fatty acids(12), and various reports alluding to the correlation between lipase activity in serum, organs and tissues(13,14) with the resistance of host species or organs to tuberculous infection. Although it would be premature to suggest that such a postulated lipase-lipid mechanism may be involved in the suppression of tubercle bacilli by histiocytes, the results reported here are suggestive and appear worthy of more intensive investigation.

Summary. Cell lysates derived from peritoneal histiocytes of normal and BCG-immunized rabbits or guinea pigs had no demonstrable direct effect on the morphology, neutral red binding capacity, viability or cytotoxic activity of virulent and attenuated strains of *Mycobacterium tuberculosis*. Histiocytic lysate in combination with Tween 80 was found to be bactericidal for strains of tubercle bacilli but not for a strain of *Escherichia coli* or a strain of *Staphylococcus aureus*. The mechanism of this activity appeared to be due to the enzymatic degradation of Tween 80 by histiocytic lipases with the formation of oleic acid. A similar antimycobacterial activity was manifested in mixtures of histiocytic lysates with crude lipid extracts of normal rabbit serum and with lipemic rabbit serum. No difference in activity was found between cell lysates prepared from histiocytes of normal and BCG-immunized rabbits or guinea pigs.

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An Intracellular Parasite Resembling a Microsporidian Associated with Ascites in Swiss Mice. (27317)

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Morris *et al.*(1) described an enzootic disease of mice characterized by ascites, hepatosplenomegaly, and the presence of an intracellular protozoan-like agent. A seemingly identical disease, previously unreported, has been maintained in our laboratory for a period of years by serial passage in Swiss mice. Some additional properties of the associated agent, brought out by our studies, are presented.

Methods. Swiss W mice of a commercial subline, which had shown an atypical response to certain carcinogens, were sent to us for examination in 1953. The mice were autopsied with findings that were clearly indicative of chronic respiratory disease. Splenic enlargement, which is not an accompaniment of that condition, was also observed. A saline suspension of ground spleens from 3 of the animals was injected intraperitoneally in Swiss weanlings from the random-bred mouse colony of the Rockefeller Institute. At autopsy on the 21st day these mice likewise showed enlarged spleens.

Separate passages with suspensions of pooled spleens and undiluted ascitic fluids, which first appeared during the second transfer, were maintained thereafter in groups of

5 Swiss mice (10 to 15 g) at intervals of 3 to 5 weeks. Unless otherwise noted the injections were made intraperitoneally with 0.1 ml of the respective inocula. In 1961 specific pathogen-free Swiss were substituted for the conventionally reared mice which had previously been used.

Results. The experimental findings in Swiss mice indicated the emergence of a specific disease entity which was readily transmissible. Abdominal distension, the first external sign with both inocula, was usually apparent during the second or third week after injection. Mice killed at this time regularly showed moderate amounts of straw colored and slightly turbid fluid in the peritoneal cavity. The ascitic reaction progressed through about the fifth week with marked swelling of the abdomen and weight gains of 15 g or more. The ascitic fluids varied from 2 to 15 ml and were sometimes viscous. The spleens were usually enlarged with weights up to 5 times normal. Hypertrophy of the livers and mesenteric lymph nodes also occurred but less frequently. At times, the livers and spleens were partially coated by a gray cellular membrane. Some of the livers also had a granular appearance due to the presence of small foci composed of lymphocytes and large mononuclear cells. A few mice, after the fifth week, showed evidence of hemorrhage indicated by reddish fluids containing many erythrocytes. There was a low but steady mortality, probably the result of continued hemorrhage. The mortality rate through the fifth week for 500 mice used in the last 50 passages was 8%.

Ascitic fluid was slowly absorbed in mice held beyond the fifth week. Splenic enlargement was not progressive but tended rather to recede. The causal agent was retained, however, for considerable periods in the absence of fluid. Spleens from 6 injected mice removed after intervals of 6 to 12 months produced typical ascites on passage. Subcutaneous and intramuscular injections were not attended by local reactions but resulted in an ascitic response which was somewhat delayed and less marked in comparison to that by the abdominal route. Ascitic fluid from mice killed during the third week was active in saline dilutions through 10^5 and heart's blood removed at this time was regularly infective. Ascitic fluid injected intracerebrally was innocuous.

The causal agent of transmissible ascites was also established in Princeton mice and maintained in them for 14 splenic passages. After the first transfer, the reactivity of these mice was significantly less than that of Swiss mice and enlargement of the spleen was often the only manifestation at autopsy. Carriage of the causal agent was indicated, however, by copious ascitic fluid in Swiss mice injected in parallel with each of the passage suspensions.

Microscopic findings. Giemsa stained films of ascitic fluid showed varying numbers of cells, chiefly lymphocytes and large mononuclears. Some of the latter contained vacuoles filled with small ovoid or rod-shaped bodies, termed descriptively oval bodies (OB). Most of the intracellular OB had a clear unstained zone at one pole while the remainder of the unit was uniformly blue. A few OB were either solidly stained or had a clear outer zone surrounding a central blue area. The latter appearance was seemingly characteristic of the agent described by Morris *et al.*(1).

Direct examination of fluids by phase microscopy, which was not done by Morris *et al.* (1), revealed morphologic features not brought out in stained films. The nests of OB were generally single (Fig. 1A) but occasionally multiple and were present only in large mononuclear cells of the macrophage type. These cells also contained numerous, shiny, lipoidal droplets. Pressure applied to an area in which parasites had been found resulted in rupture of the macrophage and the release of a wavy filament or tail from the OB. A transparent globoid structure containing one or two minute particles was attached to the opposite end of the filament (Fig. 1B and C). The dimensions were roughly 2 μ for the shell of the OB, 10 to 15 μ for wavy filaments, 20 to 25 μ for straight ones and 1.5 μ for the spheres. The release of the filament was violent and often resulted in ejection of the entire unit into the adjoining fluid. In some instances, however,

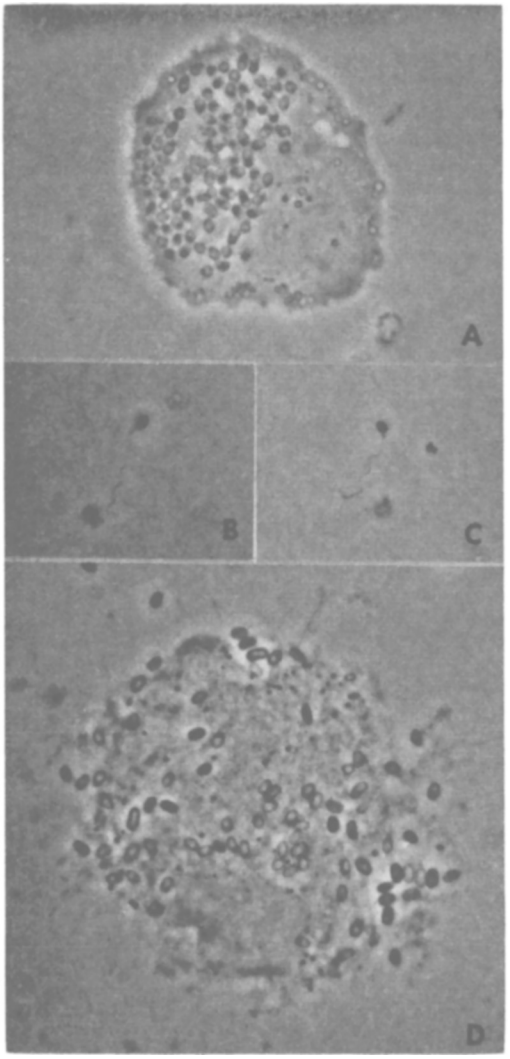

FIG. 1. Wet mounts of ascitic fluid with phase contrast ×1050. A. Intact macrophage with nest of OB. B. Extracellular OB with extruded filament and globoid structure. C. Same with detached globoid structure. D. Ruptured macrophage with OB shells and peripheral filaments.

the empty shells remained within the cell mass and the filaments protruded outwardly into the fluid (Fig. 1D). The spheres tended to separate from the filaments and float away from the OB. At the time of their release several minute particles, close to the limit of visibility, were detectable in the immediate vicinity.

Extracellular OB were non-motile and were demonstrable with certainty only in ascitic fluids. Intracellular groupings were regularly detectable in fluids removed 2 to 3 weeks after injection but even with optimal conditions only a few of the macrophages were parasitized. Their detection in fluids removed after the third week usually required preliminary concentration of the cells by low speed centrifugation. Survival of some portion of the OB in the spleen for as long as 12 months was indicated by reappearance of the entire unit on subsequent injection.

Sedimentation and filtration. Sedimentation of ascitic fluid at 1000 rpm for 15 minutes resulted in removal of both cells and OB. On resedimentation at 3100 rpm (1700 × *g*) for an hour a trace of deposit was obtained from the clear supernatant. Suspension of the deposit in a small volume of saline resulted in a slightly turbid fluid which showed numerous minute particles but no OB. The ascitic syndrome with intra- and extracellular OB was reproduced by this fluid following its injection in Swiss mice. The supernatant, after the second centrifugation, was largely free of particles and inactive on injection.

The infective particles present in ascitic fluid after low speed centrifugation were retained by a Coors No. 3 candle and filtrates were innocuous.

Discussion. It is probable that the organism under consideration is identical with the protozoan-like agent described by Morris *et al.*(1). Our additional characterization supports inclusion with the protozoa and suggests its allocation in the order *Microsporidia*. Aside from the minute particles, the morphologic features of the OB are characteristic of microsporidia as defined by Wenyon(2) and more recently by West(3). Acceptance of the present organism as a microsporidian would be of interest as none of the existing species of this order are mammalian parasites. It may well be, however, that the organism now known as *Encephalitozoon cuniculi,* a parasite of rabbits and mice, is also a microsporidian, as originally suggested by Levaditi *et al.*(4). Reexamination of this organism by phase microscopy, as a means of demonstrating filament extrusion would be of interest.

Recognition of the microsporidian-like organism as the causal agent of the murine syn-

drome seems warranted. It is undoubtedly enzootic in certain mouse colonies but neither the portal of entry nor mode of transmission are known. A minute particle capable of reproducing the entire organism appears to be the infective component.

The natural disease has been detected with certainty only in mice of the originally inbred Swiss Webster (W) strain. The so-called Bagg mice used by Morris *et al.*(1) were obtained from the Flora O'Grady colony and, as verified by her, are Swiss W. We also recovered the causal agent from the spleens of Swiss W mice secured from the dealer who supplied the originally infected animals. It was later detected in ascitic fluid which appeared in Swiss W mice of another subline, used elsewhere, in studies on murine leukemia. There is no evidence, however, that the agent is enzootic in our random-bred Swiss colony. Whatever the natural distribution of the organism may ultimately prove to be, its unsuspected presence in mice under experimentation may lead to false conclusions.

Summary. Transmissible ascites of natural occurrence in Swiss W mice is described. It closely resembles an enzootic murine disease reported by Morris, McCown, and Blount in 1956. Evidence that the causal agent is a microsporidian is presented. Ascitic fluids examined by phase microscopy show small intracellular organisms which on pressure release a filament with an attached globoid structure. An infective component of the organism may remain viable for at least a year in recovered mice.

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A Plaque Assay for Myxoma Virus Infectivity.* (27318)

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A cytopathic effect has been observed in plasma clot(1) and monolayer(2) cultures of rabbit kidney epithelial cells infected with myxoma virus. This phenomenon should lend itself readily to the development of a plaque assay and thus offer the possibility of a more quantitative approach to any studies of the myxoma virus-host cell relationship than is presently employed. The conditions suitable for such an assay on monolayer cultures of cells derived from rabbit kidney are described in this report.

Materials and methods. The South American Sanarelli strain of myxoma virus, obtained from Dr. Ralph B. Houlihan, Cutter Laboratories, was used. Virus pools were stored in glass sealed ampules at –60°C as a 10% rabbit dermal tumor suspension in Eagle's medium(3) containing 10% calf and 10% lamb serum. They were clarified before storage by low speed centrifugation and by filtration through .01 Selas bacteriologic filters.

The tissue culture systems used were an established line of suckling rabbit kidney cells (SRK) obtained from Dr. D. G. McKercher and primary rabbit kidney cells (RK) grown from trypsinized fragments of kidney tissue freshly excised from young adult animals. Both types of cultures were grown in 10 ml of medium as monolayers in 4-oz prescription bottles and consisted of mixtures of epithelial and fibroblastic cells. Growth medium for SRK cultures was composed of Eagle's medium supplemented with 2 μg/ml of hydrocortisone, 42 μg/ml arginine, 10% calf serum

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