tery of histochemical tests indicated that the hepatic globules had a nonlipid, glycoprotein composition. Other inconstant features of the liver seen on light microscopy were (i) mild focal steatosis, (ii) glycogen nuclei, and (iii) mild portal fibrosis and bile duct proliferation.

On electron microscopy, structures were identified in 5 of 11 cases (three biopsy, two autopsy) which measured 0.5 to 20 μm and matched those seen on light microscopy in shape and internal detail. Their ultrastructural characteristics were those of cytoplasmic bodies, namely, they were single-membrane bound, roughly oval, occasionally irregular and completely or incompletely filled with a homogeneous, moderately electron-dense material (Fig. 2). Some of the membranes had a beaded appearance suggesting rough endoplasmic reticulum. Occasional failure to find the globules by electron microscopy may have been due to their nonuniform cellular distribution within the liver lobule. In four of the six cases where globules were not seen on electron microscopy, they were seen by light microscopy after PAS staining.

Application of fluorescein-conjugated antiserum for human A_1AT to the liver tissues from one Pi^{ZZ} homozygote, one Pi^{SS} homozygote and two Pi^{MZ} heterozygotes revealed positively staining structures that corresponded in size, shape, and cellular localization to the globules. The fluorescence was completely blocked by prior incubation of serial sections in unconjugated antiserum to human A_1AT (liver tissues from seven controls did not fluoresce). Specific fluorescence for albumin or fibrinogen was negative; scattered fluorescing foci of gamma globulin were seen but did not coincide with the A_1AT fluorescent structure.

These observations complement the findings of Sharp *et al.* and indicate that certain genetic variants of A_1AT result in reduced levels of inhibitor in the serum with unusually large amounts of inhibitor in the liver. Intracellular hepatic globules containing A_1AT are present not only in the livers of antitrypsin-deficient patients with infantile cirrhosis, but also in the livers of antitrypsin-deficient adults with pulmonary emphysema and no liver disease. The rarity of liver disease in individuals with homozygous antitrypsin deficiency implies that infantile cirrhosis does not result directly from the abnormal intrahepatic metabolism of antitrypsin. It is possible that an additional causative factor, such as the Australian antigen (*12*), is transmitted to the fetus in families with cirrhosis. This second factor may severely damage a liver made vulnerable by the presence of A_1AT filled globules. A search for such a factor should be undertaken in these families.

More liver cells seemed to contain globules in the autopsy cases than in those specimens obtained by percutaneous biopsy, suggesting that a greater number of globules are present in individuals who are acutely ill. Of course, larger sections of liver were prepared from autopsy material, enabling easier identification of the globules. However, A_1AT is an acute-phase reactant protein, and the stimuli that normally increase the production of A_1AT by the liver may be intact in patients with a deficient variant resulting in more hepatic globules in severely ill, terminal patients. Such stimuli would be incapable of increasing the release of the deficient A_1AT variant from the liver, since neither the administration of estrogens (*8, 13*), nor typhoid vaccine (*14*) has any effect upon the serum level of A_1AT in homozygous deficient individuals. The low levels of inhibitor in the serums of these patients may result from failure of a release mechanism in the liver, associated with certain variants of the A_1AT molecule. The inherited defect resulting in A_1AT deficiency must reside within the liver, since Sharp *et al.* obtained complete normalization of the serum concentration of A_1AT following a liver transplant in one of their subjects (*9*). A releasing enzyme in the liver cell may be ineffective, or the A_1AT molecule may be unable to pass through the cell membrane when its configuration is abnormal.

We suspect that an aberrant molecular configuration of the deficient A_1AT variants impairs the release of inhibitor from the liver cell resulting in deficient levels of serum A_1AT. Both of the A_1AT variants usually associated with a deficiency in serum (Pi^Z and Pi^S) move more slowly in an electric field, suggesting that a difference in ionic charge or molecular weight may relate to their defective release from the liver.

JACK LIEBERMAN
CHARLES MITTMAN
HERSCHEL W. GORDON
Departments of Respiratory Diseases and Anatomic Pathology, City of Hope National Medical Center, Duarte, California 91010

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Gametogony of Sarcocystis sp. in Cell Culture

Abstract. *Sexual stages and cystlike bodies of* Sarcocystis *sp., a protozoan parasite found in muscles of reptiles, birds, and mammals, including man, developed in cell culture. Motile organisms, obtained from leg muscles of wild grackles, were inoculated into cell line cultures of embryonic bovine kidney. Mature micro- and macrogametes and the cystlike forms were found 30 and 42 hours after inoculation, respectively. These observations indicate that the parasite is probably a coccidium.*

Micro- and macrogametes and cystlike bodies have been observed in cultured cells inoculated with motile *Sarcocystis* sp. organisms. Previous reports (*1*) described development of *Sarcocystis* in cultured avian and mammalian cells. However, sexual stages were not found at that time and the significance of the cystlike bodies found at 48 and 72 hours was, therefore, not understood.

Motile, banana-shaped organisms were obtained from cysts in the leg and thigh muscles of wild grackles (*Quiscalus quiscula*) and prepared for inoculation into cell cultures as previ-

ously described (*1*). Embryonic bovine kidney cell line cultures in the 41st subculture and embryonic bovine trachea cells in the 36th subculture were grown in Leighton tubes by using Eagle's minimal essential medium with Hanks balanced salt solution and 10 percent fetal calf serum at 37°C. Penicillin G and dihydrostreptomycin were contained in the culture medium at concentrations of 50 unit/ml and 50 μg/ml, respectively. Cover slips were removed from the tubes 24, 30, 42, 48, and 72 hours after inoculation. Cells were examined either as fresh preparations or after they were fixed in Schaudinn's fluid and stained with either Heidenhain's iron hematoxylin or a polychrome stain (*2*). Living parasites in double cover slip preparations were photographed with a phase-contrast cinemicroscope surrounded by a heated (39°C) Plexiglas enclosure.

Multinucleate immature microgametocytes were observed within vacuoles located close to the host cell nucleus 24 hours after inoculation (Fig. 1A). Ten gametocytes averaged 7.7 by 9.2 μm and contained 2 to 12 nuclei. These correspond to the stages previously reported (*1*) as "resembling young eimerian schizonts." Of 100 stages observed at 24 hours, 82 were macrogametes and 18 were microgametocytes. The macrogametes, which corresponded to the ellipsoidal or oblong bodies previously reported (*1*), measured 5.0 by 8.3 μm (average of ten stages). In both fresh and stained preparations the nucleoplasm appeared pale and had no distinct border. The large dark nucleolus was centrally located in some specimens, but was eccentric in others. Numerous granules, which often encircled the nucleus, were present throughout the cytoplasm. These granules were refractive when observed with phase-contrast microscopy, stained deeply with iron hematoxylin, and were bright red when stained with Biebrich scarlet-orange G from the polychrome stain.

Of 100 gametocytes observed at 30 hours, 83 were macrogametes, and 17 were microgametocytes. Parasitized cells of ten contained two or three macrogametes. Occasionally microgametocytes and macrogametes were found in the same host cell (Fig. 1c). Of the microgametocytes observed at 30 hours, five contained mature microgametes (Fig. 1, B and C). The microgametes were comma-shaped and located at the periphery of a cytoplasmic mass. In fresh preparations the attached microgametes were very active, causing the central mass to rotate. This activity was apparent at 39°C, at room temperature, and immediately after cooling the preparation with several short bursts of freon spray. Free microgametes, approximately 3 μm long, were observed within the vacuole surrounding the microgametocyte and moved through the extracellular culture medium with the aid of two flagella (Fig. 1, D and E). They remained motile during a 20-minute period of observation even at the reduced temperatures. Macrogametes observed at this time (Fig. 1C) appeared similar to the macrogametes seen at 24 hours. However, some were rounded and a few had a larger nucleus with an irregular outline. The cytoplasmic granules in the macrogametes had enlarged and were located at the periphery of the gamete. Ten such stages averaged 5.0 by 7.4 μm.

Ovoid or lemon-shaped cystlike bodies surrounded by a distinct wall were first observed at 42 hours (Fig. 1F). At 48 and 72 hours, this stage was the only one found. In one 48-hour culture, ten of the cystlike forms averaged 6.6 by 8.9 μm. Granular cytoplasm usually occupied more than half of the volume and was located toward the more rounded end of the body. Sporulation, attempted by removing cultures from the incubator and leaving them at room temperature for several days, was unsuccessful.

Development of gametocytes and gametes of *Sarcocystis* in cell culture demonstrates that such stages exist in the life cycle of this parasite. The occurrence of gametes preceding the formation of the cystlike bodies suggests that these bodies may be equivalent to the oocyst stage of coccidia. Motile organisms obtained from muscles would

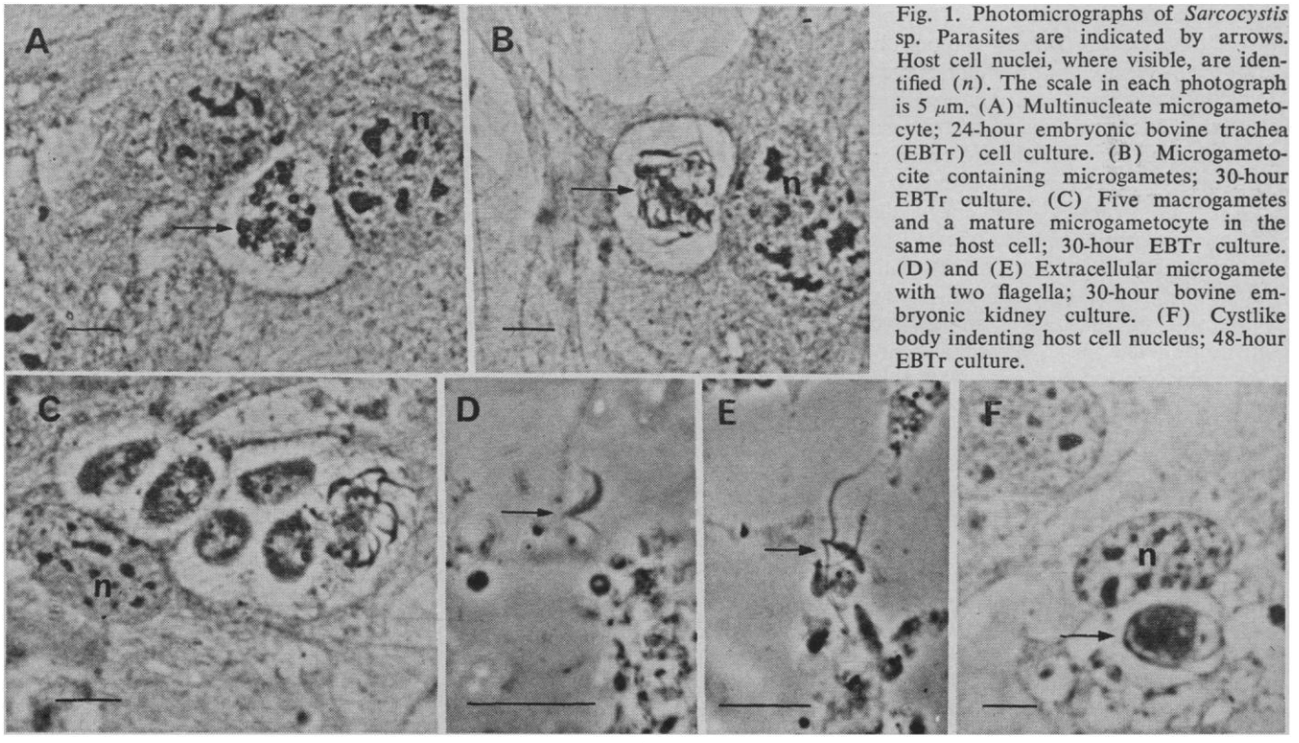

Fig. 1. Photomicrographs of *Sarcocystis* sp. Parasites are indicated by arrows. Host cell nuclei, where visible, are identified (*n*). The scale in each photograph is 5 μm. (A) Multinucleate microgametocyte; 24-hour embryonic bovine trachea (EBTr) cell culture. (B) Microgametocite containing microgametes; 30-hour EBTr culture. (C) Five macrogametes and a mature microgametocyte in the same host cell; 30-hour EBTr culture. (D) and (E) Extracellular microgamete with two flagella; 30-hour bovine embryonic kidney culture. (F) Cystlike body indenting host cell nucleus; 48-hour EBTr culture.

then represent a merozoite stage, since they form in the host by endodyogeny (*3*), and give rise directly to sexual stages in cell culture.

RONALD FAYER
National Animal Parasite Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705

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Mechanism of Gonadotropin Action in Amphibia: Involvement of Mitochondria

Abstract. *Luteinizing hormone (a pituitary gonadotropic hormone) stimulates Δ^5-3β-hydroxysteroid dehydrogenase activity in the microsomal fraction of frog testes when incubated together with the mitochondria; incubation together with the nuclei instead of the mitochondria does not result in increased Δ^5-3β-hydroxysteroid dehydrogenase activity. The increase is not induced by adenosine triphosphate, it appears to be hormone-specific, and it is sensitive to puromycin and actinomycin D. These data suggest that the mitochondrial DNA may be involved in mediating the action of luteinizing hormone in amphibian steroidogenesis.*

The controlling effect of pituitary gonadotropic hormones on steroid production in the vertebrate testis has long been recognized (*1*). Biosynthesis of gonadal steroid hormones is accelerated when gonadotropins are administered in vivo and when added to incubating tissue slices (*2*). Moreover, histochemical and biochemical data indicate that certain enzymes (the hydroxysteroid dehydrogenases) involved in steroid hormone biosynthesis show increased activity after in vivo or in vitro administration of gonadotropins (*3*, *4*). One such enzyme is the Δ^5-3β-hydroxysteroid dehydrogenase complex (3β-HSD). Testicular, ovarian, adrenal, and placental tissues are known biochemically to contain this enzyme, which irreversibly converts Δ^5-3β-hydroxysteroids to Δ^4-3-ketosteroids (*5*). Its presence has been demonstrated histochemically in all classes of vertebrates and it plays a cardinal role in the biosynthesis of many steroid hormones (*3*).

The activity of testicular 3β-HSD in amphibians is markedly increased by injections of mammalian gonadotropic hormones (*4*). Recently it also has been demonstrated that a homogenate of frog (*Rana pipiens*) testes incubated for several hours together with mammalian luteinizing hormone (LH) will show greater 3β-HSD activity in the microsome fraction than one incubated without the hormone (*6*); this demonstrates that the cells do not need to be intact in order for gonadotropins to cause increased steroid hormone biosynthesis.

One important aspect of gonadotropin-stimulated steroidogenesis which has remained largely unstudied is the site of action within the target cells, that is, whether the primary controlling site of action of gonadotropins within the steroid-producing cells resides in the cell membrane, the cytoplasmic organelles, or the nucleus. Below I present evidence —based on various recombinations of cell organelles—that LH will give an increased 3β-HSD activity in the microsomal fraction of frog testes in the presence of the mitochondria but not the nuclei. Evidence is further presented which idicates that this increase in 3β-HSD activity involves protein synthesis (puromycin-sensitive) and may be mediated via the mitochondrial DNA (actinomycin-sensitive).

Testes from grass frogs (*Rana pipiens*) were excised and weighed. The gonads from about 10 to 15 individuals were pooled over ice and then homogenized at 0°C with a motor-driven glass-Teflon tissue homogenizer. The homogenizing medium consisted of 0.02*M* phosphate buffer (*p*H 7.6) and 0.25*M* sucrose, and sometimes contained mercaptoethanol (0.001*M*); the final tissue concentration was usually 5 percent (wt./vol.). Standard differential centrifugation procedures were used to separate the organelles: pellet from 10 minutes at 200 to 800*g* = nuclei; pellets from 10 minutes each at 800 to 5,000*g* and 5,000 to 24,000*g* = mitochondria; supernatant from 10 minutes at 24,000*g* = microsomes and dissolved substances. Organelles were resuspended in homogenization medium, recombined (see Figs. 1 and 2 and Table 1), and incubated in a medium consisting of homogenizing solution (90 percent) and calf serum (10 percent) and the various test substances. Incubation was carried out in 25-ml erlenmeyer flasks in a Dubnoff incubator (25 oscillations per minute) at 28° to 30°C for 4 to 6 hours under air. The contents were then centrifuged at 24,000*g* for 10 minutes and the supernatant was assayed for 3β-HSD activity as described (*4*).

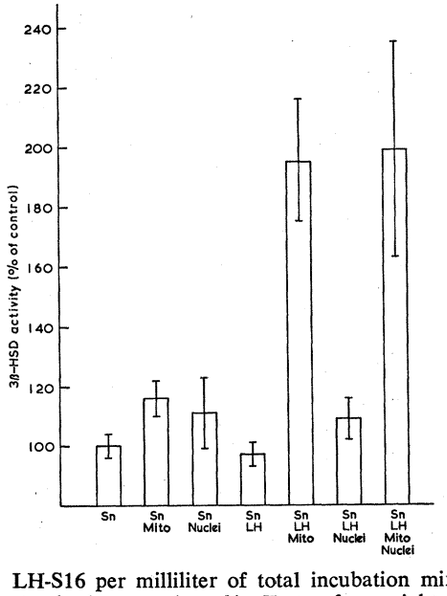

Fig. 1. The role of mitochondria and nuclei in mediating the stimulatory effect of LH on 3β-HSD activity in *Rana* testicular microsomes. Testes were homogenized (5 percent, wt./vol.) in the following medium: 0.02*M* potassium phosphate buffer (*p*H 7.4), 0.25*M* sucrose, and 0.001*M* mercaptoethanol. Organelles were isolated by differential centrifugation. The microsomal fraction was recombined with the mitochondria or nuclei (each equivalent to an amount from 100 mg of fresh tissue) and then incubated in homogenization medium containing calf serum (10 percent by volume). Final total incubation volume was 3.0 ml. All operations except incubation and final assay were at 0° to 4°C. Incubation in a Dubnoff incubator was at 29°C for 6 hours. The 3β-HSD activity was determined on the 24,000*g* supernatant by the spectrophotometric technique described previously (*4*). Sn = supernatant, 24,000*g* for 10 minutes. Mito = mitochondria. LH = 65 μg of ovine NIH-LH-S16 per milliliter of total incubation mixture. Bars and lines represent means and standard errors ($n = 3$). Testes from eight animals were pooled and each assay was run three times. One hundred percent is equal to approximately 2.5 nmole of the reduced form of nicotinamide adenine dinucleotide ($NADH_2$) per gram per minute formed between the second and twentieth minutes.