SARCOSPORIDIOSIS OR TOXOPLASMOSIS IN MAN AND GUINEA-PIG *

B. H. KEAN, MAJOR, M.C., A.U.S., and ROBERT G. GROCOTT, B.S.
(From the Board of Health Laboratory, Gorgas Hospital, Ancon, Canal Zone)

INTRODUCTION

In many of the texts on human parasitology is a chapter entitled "Parasites of Undetermined Nature," and according to Craig and Faust [1] "Those belonging to the Sarcosporidia and to Toxoplasma are the most important . . ."

The exact taxonomic status of the Sarcosporidia has not been determined. The order Sarcosporidia, Bütschlii, 1882, containing only one genus, Sarcocystis, Lankester, 1882, has been considered closely allied to the Cnidosporidia [2] or Neosporidia,[3] but Wenyon [4] disapproved of this classification. Belding [5] included the order Sarcosporidia in the subclass Acnidosporidia (characterized by the production of simple spores during the life of the sporozoon), class Sporozoa. Approximately 50 species of Sarcocystis have been recorded in mammals, including man, rat, mouse, sheep, cattle, pig, horse, rabbit, dog, cat, deer; birds; reptiles; and recently in fish.[6] It is probable that there are fewer species, although Alexeieff's [7] suggestion that only one species exists may be too radical.

The epidemiology and life cycle of Sarcosporidia are incompletely known. The evidence suggests that, following the consumption of infected food, sporozoites are liberated from the adult cysts, penetrate the intestinal epithelium, reach the lymphatics or possibly the blood stream, and eventually localize in the skeletal muscles. Animals on the whole are tolerant to the presence of sarcocysts, although various debilities have been associated with the infection (see Scott [8] for review). Pfeiffer [9] first demonstrated that emulsions of the parasite of sheep, *Sarcocystis tenella*, when injected subcutaneously, can kill mice and guinea-pigs. The name of "sarcocystin" was applied to this toxic substance by Laveran and Mesnil.[10]

In his authoritative monograph on the Sarcosporidia, Babudieri [11] accepted the following as authentic reports of human sarcosporidiosis:

Baraban and St. Remy [12]	1894	Manifold [16]	1924
Vuillemin [13]	1902	Lambert [17]	1927
Darling [14]	1909	Vasudevan [18]	1927
Darling [15]	1920	Bonne and Soewandi [19]	1929
To these may be added:			
Feng [20]	1932	Hewitt [22]	1933
Price [21]	1933	Gilmore, Kean and Posey [23]	1942

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We are in agreement with Babudieri [11] in regard to these cases which he regarded as authentic (within the limitations of the discussion in this paper) and also with his rejection of many reported cases because he considered them incorrect, doubtful, or lacking in sufficient data for review. The latter group includes the cases of Lindemann, Leuckart, Koch and Gaffky, Rosenberg, Kartulis, and others. (See Babudieri for review.) The case of Kartulis [24] has in the past been rejected by some authorities and credited by others. We are inclined to believe that this is an authentic case, but proof at this date seems impossible.

The case of "bone sarcosporidiosis" reported by Cone [25] must be questioned. In 1928, Naidu [26] published a record of a patient who probably was the same one described by Vasudevan [18] in 1927. The infection observed by Hertig [27] in the myocardium of an infant has been redescribed by Pinkerton and Weinman [28] as toxoplasmosis. Price [21] noted cases of "Scoglia" (probably Scaglia [29]) and of Askanazy,[30] and Rivas [31] mentioned those of Nancy and A. J. Smith, but we have been unable to find records of these cases.

Morphology of the Parasites Hitherto Reported as Sarcosporidia in Man

Although the morphology of the Sarcosporidia varies considerably depending upon the species(?), host, and age, the following description by Craig and Faust [1] of the human parasite, *Sarcocystis lindemanni*, Rivolta, 1878, is generally applicable:

"When fully developed, *S. lindemanni* consists of a cylindrical, elongated or fusiform body, hyaline in appearance, with more or less pointed ends, lying in the affected muscle fibers. It is enclosed in a membrane and contains myriads of round and crescent-shaped spores. In 1843, Miescher found these bodies in the muscle fibers of mice; since that time they have been known as 'Miescher's tubes.' They vary much in size, from forms measuring as much as 5 centimeters in length to others so small that it requires a microscope to detect them. When visible to the naked eye they appear as minute white streaks within the muscle fibers. When removed from a muscle fiber each parasite consists of a cylindrical whitish tube with somewhat pointed ends and a finely lobulated surface. The membrane forming the outer coat of the organism may show a radial striation and from this membrane firm prolongations extend, dividing the tube into separate compartments, of which the outer contain round cells, while in the inner, fully developed compartments there occur the characteristic crescentic bodies called spores. Rounded, ovoidal, elongated or sickle-shaped spores are produced which are known as 'Rainey's corpuscles.' The spores measure from 12 to 16 microns in length and from 4 to 9 microns in breadth; when properly stained they are seen to contain an elongated nucleus situated near the more rounded end of the spore. This nucleus consists of a nuclear membrane and a central karyosome. Many thousands of spores are contained in a fully developed parasite."

In 1894 Baraban and St. Remy [12] described the first case of human sarcosporidiosis which has been universally accepted as authentic. The

parasites were found in the laryngeal muscles of a man executed by hanging. The parasites were large, measuring 1.6 mm. by 0.17 mm., and distended the muscle fibers. The capsules were thin, but striated. Room compartments contained individual sporozoites measuring 8 to 9 μ in length. No inflammatory reaction was present.

Darling,[14] in 1909, recorded what he considered the third instance of human sarcosporidiosis. He was unaware, apparently, of Vuillemin's [13] report but accepted at that time the case of Kartulis.[24] The parasites were found in the biceps of a Negro ill of typhoid fever. Darling's observations may be recorded in detail:

"Here and there were oval or round dotted bodies about the width of a striated muscle fiber, their length being about twice that. The muscle fiber was not distended by the presence of the dotted body. One oval body measured 0.084 mm. in length and 0.027 mm. in width. One of the bodies in cross section was circular and was 0.021 mm. in diameter. The oval or round bodies were strongly contrasted from the eosin-staining muscle fiber by the very pronounced blue stippling of the former. This stippling was seen under the high power to be due to the nuclei of small oval sporozoites. In this section there was some hyaline change in the muscle fibers, both in the infected and the non-infected fibers. Occasionally within the capillaries near one of the oval bodies there was a slight increase in the number of polymorphonuclear leucocytes and there were a few foci of acute myositis involving a single fiber. Some of the specimens showed a cross-section of the sporozoön, in which it was seen that the latter was just within the sarcolemma. No matter how wrinkled or distorted the muscle fiber might be, the sporozoön, with its very thin refractile membrane, was seen to preserve its circular or oval outline. Under the highest powers the sporozoön was seen to be made up of hundreds of little oval vesicular bodies having a round nucleus at one end. The sporozoite took the eosin irregularly and appeared to be vacuolated. The little sporozoites were decidedly vesicular and were either round or oval. All had one nucleus and very rarely two nuclei placed at opposite ends of the short axis of the sporozoite. The sporozoites were closely packed within the mother capsule or membrane, yet without any arrangement suggesting a room system. The measurement of sporozoites in the section was: length 4.25 microns, width 1.75 microns."

It was obvious to Darling [14] that the parasite he observed differed in several respects from that described by Baraban and St. Remy [12] and also from animal Sarcosporidia such as *Sarcocystis tenella* (in sheep) (Fig. 4) and *Sarcocystis miescheriana* (in pigs) (Fig. 5). Darling believed that the parasites in his human case differed from those in animals because man was a strange host and hence the parasite was unable to complete its life cycle.

The experiments of Darling [32] presented what has been considered proof of his theory. He fed 6 guinea-pigs rat muscle naturally infected with *Sarcocystis muris,* and found that 2 pigs killed 6 months later had parasites in the skeletal muscles similar to those seen in his human case. These uncontrolled experiments were supposed to have confirmed the work of Negri,[33] who fed 11 guinea-pigs infected rat muscle and found parasites in 9. No parasites were found in 12 control pigs. Negri was

impressed by the differences between the parasites in his experimental animals and in rats, and attributed the morphologic change to the fact that the parasites were in a strange host, a theory popularized by Darling. Both Negri and Darling used the guinea-pig as their experimental animal because they believed that this animal was not naturally infected with Sarcosporidia.

Our review of the reported cases of human sarcosporidiosis has revealed that they may be divided into two groups: Group I in which the parasites, cyst and individuals, are large, with well defined, striated cyst walls and inner room compartments. This parasite resembles the Sarcosporidia of animals such as sheep and pig. In this category belong the cases of Baraban and St. Remy,[12] Vuillemin,[13] Bonne and Soewandi,[19] Feng,[20] Price,[21] and Hewitt.[22]

Group II is characterized by the smaller size of the cyst and its individual members, a delicate, ill defined, nonstriated capsule and the absence of internal septa. Here may be placed the two cases of Darling[14, 15] and those observed by Manifold,[16] Lambert,[17] Gilmore, Kean, and Posey.[23]

Until a few years ago the following case would surely have been listed as another instance of sarcosporidiosis and, according to the foregoing classification, would belong to group II.

Report of Case

Patient A. W. (Gorgas Hospital chart no. 530409, Board of Health Laboratory autopsy no. 14230) was a Negro housewife, 48 years old, who died in Gorgas Hospital, Ancon, Canal Zone, on August 17, 1943, 4 hours after admission for acute dyspnea and retrosternal pain.

Background. The patient was born in Jamaica, British West Indies, and lived there until the age of 25. The family history was not contributory. At the age of 25 she went to Bocas del Toro, Republic of Panama, where she worked as a seamstress for 12 years, then spent some years in Almirante and Puerto Armuelles, Republic of Panama, and came to the city of Panama about a year before her death. Her health had generally been good. She had had a child which died in infancy before she left Jamaica. She married in Bocas del Toro at about the age of 28 years and had two abortions in the early months of pregnancy.

A year before her death she was admitted to The United Fruit Company Hospital in Puerto Armuelles, Republic of Panama, where a colpotomy for pelvic abscess was performed. Convalescence was uncomplicated. No history of antiluetic therapy was obtained. Her food habits were not remarkable and were typical of Jamaicans. She ate no raw meat. Her only obvious contact with animals was with a dog, a household pet not available for examination. It was known that the house in which she resided in Panama during the last year of her life was infested with rats, and contamination of food was possible. Her husband was living and well, and declined to permit a muscle biopsy.

History of Terminal Illness. Three weeks before admission the patient became ill, complaining of epigastric pain, exertional dyspnea, orthopnea, and swelling of the right leg. She continued her household duties for a fortnight, however, but then took to bed. On the day of admission, sharp, severe, substernal pain radiating to

the back, marked dyspnea, abdominal distention, profuse diaphoresis, and questionable fever brought her to the hospital.

Physical Examination. The patient was a somewhat obese Negro woman in acute distress. Temperature was 97.0° F.; pulse rate, 120 per minute; respiration, 32 per minute; blood pressure, 120 mm. Hg systolic and 90 mm. Hg diastolic. A systolic murmur was heard at the apex of the heart, but no other abnormalities were noted upon physical examination of the heart and lungs. No peripheral edema was present.

Course in Hospital. One hour after admission the retrosternal pain became even more severe and she vomited. She was given morphine sulphate, grains 1/6, without relief. Two hours after admission she appeared to be in extreme shock. Her skin was cold and clammy, the blood pressure could not be obtained, the heart sounds were poor in quality and the cardiac rhythm became totally irregular. She died 4 hours after admission without rallying. No laboratory procedures were conducted. The clinical diagnosis was coronary occlusion.

AUTOPSY FINDINGS

A complete autopsy, including examination of the head, was performed 11 hours after death. The anatomic diagnoses included: parasitization of heart (sarcosporidiosis or toxoplasmosis); cardiac hypertrophy (360 gm.); chronic interstitial myocarditis, slight; area of focal encephalomalacia of brain, small; chronic diffuse thyroiditis (Hashimoto type); hemorrhages of thymus, small, focal, terminal; acute and chronic passive congestion of liver; fibrous perisplenitis, old; fibromyomata of uterus; chronic pelvic peritonitis; chronic salpingitis, bilateral; atrophy of ovaries; obesity, moderate; general arteriosclerosis, slight; post-mortem degeneration of pancreas and kidneys, moderate. The exact cause of death was not determined nor could the pathologic findings be correlated satisfactorily with the clinical course.

Since the parasites were observed only within the heart, description will be limited to that organ. No skeletal muscle was taken for examination. No parasites were found in 167 sections of the brain.

Gross Examination of the Heart

No excess pericardial fluid was present. The heart was slightly enlarged, weighing 360 gm. after removal of blood clot from the chambers. The apex was located in the fifth interspace, 11.0 cm. to the left of the midsternal line. The epicardial surface was smooth and free from adhesions to the pericardium. A moderate amount of subepicardial fat was present. The myocardium was uniformly firm and reddish brown. No areas of myocardial necrosis or fibrosis were recognized. The endocardium was smooth and glistening. The valve leaflets were delicate and appeared competent. No lesions of the aortic valves suggestive of syphilis were present. Coronary sclerosis was slight; no occlusion was observed.

Microscopic Examination of the Heart

Thirteen blocks were taken from the interventricular septum, the left ventricular wall, and the right ventricular wall. Sections of the auricular walls were not taken. From these thirteen blocks, 365 sections were prepared. In six of the blocks, including all taken from the right ventricular wall, no significant abnormalities were noted. The subepicardial fat was free of inflammatory reaction. The myocardial fibers tended to be large and contained a small to moderate amount of yellowish perinuclear pigment. A few small patches of myocardial fibrosis were found. The endocardium was not thickened and no verrucae were seen.

In seven sections of the left ventricular wall there were found nine parasitic cysts. Since all of these cysts were similar in character, it may be sufficient to describe a typical one (Fig. 1).

Within a single swollen muscle fiber, cut in cross or slightly tangential section, was a cyst measuring 60 by 50 μ. The cyst was packed with numerous elongated, slightly elliptical bodies which, for purposes of convenience in description, were called sporozoites. These bodies averaged 5 μ in their greatest dimension and had nuclei approximately 1 μ in diameter generally located at one pole. The cyst possessed a delicate capsule, or membrane, which stained poorly in routine hematoxylin and eosin sections, but in van Gieson preparations stood out as a shiny, refractile structure. It could not be ascertained whether the capsule was of parasitic or muscle-fiber origin. No striation of the capsule and no internal septa could be demonstrated. Toward the periphery of the cyst, just beneath the capsule, elongated sporozoites were not seen. Instead, there was a row of tiny nuclei representing, possibly, immature sporozoites.

The following were measurements of other cysts: 50 by 27 μ; 105 by 47 μ; 80 by 52 μ; 35 by 26 μ. Attempts to section, in serial fashion, some of the parasites were productive of the following results. In most instances serial sections could not be obtained, for the parasites were found in only one slide. In one instance, 15 consecutive sections, cut at intervals of 5 μ, included the same cyst. It was estimated, therefore, that the size of this cyst was at least 75 by 35 by 25 μ. In another instance it was possible to obtain 9 consecutive sections, each containing the same cyst, and from the method of preparation of this material and from a study of the sections it appeared likely that the entire parasitic structure was included in the material studied. The dimensions of this cyst were 105 by 47 μ.

A striking feature of the sections was the virtual absence of inflam-

matory reaction in the myocardium adjacent to the parasites. In fact, in only one section of the left ventricular wall could an area be found in which there was a distinct chronic inflammatory reaction with infiltration by lymphocytes. Scattered within other sections, however, there could be found a slight inflammatory reaction which bore no direct relation to the presence of the cysts. No spirochetes were found in sections stained by Levaditi's method.

Several of the cysts were ruptured by microdissection of stained material and the individual parasites studied. These averaged 5 by 1 μ and were characterized by an elongated, elliptical or crescentic shape, and by proportionately large nuclei which, for the most part, were located at one pole and produced a bulge in the outline of the sporozoite. The illustrations published by Darling [32] of the sporozoites seen in his "experimental guinea-pig sarcosporidiosis" are accurate representations of the individual sporozoites in this human material.

Parasites in Guinea-Pig

In 1942 Gilmore, Kean, and Posey [23] reported the presence of parasites in the heart of a Panamanian girl, 12 years old, which morphologically appear identical with those in the current case. At that time, following suggestions by Augustine [34] and Weinman,[35] serious consideration was given to the possibility that the parasite was Toxoplasma. The parasite was classified as Sarcosporidia, however, for various reasons which are recorded in the report; among those reasons was the fact that the parasite was indistinguishable from those in Darling's [15, 32] illustrations and those of subsequent cases,[16, 17] the diagnosis of which had not been questioned.

When the current case appeared we determined to investigate the muscles of guinea-pigs, for if Darling were wrong in his classification, then all cases in group II (page 470) were in an incorrect category. It did not seem reasonable that Sarcosporidia, which is so widespread in its distribution, should be absent in the guinea-pig.

Between September 10, 1943, and November 13, 1943, the skeletal muscles of 60 laboratory guinea-pigs, *Cavia cobaya,* were examined. These pigs were imported from the United States where they had been purchased from a commercial dealer; presumably they had not been used previously for experimental purposes. They were kept under the usual laboratory conditions until sacrificed for complement. No muscle parasites were recognized grossly. Blocks of thigh and abdominal muscles of all were fixed in formol-alcohol, and prepared by the usual paraffin method, with hematoxylin and eosin staining.

In 5 of the 60 animals parasites resembling those described and illustrated by Negri [33] and Darling [14] were found in the skeletal muscles. Complete autopsies were done on 2 of these 5, the bodies of which had been saved in the ice box. In 1 of these 2 guinea-pigs parasites were found only in the thigh muscles. Examination of the masseter, pectoral, abdominal, and tongue muscles, and of brain, heart, lungs, liver, spleen, and kidneys was negative. In the other pig parasites were found in thigh and paravertebral muscles, brain, and kidney; no parasites were noted in sections of heart, lungs, liver, spleen, tongue, esophagus, and trachea.

MUSCLE

The parasites were not significantly different in any of the 5 pigs in which they were found. About half of the muscle sections of these pigs had cysts. The parasites were more numerous in sections of the thigh than in the other muscle groups such as abdominal, pectoral, and masseter. The greatest number found in any one section was three well formed cysts (Fig. 2). The cysts were located within individual muscle fibers, generally in an eccentric position. In a few instances the cysts seemed to be located between muscle fibers.

Cross and tangential sections of cysts varied in size from 20 by 26 μ to 48 by 18 μ. Since none of the cysts was cut longitudinally, their length was not determined. In one instance it was possible to obtain six serial sections cut at intervals of 5 μ, which would make the cyst at least 30 μ long. Most of the cysts were cut in cross section and appeared as circular nests; others were cut obliquely and were ovoid in shape. The parasitized muscle fibers were swollen and a few showed hyaline degeneration. Each cyst possessed a thin, delicate capsule or limiting membrane measuring approximately 1 μ in thickness. This membrane could be seen best in sections stained with van Gieson's picro-acid fuchsin by which it was stained red and stood out in marked contrast to the greenish yellow fiber. Sarcolemma, however, possessed the same tinctorial properties. We could not determine whether the capsule was of parasitic or muscle origin.

Each cyst was composed of, or filled with, many tiny bodies which, for purposes of convenience, were termed sporozoites. Under the low power objective the sporozoites were noted as fine basophilic stippling within the cyst. Under higher magnification the sporozoites were seen to be elongated. They were so closely packed that counting was difficult but it was estimated that each cyst contained about 150 sporozoites. In some of the cysts the bodies were disposed in a haphazard fashion, whereas in others they tended to collect in groups. Toward the periphery of the cysts, the elongated bodies were not present. Instead,

tiny spherical or ovoid basophilic bodies resembling nuclei of the sporozoites were noted.

In order to study the individual sporozoites, the following technic was employed. The coverslip was removed from a stained section and a drop of balsam was placed on the tissue. Practically all of the tissue surrounding a cyst was dissected away and the débris was flushed off with xylol. A tiny drop of balsam was placed directly upon the cyst and the coverslip returned. Pressure on the coverslip was applied. This was sufficient to rupture the cyst capsule and the extruded individual sporozoites could then be examined under the oil immersion objective and measured. The dangers of regarding the measurements of sporozoites treated in such a fashion as *in vivo* dimensions must be obvious.

In these preparations the sporozoites measured 5 by 1 μ. Some were crescentic or banana-shaped. Most of them tapered at both ends, but several were bluntly rounded at one pole. A dark-staining nucleus was present near the tip of each parasite, and in many it was found to occupy the extreme distal part of the sporozoite. In these sporozoites the nucleus was found to accommodate itself to the taper of the corpuscle and appeared as a dark-staining, roughly triangular body with a somewhat rounded base. The nuclei in other sporozoites were found to be nearer the center, but it was difficult to find any with a centrally placed nucleus. A section stained by Heidenhain's iron hematoxylin technic showed the nuclear chromatin to be arranged chiefly around the periphery of the nucleus. No definite intranuclear structures were ascertainable. The cytoplasm of some of the corpuscles was distinctly granular, and vacuoles were present in a few of them. The morphologic characteristics of the cysts and of sporozoites appeared identical with those so beautifully illustrated by Darling.[14, 32]

BRAIN

Parasites were found in the brain of 1 of the 2 guinea-pigs upon which complete autopsies were done. The cysts (Fig. 3) were ovoid or circular in outline and measured from 20 to 25 μ in diameter. No outer membrane or capsule could be recognized and the cysts seemed to be limited only by the surrounding parenchyma. As many as 80 sporozoites could be counted in some collections; these resembled those found in the muscles, but appeared scattered in an ill defined, faintly basophilic ground substance. There was no surrounding cellular reaction, but the leptomeninges showed a slight chronic inflammatory exudate. These cysts resembled the illustrations of toxoplasmosis in guinea-pigs (Markham [36]), wild rats (Perrin, Brigham, and Pickens [37]), and mice (Weinman [38]).

KIDNEY

Within the lumen of a renal tubule was a circular nest of parasites measuring 35 μ in diameter. This nest was composed of 50 or 60 nucleated bodies scattered in faintly basophilic stroma. Nearby was a collection of 7 smaller cysts ranging from 15 to 20 μ in diameter. Each of these contained 10 to 15 nuclear structures scattered in a similar stroma. These aggregates resembled those found in the brain and muscles, but there was less differentiation of the internal structure. Post-mortem degeneration was considerable and may have played a rôle in obscuring the inner structure of these cysts. There was no surrounding inflammatory reaction.

COMMENT

In the absence of inoculation and serologic studies, absolute identification of the parasites in the current human case and in the guinea-pigs is impossible. On morphologic grounds, however, the following statements appear warranted:

1. The parasites in our human case are similar to those described by Darling and others (group II) as Sarcosporidia.

2. The classification of these parasites as Sarcosporidia has been based mainly upon Darling's experiment. We found parasites occurring spontaneously in guinea-pigs similar to those which Darling thought he had transmitted experimentally. Negri's work [33] requires confirmation.

3. The parasites in both group II and in the guinea-pigs resemble Toxoplasma more than Sarcosporidia. Toxoplasmosis has been reported in guinea-pigs by Mooser,[39] Markham,[36] and Sabin and Olitsky,[40] but parasites in peripheral skeletal muscle were not mentioned. A detailed discussion of Toxoplasma in man need not be presented for several articles have summarized current knowledge (Pinkerton and Weinman,[28] Wolf, Cowen, and Paige,[41] etc.). The cysts, both in group II and in guinea-pigs, are somewhat larger than those generally described in chronic toxoplasmosis,[37,38] possibly because they are present in the more abundant cytoplasm of striated muscle.

4. Although the parasites in group II of the human cases and in the guinea-pig appear similar, it has not been established that they are identical. In fact, there is no proof that the parasites in the muscles, brain, and kidney of the guinea-pig are the same.

It is of some interest that three instances of this type of human infection should have been found in one laboratory when so few cases have been recorded all over the world. Darling [14] reported in 1909 the parasites in the biceps muscle of a patient ill of typhoid fever. The

second case (Gilmore, Kean, and Posey [23]) was found in 1941 while searching for *Trypanosoma cruzi* in the heart of a child. In the current case many blocks of heart muscle were taken because Chagas' disease was suspected at the autopsy table but the parasites were first seen by a technician (Mr. J. M. Benevides) who picked up a slide to check its stain. (Darling's second case [15] was from the Federated Malay States.) *

Summary

1. The literature on human sarcosporidiosis was reviewed and the reported cases were divided into two groups:

Group I, in which the parasites were characterized by the large size of the cysts and sporozoites, striated capsules, and internal septa. This group resembled animal Sarcosporidia such as *Sarcocystis tenella* (sheep) and *Sarcocystis miescheriana* (pig). These were regarded as authentic cases of human sarcosporidiosis.

Group II, in which the parasites were characterized by smaller size of the cyst and sporozoites, and absence of striated capsule and internal septa. These cysts resembled parasites which Darling thought he had transmitted to guinea-pigs by feeding them rat muscle infected with *Sarcocystis muris*.

2. A case is reported in which parasites in the heart of a Negro woman belonged in group II.

3. The spontaneous occurrence in the skeletal muscle of 5 of 60 guinea-pigs of parasites morphologically indistinguishable from those which Darling claimed to have transmitted experimentally was observed. The presence of parasites resembling Toxoplasma in the brain and kidney of one of these pigs suggested strongly that the muscle parasites were also Toxoplasma.

4. Since the classification of group II as Sarcosporidia stemmed from Darling's questionable experiment, it was considered probable that the parasites in group II were Toxoplasma rather than Sarcosporidia.

5. In the absence of serologic and inoculation experiments, final classification of the parasites was not attempted. The possibility that the parasites in group II and in the muscle of guinea-pigs were neither Sarcosporidia nor Toxoplasma was not excluded.

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* Since this paper was submitted for publication, parasites resembling Toxoplasma were found in sections of the brain and heart of a child upon whom an autopsy was performed in this laboratory in 1936. (Tomlinson, W. J. Human chronic toxoplasmosis. *Am. J. Clin. Path.* In press.)

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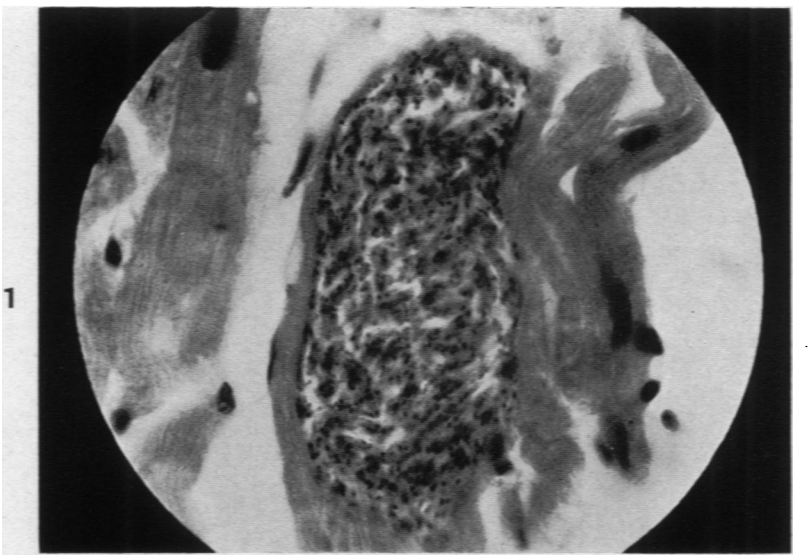
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 80

FIG. 1. Cyst in human cardiac muscle. (Patient A. W.) × 850.

FIG. 2. Cyst in thigh muscle of guinea-pig. Resemblance to human parasite in Figure 1 may be noted. × 850.



1

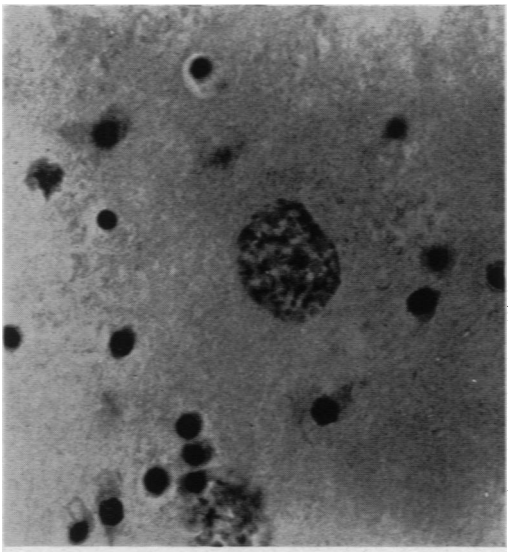
2

PLATE 81

FIG. 3. Cyst or pseudocyst resembling Toxoplasma in brain of guinea-pig. A second cyst (out of focus) is present at the lower border of the field. × 850.

FIG. 4. *Sarcocystis tenella.* Parasite in heart of sheep, showing large cyst and individual sporozoites. Internal septa and a well defined capsule can be seen. × 850.

FIG. 5. *Sarcocystis miescheriana.* The cyst in cardiac muscle of a pig (not guinea-pig) has a thick striated capsule. × 850.



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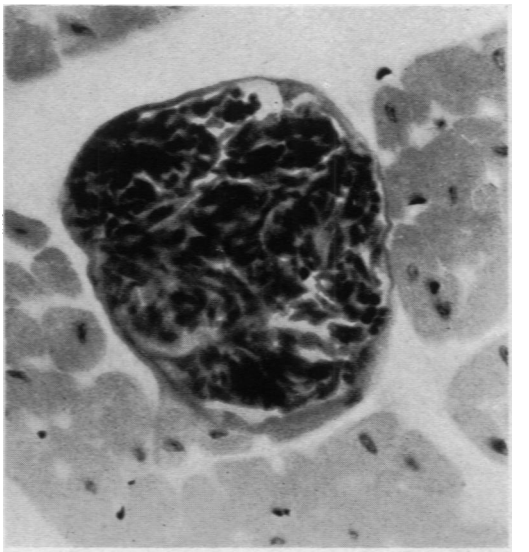

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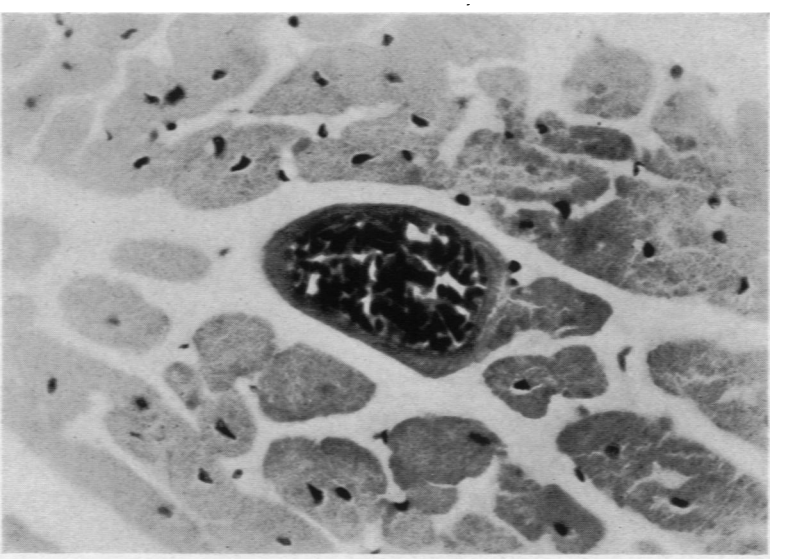

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