Sarcocystis: Development in Cultured Avian and Mammalian Cells

Abstract. Sarcocystis, *a protozoan that parasitizes muscle tissue of reptiles, birds, and mammals, including man, developed in avian and mammalian cell cultures. Motile banana-shaped organisms, released from cysts in grackle muscle, entered cells and transformed into enlarged ellipsoid or oblong stages, which developed into either multinucleate or cyst-like stages, or both.*

Some species of the protozoan genera *Besnoitia, Eimeria, Plasmodium,* and *Toxoplasma* have been cultured through part or all of their life cycles in vitro, but the many attempts to culture *Sarcocystis* (*1, 2*), a closely related parasite, have not been successful. The development of *Sarcocystis* sp. in cultured cells is reported here. I will refer to stages not previously reported descriptively rather than give them specific names at this time.

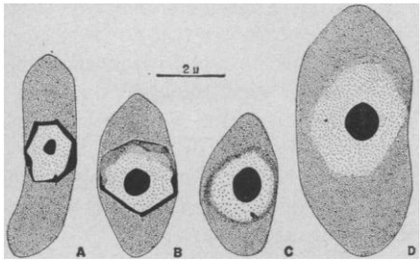

Fig. 1. Semidiagrammatic representation of nuclear changes associated with transformation of intracellular *Sarcocystis* organisms. (A) Banana-shaped organism with vesicular nucleus, well defined peripheral chromatin, small nucleolus; 3-hour MDCK cell culture. (B) Body of organism shorter and wider; peripheral chromatin thicker, paler, and less distinct in areas, nucleus and nucleolus enlarged; 6-hour MDCK cell culture. (C) Organism shorter than at 6 hours, peripheral chromatin nearly dispersed, nucleolus enlarged; 12-hour ECK cell culture. (D) Ellipsoid organism, peripheral chromatin dispersed, enlarged nucleus is no longer vesicular; 24-hour ECK cell culture.

Motile banana-shaped organisms were obtained by crushing cysts in aseptically excised pieces of leg or breast muscle from wild grackles (*Quiscalus quiscula*) with a glass tissue grinder. The parasites, suspended in 2 to 3 ml of Eagle's basal medium with Hanks balanced salt solution (HBME) plus 10 percent fetal calf serum, were poured through several layers of sterile gauze to remove tissue debris and centrifuged at 290*g* for 5 minutes. The pelleted parasites were then resuspended in tissue culture medium and, in the course of 14 experiments, inoculated into six cell types (Table 1). These included cell line cultures of embryonic bovine kidney (EBK), embryonic bovine trachea (EBTr,) and Madin-Darby canine kidney (MDCK), as well as primary cultures of embryonic chicken kidney (ECK), embryonic chicken muscle (ECM), and embryonic turkey kidney (ETK). The ECK and ETK cells were grown in a mixture of 90 percent Hanks balanced salt solution (BSS), 5 percent lactalbumin hydrolyzate (2.5 percent solution in Hanks BSS) and 5 percent fetal calf serum. All other cells were grown in HBME with 5 percent fetal calf serum except for one experiment in which ECM cells were grown in Medium 199 with 10 percent fetal calf serum. In each experiment, approximately 500,000 cells, suspended in 2 ml of culture medium, were pipetted into the Leighton tubes. These tubes were incubated at 37°C for 24 hours before inoculation with the parasite; they were maintained at this temperature after inoculation for the length of each experiment.

Cover slips were removed from the tubes 1 or 3, 6, 12, 24, 30, 48, 72, and 96 hours after inoculation of the parasites, except for those containing ETK cells. Cover slips were removed from the latter daily for 6 days after inoculation. The cells were examined as fresh preparations or fixed in Schaudinn's fluid and stained with Heidenhain's iron hematoxylin. Live parasites, in double cover-slip preparations of EBK, EBTr, and MDCK cells, were filmed with a phase-contrast cinemicroscope which was surrounded by a heated (39°C) Plexiglas enclosure.

Live extracellular *Sarcocystis* organisms observed in EBTr and MDCK cell cultures 1 hour after inoculation underwent flexing, gliding, and pivoting movements. These movements have also been recorded for extracellular *Besnoitia* and *Eimeria* organisms in cell culture (*3, 4*).

The banana-shaped organisms entered all cell types except ECM cells. Live organisms in MDCK cells (Fig. 2A) repeatedly moved forward and backward within the cell cytoplasm, approximately 1 μm in each direction. From one to five organisms, often located adjacent to the host cell nucleus, were seen in a single cell. Twenty such organisms in ECK cells, fixed 3 hours after inoculation, averaged 2.4 by 5.0 μm and 20 extracellular organisms in the same preparation averaged 1.5 by 5.4 μm.

At 6 and 12 hours after inoculation, intracellular organisms were shorter than those observed at 1 to 3 hours. In ECK cells at 12 hours, 20 organisms averaged 2.1 by 4.8 μm.

Organisms examined at 24 hours

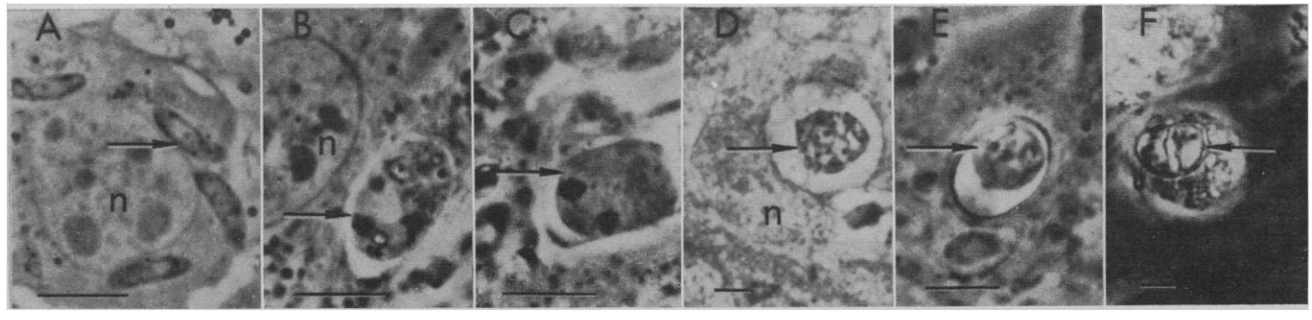

Fig. 2. Photomicrographs from 16-mm motion picture film of live intracellular *Sarcocystis* organisms except for (D) in which the organism has been fixed and stained. Parasites are indicated by arrows. Host cell nuclei, where visible, are identified (*n*). The scale in each photograph is 5 μm. (A) Banana-shaped organisms; 1-hour MDCK cell culture. (B) Ovoid stage in vacuole in host cell cytoplasm; 24-hour EBTr cell culture. (C) Organism undergoing nuclear division; two dark enlarged nucleoli; 48-hour EBTr cell culture. (D) Multinucleate stage in vacuole in ECK cell cytoplasm; 48 hours. (E) Cyst-like body containing sphere of granular cytoplasm; 48-hour EBTr cell culture. (F) Cyst-like body containing two elongate forms; 48-hour EBTr cell culture.

Table 1. Intracellular stages of *Sarcocystis* observed in various types of cultured cells; × indicates presence of intracellular stage listed.

Cell type	Serial passage	Experiments (No.)	Leighton tubes per experiment (No.)	Organisms inoculated per tube (No. × 1000)	Intracellular stages				
					Uninucleate			Multinucleate	Cyst-like
					Banana-shaped	Ellipsoid	Round		
EBK	25	3	7	300, 500, 1600	×	×	×	×	×
EBTr	36	3	7	300, 500, 500	×	×	×		×
MDCK	52	3	7	300, 500, 1600	×	×			
ECK	Primary	2	7	3000	×	×	×	×	
ECM	Primary	2	7	3000					
ETK	Primary	1	6	200	×	×	×	×	

were large ellipsoidal or oblong bodies. Twenty of these in ECK cells averaged 3.8 by 7.2 μm. Changes in the parasite nucleus were observed before and after this transformation process in specimens fixed at 3, 6, 12, and 24 hours (Fig. 1). These changes are similar to those described for the transformation of *Eimeria bovis* sporozoites into trophozoites (*4*). In both stained and live specimens (Fig. 2B), the cytoplasm of the ellipsoid-oblong parasite contained many small granules not observed in other stages. Some living parasites rapidly rotated clockwise on their longitudinal axis, stopped, and rotated counterclockwise. This sequence was repeated several times.

At 30 hours, intracellular organisms in EBK, EBTr, ECK, and ETK cell cultures were nearly round but otherwise appeared very similar to the ellipsoid-oblong form. Twenty organisms in ECK cells averaged 5.2 by 7.2 μm. Living specimens rotated clockwise and counterclockwise for several rotations in each direction or alternatively reversed directions, moving through an arc of 90°.

An organism undergoing nuclear division was observed in EBTr cell cultures at 48 hours (Fig. 2C). Two- to nine-nucleate stages, resembling young eimerian schizonts, were found as early as 30 hours in ECK cells and in EBK, ECK, and ETK cells at 48 and 72 hours (Fig. 2D). Twenty such forms in 48-hour ECK cells averaged 6.5 by 7.5 μm.

Intracellular bodies observed in EBK and EBTr cell cultures at 48 and 72 hours were bounded by a thick wall which imparted a cyst-like appearance. Twenty such forms averaged 6.5 by 8.5 μm. Most of these cyst-like bodies were ovoid or lemon-shaped. A sphere of what appeared to be granular cytoplasm occupied more than half the volume and was located toward one end of the body (Fig. 2E). A less dense material was present between the sphere and the other end of the body. A few bodies contained two inclusions, some resembling the banana-shaped organisms (Fig. 2F).

Although caution must be exercised in interpretation, it is interesting to note that the cell types used so successfully for the cultivation of eimerian species (*5*), which parasitize the intestinal tract, have also supported development of *Sarcocystis*, and that *Sarcocystis*, which has been found only in muscle tissue, did not even enter cultured muscle cells. Recent studies on *Toxoplasma gondii* (*6*) indicate that this parasite develops, in the intestine of cats, through stages identical with those of coccidian parasites. The possibility that intestinal stages occur in the life cycle of *Sarcocystis* has long been suspected on the basis of studies on infection by fecal contamination (*1*). The finding of nuclear and cytoplasmic transformation as well as multinucleate stages similar to those in *Eimeria* further suggests that *Sarcocystis* may be closely related to this genus. The significance of the cyst-like bodies is not known. Their appearance is similar to the oocyst stage of *Eimeria*, but they are much smaller and identification of preceding sexual stages, as in oocyst development, has not been concluded.

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References and Notes

1. J. W. Scott, *Univ. Wyo. Agr. Exp. Sta. Bull.* **259**, 1 (1943).
2. N. D. Levine, *Protozoan Parasites of Domestic Animals and of Man* (Burgess, Minneapolis, 1961), p. 322.
3. R. Fayer, D. M. Hammond, B. Chobotar, Y. Y. Elsner, *J. Parasitol.* **55**, 645 (1969).
4. R. Fayer and D. M. Hammond, *J. Protozool.* **14**, 764 (1967).
5. W. H. Patton, *Science* **150**, 767 (1965); D. M. Hammond and R. Fayer, *J. Parasitol.* **54**, 559 (1968); D. J. Doran, *Proc. Helm. Soc. Wash.* **37**, 84 (1970).
6. W. M. Hutchinson, J. F. Dunachie, J. Chr. Siim, K. Work, *Brit. Med. J.* **1**, 142 (1970); H. G. Sheffield and M. L. Melton, *Science* **167**, 892 (1970); J. K. Frenkel, J. P. Dubey, N. L. Miller, *ibid.*, p. 893.
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Lymphocytic Responses to Streptococcal Antigens in Glomerulonephritic Patients

Abstract. *The lymphocytes from patients with progressive glomerulonephritis showed significant inhibition of cell migration in the presence of group A streptococcal particulate antigens. Marked increases in the level of DNA synthesis of these lymphocytes were also observed after contact with these antigens. Lymphocytes from patients with unrelated renal disorders exhibited minimum reactivity to streptococcal antigens.*

The exact nature of the pathological mechanisms involved in progressive human glomerulonephritis remains obscure (*1*). While studies in both experimentally induced glomerulonephritis and acute post-streptococcal nephritis in man suggest that antigen-antibody complexes are mediators of the renal damage (*2–5*), evidence of the continuing participation of complexes in progressive nephritis are more difficult to obtain. In experimentally induced progressive glomerulonephritis there is a gradual attrition of glomerular-deposited, immune complexes after cessation of administration of the antigens (*6*) and immunofluorescent studies with streptococcal antiserums of renal biopsy specimens in man have failed to demonstrate streptococcal products in the renal lesions in these patients (*4*, *7*). In one of these studies (*4*), fluorescein-labeled streptococcal antigens did bind to areas of "fixed" gamma