (1916) also found a protozoan cyst in the digestive tube of a mouse whose faeces had, by ingestion, infected with sarcosporidiosis a lot of well mice and he suggested that this was possibly a stage of *S. muris*. In a later work (1918) Nègre states that experiments proved the existence of an infective stage in the faeces of mice infected with the muscle parasite. This appears 15 days after ingestion of infected muscle and disappears about the 75th day. The maximum power of infection was from the 20th to the 50th day. This stage resists dessication for a month. Heating the faeces for 30 minutes to 60° C. did not inhibit the infective power, but this was diminished by heating for 15 minutes at 60° C; heating to 85°-90° destroyed infective power. He used young mice weaned from their mothers, and found they were more liable to infection than adults. Isolation of mice that had eaten infected
muscle caused a distinct diminution of the number of infections. From appearance in the muscle to full development of the sarcocysts he found to be 45-50 days. At the end of 90 days the muscle was most infective; after this period infectiveness diminished.

We have found no records where sarcosporidia have been transmitted to foreign hosts by feeding food contaminated with faeces. This should provide an interesting series of experiments. Nègre made the interesting observation that more mice are infected by eating muscle if confined together than if isolated. This suggests the importance of the resistant intestinal stage, as a means of natural infection in an animal that is known to be cannibalistic in its habits. Ingestion of food contaminated with this intestinal stage is probably the usual method by which *S. muris* is transmitted from one mouse to another.

*No intermediate host necessary.* We have shown repeatedly that keeping lambs in a screened cage, free from insects, mice or other possible intermediate hosts, does not prevent infection. The only apparent possible sources of infection were the faeces of ewes confined with the lambs. The hypothesis advanced by Minchin that carnivorous animals act as intermediate hosts must be discarded. Also, the hypothesis must be abandoned that the parasites are eaten after death of the host by some carrion-feeding animal, whether vertebrate or invertebrate, and the spores set free by digestion and so contaminate the food of herbivora. Scott (1915) showed that infection with *S. tenella* failed to occur as the result of feeding lambs on grass that was contaminated with feces from a dog which had previously fed on infected muscle. Again (1920a), it was shown that raising lambs in a screened cage with their ewes, free from insects, mice or other possible intermediate hosts, not only failed to prevent infection but increased the percentage of infection. Darling (1915) advanced the hypothesis that sarcosporidia of herbivorous animals are aberrant forms of Cnidosporidida that are normally present in the intestines of insects. Scott, (1920b) after a comprehensive series of experiments found it necessary to reject this hypothesis as untenable. Wasielewski (1896) on account of the delicate nature
of the spores advanced the theory that an intermediate host is required, as in the case of the malarial parasite, to convey the parasite from one host to another. The fact that the percentage and degree of infection increased in lambs that were closely confined with their ewes in screened cages in the absence of an intermediate host, furnishes conclusive evidence that Wasielewski's hypothesis is incorrect. Fiebiger (1910) on the basis that the spores are known to die in the cysts and that they had never been observed to escape into or from the body, argued that spreading would be possible only through the eating of flesh by another animal and the parasites set free. Vanni (1932a), on the ground that the sarcocysts are not reproducible in the muscles, asserted that the passage of the spore through an intermediate host is consequently necessary. Later (1932b) he fed spores of *S. tenella* to the flesh fly, *Sarcophaga canaria*, and to the blow fly, *Calliphora vomitoria*, and demonstrated metacyclic forms in the digestive tube and faeces of these flies, which he thought were developmental stages of *S. tenella*. He fed faeces of infected *Sarcophaga* to two rats, and faeces of infected *Vomitoria* to two rats. One of the latter developed sarcosporidia in the muscles 40 days later. As rats fed with spores taken directly from sheep did not become infected, he concluded that *Callyphora* is the intermediate host of *S. tenella*. While the author states that he has been careful to exclude flagellates and other natural infections of flies, the impression remains that the metacyclic forms, particularly the flagellates, may have been natural parasites of the flies. There is also the suspicion that the one rat found infected had become infected from another source.

Our experiments were so conducted that mice, sheep ticks, mosquitoes, flesh eating flies, blood-sucking as well as non-blood-sucking insects, were all eliminated as possible intermediate hosts. Any hypothesis involving an intermediate host must therefore be rejected.

**Nature of successful infections.** We have seen that successful infections have always occurred under circumstances that agree with the hypothesis of infection by fecal contamination of food. Some infections have always occurred independent of ex-
periments specifically designed to test other hypotheses. On August 24, 1914, I scattered infected sheep muscle as follows: (1) in a pond, located near a swamp in a pasture in which 18 lambs with their ewes were pastured. (2) In a dry pasture in which 23 lambs with their ewes were pastured. In the first group 10 lambs (55%), and in the second group 5 lambs (21%) became infected with *S. tenella*. It was evident that wet or swampy conditions favored infection. In numerous experiments conducted by Scott from 1915 to 1918, designed particularly to test the theory of an intermediate host, it was found that infection had not been controlled, and went on independently of various controlled conditions and treatments given. It was noted, however, that all lambs allowed to graze with their ewes became infected, and that lambs kept in a dry, barren lot, and fed last season's hay in racks were lightly infected. Scott and O'Roke (1920) showed that infection with *Sarcocystis tenella* takes place independent of the presence or absence of insects, and in the absence of a carnivorous animal; that the percentage and degree of infection are greater in wet pastures than in dry pastures and greater than in dry lots where grain and hay are fed; that here restricted range increases infection. In 1921, the writer isolated a winter lamb when less than one week old and raised it by hand on cow's milk; later it was grazed in a moveable pen. When killed the following winter it was found heavily infected. It became infected before it was removed from the ewe, or perhaps the spores were carried on shoes from an infected area. The heavy infection is probably to be accounted for by close confinement and auto-reinfection. As noted above heaviness of infection can be greatly increased by feeding contaminated grass, especially if the lambs are confined to very narrow grazing limits. As the result of experiments in 1922-25, the writer found that lambs closely confined (in a pen, screened cage, or dry lot), or with restricted grazing of any kind, become more heavily infected with *S. tenella* than lambs with wider range and unrestricted grazing. In 1937, the author, after a protracted drouth period in this region lasting six years, noted the growing scarcity of sarcocysts in range sheep.

As mentioned previously mice can be infected more easily and more successfully by contaminating their food with faeces of
infected mice than by feeding infected muscle. Nègre (1918) found that young mice were more liable to infection with *S. muris* than adults, and the isolation of mice that had eaten infested muscle caused a distinct diminution in the number of infections. This result indicates the importance of the faeces-contamination theory in the life history of *S. muris*. In all cases of infection by feeding with *S. muris* or *S. tenella* the resulting forms usually have been small and abortive. Conversely, infection by means of fecal contamination is more successful, produces heavier infections, and the resulting organisms are normal in rate of growth, size and appearance. While the exact nature of this part of the life cycle has not been demonstrated, and may not have been observed, there no longer is any reason to doubt the existence of an infective intestinal stage in *Sarcocystis tenella*.

*Age of naturally infected animals and seasonal infection.* Manz (1867) found no cysts in swine before August; he repeatedly saw them from August to October, but almost all were of small size. Morot (1886) found that the maximum size of cysts present in sheep increased with age, though smaller sizes were also present in the older sheep. Pfeiffer (1891) found the youngest cysts present in spring, and in young sheep, but not in sucking lambs. The following year he reported the same was true of swine. Bertram (1892) found both small and large sarcocysts in old sheep, but only small ones in 8 months old lambs, and none in embryos. Bergmann (1902, 1913) made extensive investigations of age of the host, per cent infected at different ages, earliest age infected, time of year of invasion, and location in the body, of cattle, sheep, swine, horses and reindeer. In 100 lambs from one and one-half to two and one-half months of age, *S. tenella* was found in only 8. In 342 lambs, about three months old, 20% were infected with sarcocysts. *Sarcocystis tenella* was found earliest and most abundantly at the lower end of the gullet in the striated muscle fibers. On the basis of this primary invasion site, noted in horses and cattle as well as sheep, he believes that Sarcosporidia are taken in with food of a plant nature, enter the walls of the digestive tube and are carried through the lymph and blood streams to different parts of the body where they enter.
the muscle fibers. His records indicated that summer was the most favorable time for invasion. The youngest animals found infected by Bergmann were, a lamb, 6 weeks; a calf, 6 weeks; a pig, 10 weeks; a colt, 10 months. Alexieff (1913a) found no Balbiania at Paris after Easter. Viljoen (1918) found sarcocysts in calves, 8, and 2 months old, and in a foetus, but not in a calf 4 months old. The same year Walker found sarcocysts in 26 out of 27 sheep in South Africa, the one exception being a 6 weeks lamb. As the result of experiments and the examination of the size of sarcocysts in sheep and lambs in the years 1915 to 1917, I observed that the smallest sarcocysts were found on the Laramie Plains only in summer and early fall, and came to the conclusion that in this region infection took place during this period and not at other seasons. Scott (1918b) measured the size of sarcocysts in lambs and ewes of known ages raised on the Laramie Plains. It was found that the average size of the parasite increases with the age of the lamb, and that the ratio of the size of the smallest to the largest sarcocysts increases for a time and then decreases. Young stages were found in both ewes and lambs in summer and autumn, but not in the winter or spring. Hence infection was discontinuous. No sarcocysts were found in lambs under 10 or 12 weeks of age, and measurements of sarcocysts in ewes of known age showed that infection occurred in successive seasons. Conversely, within limits one could tell the age of sheep by the number of seasonal infections found in the heart muscle. In 1920, a group of seven lambs was pastured at successive intervals, usually one at a time, from July 10 to September 20, in a small grassy pen. The object of the experiment was (1) to determine at what time during the summer infection is most likely to occur, and (2) how long a time must elapse after grazing begins in a pasture before the pasture becomes infective. Lamb No. 3 (pastured July 24-August 13), No. 6 (August 28-September 20), and No. 7 (September 14-18) were the most heavily infected in the order named. These results are not conclusive, though the latter part of the 72 day period appeared more favorable for infection. The controls, kept closely confined in a screened cage, showed a higher degree of infection than any other group. All
results favored the conclusion that no intermediate host is necessary, and that natural infection takes place by contamination of food with infective faeces.

This seasonal infection is probably due to some factor in climate, for Bergmann (1902) in Sweden reported infection in swine in all months of the year, though the highest percentages of infection occurred in July, August, September and October, and the lowest in December and January. Fantham, in yearly reports (1920-1923), working near Pretoria, South Africa, in the southern hemisphere, found that during June, July, August, September and early October spores were rarely seen in smears from the heart, while during November to May spores were regularly seen, usually numerous. Other scattered observations tend to confirm the results obtained by Bergmann, Scott, and Fantham. Rademaker (1923) found cattle over 2 years of age 98% infected. Nakanishi (1929) found that oxen from five to nine years old, enclosed in a stall from one to five years, were always heavily infested; that some adult swine over one year of age were infected, but pigs showed no sarcocysts. Nikolsky (1931) reported as follows: "A dead fetus with subcutaneous bloating was removed from a cow by embryotomy of the calf. *** In the examination of smears from the lungs and spleen the sarcosporidian Sarcocystis hirsuta Moule, 1885, was discovered. *** The parasites lay free outside the cells one or two in a field of view." The author concludes that these parasites could have arrived at this location only through the blood stream, and that animals infected with Sarcosporidia are able to transmit the same to their offspring. While this appears to be a case of transmission of spores through the placental membranes, it occurred under abnormal conditions, and there is no proof that such transmission would occur except infrequently, under normal conditions. One should know the conditions under which the embryotomy was performed, and was there a possibility of contaminating the smears with maternal blood.

In general seasonal infection seems to be prevalent, but Bergmann found this did not hold for swine, though infection in winter and spring was diminished. Perhaps close confinement in small sties may have had some influence on continuous infection. In
view of the delicate nature of the spores, it would not be surprising to find in some parts of the world local conditions that nullify or render inoperative the climatic factor or factors that ordinarily produce the phenomenon of seasonal infection. With changing climates and changing local conditions, something of this kind is to be expected.

Seasonal infection apparently has some relation to the age at which young animals become infected. For example, on the Laramie Plains, lambs born in winter or very early spring become infected at an older age than lambs born later in the spring. This probably applies to infection with Sarcosporidia in other animals as well as in the sheep.

Path of Natural Infection. Pfeiffer (1888) in discussing natural infection of the sheep stated that the failure to produce infection by inoculation argues for infection by way of the alimentary canal, in spite of negative feeding results in dogs, etc. Stiles (1891) after numerous negative feeding experiments expressed the belief that the spores "must undergo some change outside before it can infect another animal." This may now be accepted as a fact. It has been noted that Bergmann found sarcocysts at the earliest age and most abundantly in the striated muscles at the lower end of the gullet, but they were not present in the smooth muscle fibers of the stomach only 4 centimeters away. Later the sarcocysts appeared in other locations in the body. Scott (1918b) reported that infection occurred independently of the conditions present in his experiments, but that it was more restricted among lambs fed from a rack in a dry, bare lot than in lambs raised in pastures. Later (1920a) he raised lambs with their ewes in a screened cage, and found that these lambs became more heavily infected than under pasturing conditions, though the cage was kept free from all rodents, sheep ticks, mosquitoes, flies and all other insects except a few transient gnats. From this and other experiments he concluded that infection naturally takes place by way of the alimentary canal, by means of food recently contaminated with fresh faeces from an infected sheep or lamb.

Under these circumstances the life cycle appeared to be direct, since only one host was necessary. Scott and O'Roke (1920)
reported that lambs are more certain to become infected and the number of sarcocysts per unit of muscle greater, if the lambs are kept closely confined with their ewes in a screened cage than if allowed to run free in a dry lot or in pastures. Scott (1930) stated that lambs raised under crowded conditions become more heavily infected than those that have a wider range, whether in pasture, dry lot, or elsewhere. Considering all these facts there appears no reason to doubt that food contamination is the usual method by which Sarcosporidia are transmitted to new hosts. Other observations have favored this view. Mason (1910) noted that in camels mature sarcocysts are most numerous in the oesophagus and heart. Chiwy and Colback (1926) concluded from a study of cattle that infection apparently takes place by way of the alimentary canal. Schlegel (1920) states that observations on young goats show the tonsils to be the chief portal of entrance. Brooks (1903) was convinced from the character of the disease, as seen in the New York Zoological Park, that it is transmitted from animal to animal by the grass they eat or through the water supply. Bergmann (1913) came to the conclusion, from data available, that sarcosporidia of herbivora are taken with food of a plant nature. Marullaz (1920) after feeding muscle infected with *S. muris* to young mice, claimed that he recovered the spores in the epithelial cells of the small intestine in one and three-fourths hours after the experiment, in the form of oval elements lodged in the upper part of the cell. From the second hour the spore begins to develop two nuclei. He frequently found in the intestinal lumen the presumed sarcosporidia, free or enclosed in the sloughed off epithelial cells, binuclear forms, similar to the cells which one observed within the covering membrane of the villi. These elements were not those used to produce infection of the animal, for it is toward the tenth day after infection that they are most numerous. The supposed Sarcosporidia remaining in the cells complete mitosis, but he has seen others at the disaster stage, from the twenty-fourth hour penetrate into the lymph spaces of the tissue supporting the villi. He failed to follow these elements in the lymphatic vessels and ganglia, but in the liver he found, rarely, from the eleventh day small oval elements, free or enclosed in
the hepatic cells that he regards as stages in the development of the spores. Similar elements fewer in number were found in the spleen, but these were always extra-cellular. By examining muscles between the 44th and and 55th day after ingestion he found elements analogous to those found in the liver, located in or penetrating the muscle fibers. Likewise he observed a round, intramuscular element, 5μ in diameter enclosing 8 corpuscles arranged as a rosette, which he regarded as the beginning of a sarcocyst. The figures of Marullaz illustrating these forms are not convincing, and there is no direct proof that the parasitic forms found in the epithelial cells and in the lumen of the intestine are Sarcosporidia. The writer after feeding *S. tenella* to young rats (1927) found similar parasitic bodies in the epithelial cells, in the lumen of the intestine between the villi, and in sub-epithelial locations.

At first it was supposed that these represented amebobulae that had escaped from the sarcosporidial spores in the intestine. However, control animals that were kept for a long period, and additional subsequent experiments, showed that sarcocysts did not develop in the muscles at a later period. While Marullaz's experiments should be repeated, to prove or disprove his claim, at this time it appears that he was working with some unknown epithelial parasite, and possibly lymphocyte cells in certain other locations. Erdmann (1910) states that in the intestine of mice, after feeding *S. muris*, the spore membranes rupture and liberate small ameboid bodies which enter intestinal cell and for a few days undergo multiplication. However (1914), she hesitates to say these forms are Sarcosporidia, for they disappear and sarcocysts do not appear in the muscles until 40 days later. At one time the writer thought he had discovered an ameboid form escaping from one end of a spore of *S. tenella*. It turned out to be a parasitic amebobula that was closely applied to or trying to enter one end of a degenerating spore. Arai (1925) fed mice with fresh spores of *S. tenella*. After two hours the spores were found the entire length of the small intestines, but most abundant in the upper parts, and some in the upper part of the large intestine. Already morphological changes were beginning, and at four hours marked degeneration changes were taking place, and at six hours no
spores were found in the alimentary canal. After two hours the unchanged spores were found penetrating the epithelium between the cells and into the sub-epithelial spaces. At four hours the spores were found in the gut tissue, but none at a later time. He found spores in tail blood five hours after feeding, but not at one, two, or three hours; also in blood from the heart six hours after feeding. No further spores were found in the blood up to 35 to 50 days after feeding though the muscle then contained unripe cysts. Scott (1929 and 1930) fed Balbiania to young rats and recovered the unchanged spores in the blood 1½, 3, 4¼, 5 and 6 hours after feeding, but not at an earlier or later time. The spores remaining in the intestine underwent digestion in the course of a few hours.

There is no part of the life-history of Sarcosporidia more obscure than the path of natural infection. Arai and Scott have both shown that spores of S. tenella can pass from the intestinal lumen through the epithelium into the lymph spaces and finally into the blood vessels unchanged. This proves that the ripe spores can migrate through tissues. However, in both cases the spores were fed to a foreign host, and the fact that the spores appeared in the blood in the same form in which they are found in the blood after rupturing of the sarcocyst, suggests that this may not be a natural process of infection. The fact that sarcocysts did not appear in the muscles at a later date also argues against this migration of spores as being a natural method of infection. On the other hand there is no direct proof that the forms described by Erdmann, Crawley, and Marullaz are derived from the spores of sarcosporidia, and these forms disappear from 35 to 50 days before any sarcocysts appear in the muscles. The writer has clearly demonstrated that there is an intestinal stage of S. tenella that is capable of contaminating food and infecting other hosts, of the same species, or reinfecting the same host, but how or in what form it passes from the intestine to the muscle is still uncertain.

In view of the difficulties involved several writers have suggested that in the case of herbivores natural infection is possibly transmitted by means of biting insects. Sergent (1921) found
sarcosporidian spores in a droplet of blood obtained by piercing the jaw of a calf. This was the only instance in which he was successful in finding spores under the above conditions. In a further report (1922) he expressed the belief that a blood sucking insect acts as a host to transmit the sarcocysts of bovines. In view of the extreme scarcity of spores in the blood, and in view of the established facts in favor of transmission by way of the alimentary canal, there appears to be no substantial reason for favoring this hypothesis. Missiroli (1928) found a falciform parasite in the thoracic muscles of *Anopheles maculipennis*, and advanced the theory that it was a sarcosporidian and thought it confirmed the view that the parasite is transmitted between cattle by some blood-sucking animal. His figures, however, indicate that the parasite he was dealing with had a very different structure from the spores of Sarcosporidia. Scott (1920b) showed that no intermediate host is necessary, and that the life history is direct, without the aid of any biting insect.

*By way of the placenta.* For want of a more satisfying explanation, the suggestion has been made that infection of young animals takes place by way of the placenta. McGowan (1923) expressed the belief that *S. tenella* is transmitted from mother to lamb, *in utero*, or in milk. His experiments, however, do not exclude other possible methods of infection. Bertram (1892) and Scott (1918b) found no sarcocysts in embryo lambs. Smith (1905) bred two heavily infected mice in the fall of 1901. The resulting litter of three young were kept until January, 1902, when they were three-fourths grown, but when killed they were not infected. It would be possible for spores in the blood to migrate through the placenta and infect the young mouse or lamb, a path occasionally followed by certain other organisms. However, in the absence of any direct evidence with the two exceptions of foetal infection mentioned, and with the established fact that sarcocysts cannot be found in young lambs until they are 6 to 12 weeks of age, this theory does not furnish an adequate explanation of natural transmission, and need not be regarded seriously.

Inoculation experiments have been tried by numerous investigators. Kasparek (1895) injected *S. tenella* from *Balbiania*,
sub-cutaneously into white mice and into guinea pigs. He ob-
served sporozoites, shortly changed in form, in the blood stream. He injected the contents of a cyst sub-cutaneously into a mouse which died after 24 hours; as he examined the heart blood he found forms similar to the original spores. In the same way he inoculated three guinea pigs. The first guinea pig died in 36 hours, with negative results. Blood from the ear vein of the second guinea pig contained sporozoites at the end of 4 hours, but none at the end of 12 hours. The third guinea pig had spores in the blood at 4 and 5 hours after injection, but none later; this animal was killed after 30 hours, but no spores were found in the blood, in the spleen or at the site of injection. He was uncer-
tain whether the spores migrated, changed their form or were destroyed. Reick (1888), under aseptic conditions, injected sarco-
cysts from the oesophagus of sheep and horses, sub-cutaneously into dogs and rabbits; the dogs were unharmed, but the rabbits died within 24 hours. Pfeiffer (1888) failed to produce infection by injecting an emulsion of *S. tenella* in sheep serum, into mice, rabbits, and lambs. Fatal results were produced in rabbits, but the injection was harmless to mice and lambs. Smith (1901) injected sporozoites sub-cutaneously, but states that they did not seem to infect. Nègre (1907) failed to infect mice with sarcocysts by inoculating spores under the skin or in the peritoneum. Other experiments with inoculations, which produced fatal or pathological effects, will be discussed in another place. In general, the results obtained from inoculation experiments have been negative or pathological, and no evidence has been found favoring the insect theory of transmission of sarcosporidia.

A part of the life history that is entirely unknown is the passage from the alimentary canal to the muscles. This subject has been discussed in part under the heading, the path of natural infection. No one has explained why these parasites have a special affinity for striated muscle. The only logical path to reach such locations is through the blood stream, perhaps including the lymph system. As stated before, all the experimental evidence indicates that natural infection takes place by the passage of the parasite through the epithelial lining of the digestive system. Babudieri
(1932) states that the infective stage is unknown. Schlegel (1918) claimed that the tonsils are the chief portals of infection in goats. The discovery by Bergmann that sarcocysts of *S. tenella* are found first in the muscles of the oesophagus before they are found in other muscles, suggests the idea that the organisms may pass directly through the walls of the oesophagus into the muscular layers, while those that enter the blood stream require a longer period to reach the striated muscles. Or, it may be that Bergmann was mistaken in his observation as to the earliest infections. Elsewhere we have discussed some of the attempts to follow the passage of the parasite from the digestive tube to the muscles. The investigator who develops a technique to follow the parasite during this stage will clear up a very important part of the life history which at present is unknown. An explanation of Crawley's mistake in describing the development of gametes within the epithelial cells of the mouse was found by Scott in 1928, and reported in 1930.

Smith (1901) noted the long latent period between infection and the growth of the sarcocyst in the muscle, and affirmed that the parasite requires this time to store up energy for rapid growth and multiple division. He rejected the view that there was a sexual stage on the ground that he failed to find these stages, and the general results of his experiments. No one knows the location of the parasite during this latent period. It may be resting in the muscle cell, unrecognized because of its similarity to a lymphocyte or some other cell. Or, it may be located in some other part of the body undergoing transformation, as suggested by Erdmann, Crawley, and Marullaz. Crawley (1914) after experimental feeding, found that the spore in the intestine of the mouse becomes active, displays energetic twisting and boring movements by which it forces its way into a cylinder cell of the intestinal epithelium, and there comes to rest, within two and one-half hours, possibly earlier after feeding. Banana-shaped spores were "found both free in the lumen and in the cylinder cells in mice killed at appropriate periods after the inoculative feed." Up to this point his observations on *S. muris* agree rather closely with those of Arai and Scott on *S. tenella*. He continues, "Besides
these, however, others occur. * * * oval bodies generally about half as long as the typical spore.” In some of the intra-cellular cases these forms had undergone conspicuous changes, which Crawley (1916) interpreted as the development of male and female gametes, which afterward unite in fertilization. As Scott pointed out in 1930 this supposed sexual differentiation was based largely upon erroneous observation. Crawley admits the close similarity of some of these forms to the development of Coccidium schubergi, and Wenyon (1926) comments that Crawley's figures are unconvincing and some of them might equally well represent degenerating parasites, while others might conceivably be stages in the development of Eimeria falciformis, a common intestinal parasite of mice. The figures of Marullaz (1920) are equally unconvincing, and a close reading of the context leaves one with the impression that his series of observations were not necessarily connected with the spores of sarcosporidia, and there is evidence that he was dealing in part with another parasite, and perhaps with lymphocytes within the liver and spleen. Whether there is sexual reproduction in the intestine before invasion, or in the tissues after passing through the intestinal epithelium, or any other type of multiplication is still an open question.

SUMMARY

1. The Sarcosporidia consist of the parasitic protozoa found in the muscles and connective tissue of mammals, birds and reptiles. The geographical distribution of some species is known to be almost world wide.

2. The genus Sarcocystis includes those forms found in striated muscle. The severity of infection with S. tenella depends upon the age of the animal, its food, restriction of feeding habits, climate, moisture, the season, and probably the constitution or tendency toward natural immunity of the individual attacked.

3. Sarcosporidia were first observed in mice in 1843. The life history is still incompletely known. More is known of the life history of Sarcocystis tenella of the sheep than of any other species, with the possible exception of S. muris of mice. This paper aims to include the experiments bearing on the life history,
conducted at this experiment station, and to bring together our knowledge of the life history of Sarcosporidia, particularly *S. tenella*.

4. A sarcocyst of *S. tenella* begins as a one-celled amoeboid parasite within a striated muscle cell. The next stage observed consists of two apparently naked amoeboid cells, each with one nucleus. These sporoblasts increase by repeated divisions, and a young sarcocyst contains a number of rounded cells from 4 to 8 micra in diameter, each with a nucleus surrounded by cytoplasm, and all enclosed by a cyst wall built in part from the muscular and connection tissues of the host. Rarely, it appears that the sporoblasts at this stage may break up, wander out, and start new infections in other muscle cells. Or, they may possibly escape by way of the alimentary canal, and become the infective intestinal stage.

5. As growth progresses, the older sarcocysts are divided into chambers by secondary walls which in *S. tenella* are apparently derived from the parasite. The rounded cells (sporoblasts, pansporoblasts, prosporoblasts) continue to divide and later transform into ellipsoid and then into banana-shaped spores, which may increase by binary fission. In very old, large sarcocysts (*Balbiania*), the centrally located spores degenerate and disappear.

6. The cyst wall of many, perhaps most, of the mature sarcocysts breaks down, the spores escape and find their way into the blood stream. From the blood the spores apparently pass into the digestive tube and escape from the host in feces. Whether they change their form in this migration is not known. Feeding experiments indicate that the spores of *S. tenella* undergo some change before they can infect other sheep.

7. The mature spores of *S. tenella* are capable of active locomotion and boring movements, when subjected to treatment with bile, pancreatic juice, centrifuging, the products of fermenting muscle, and thermal stimuli in certain media. This action may play an important part in the life history, but at the present time its significance is not clear.
8. Spores have been found in faeces and in nasal secretions of the sheep, and it has been experimentally proved that the faeces of infected sheep contain an infective stage, which by contaminating food, transmits the parasite to a new sheep host. It has also been conclusively proved that no intermediate host is necessary.

9. At certain times and under certain conditions there is an infective intestinal stage which by contaminating grass or other food produces new infections in the same or other sheep by way of the alimentary canal. The protozoan cyst found by Nègre (1910) in the digestive tube of a mouse whose feces had infected by ingestion, a lot of well mice, may have been the infective stage of S. muris. This remains to be demonstrated.

10. From the time of infection, and presumed penetration by the parasite through the epithelium lining of the digestive tube until the sarcocyst starts its growth in the muscle cell there is a long latent period, usually estimated at not less than six weeks in S. muris. Nothing definite is known of the life history during this period. It is presumed that the parasite reaches its final location in a striated muscle cell through the blood stream.

11. This bulletin aims to bring together our knowledge of the life history of Sarcosporidia, based on the long series of experiments and observations carried out on Sarcocystis tenella at this experiment station, and a critical examination of the work of others who have contributed something to the life history of the group. The merit of the work done at the Wyoming Experiment Station consists in proving that the life history of Sarcocystis tenella is a direct one and does not require an intermediate host; in disproving other theories that have assumed the necessity of an intermediate host; and in pointing out spurious observations and erroneous hypotheses of some other investigators. It is believed that this is the most comprehensive study of the life history of Sarcocystis tenella that has yet been made. The economic importance and toxic effects produced will be treated in another bulletin.
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