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Experimental Transmission of a Murine Microsporidian in Swiss Mice

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The production of ascitic fluid and splenomegaly on intraperitoneal injection in weanlings was used as a test for microsporidia after introduction by other routes and in other loci. Oral and cerebral administration was followed only by enlarged spleens which reproduced the ascitic response on passage. Microsporidia were demonstrable by phase microscopy in all fluids. Positive findings were also obtained with liver, kidney, brain, lungs, blood, and urine. Intramuscular and intranasal injection were occasionally followed by ascites, but splenomegaly again predominated. The results of contact experiments indicated that the organisms were not readily communicable either in weanlings or nurslings. Relation of the microsporidian to *Encephalitozoon cuniculi* (*Nosema cuniculi* Lainson et al.) is discussed.

A murine protozoon which produced ascites and splenomegaly on intraperitoneal injection in mice was described in 1956 by Morris et al. (6). A similar organism had been under observation in our laboratory, and in 1962 we presented evidence of its morphological resemblance to protozoa of the order *Microsporidia* (7). These organisms were known to be widely dispersed in nature but had been found only in nonmammalian hosts. Our findings were confirmed and extended by Lainson et al. (3) and by Weiser (10). The rodent species are now classified in the genus *Nosema* but will be referred to here as microsporidia. Observations made over a period of several years on their experimental transmission and distribution in Swiss mice are brought together in this paper.

MATERIALS AND METHODS

The microsporidian under study was originally recovered in 1953 from naturally infected mice of an outside source. It was readily established in Swiss mice by passage and has been maintained continuously in weanlings from the specific pathogen-free (SPF) colony of the Rockefeller University. Transfers were made in groups of 5 mice of either sex and 12 to 15 g in weight. They were injected intraperitoneally with 0.1 ml of pooled and undiluted ascitic fluids or of spleen suspensions (about 10%) prepared in saline with a Ten Broeck type tissue grinder. The mice were killed with ether at varying intervals and autopsied. Ascitic fluid pools were regularly examined by phase-contrast microscopy for microsporidia. Intracerebral injections were made with 0.02 to 0.03 ml of fluid, containing 1,000 units of penicillin per ml, in 10- to 12-g mice anesthetized with ether. Nasal instillations were also made in anesthetized mice by placing 5 to 6 drops of ascitic fluid or lung suspension on the external nares. Intramuscular injections were made in the right hind leg with 0.05 to 0.07 ml of inoculum. Oral administration was conducted by letting the mice suckle fluid from a capillary pipette. Blood samples were removed from the heart after ether anesthesia, and about 0.25 ml was added to an equivalent volume of heparin-saline. Infant mice varied in age from 1 to 5 days and were reared by their respective mothers. All infected animals were held under observation in isolation quarters.

RESULTS

Intraperitoneal transmission. Some of these observations were reported earlier (7) but have been extended. Mice of the passage series regularly showed abdominal distension 2 to 3 weeks after injection with either fluids or spleen suspensions. The characteristic bulge is shown in Fig. 1. The ascitic fluid was usually straw-colored but occasionally tinged red with blood, slightly turbid, and sometimes viscid. It varied from 2 to 5 ml in volume. Ascites was accompanied by moderate enlargement of the spleen and, less often, of the liver. The ascitic and splenic response reached a maximum about the 5th week and then gradually subsided. The peak volume of fluid was 15 ml and weight of the spleen, 600 mg. The average spleen weight for five injected mice killed after 37 days was 454 mg and for five normal ones of the same age, 114 mg.

Examination of ascitic fluids in wet state with a phase-contrast microscope at a magnification of 1,000 revealed chiefly lymphocytes and macrophages. The small spores (2 μ) of the microsporid-

ian were usually intracellular and found only in the latter cells. Infected ones were sparsely distributed and were commonly less than 5% of the total number. The ovoid spores varied from 2 to 50 or more per cell and were contained within cytoplasmic vacuoles. They were rarely seen after the 5th week, although the fluid was active on passage. Infected cells ruptured on application of light pressure to the cover slip. The mature spores emerged explosively and extruded a wavy or straight filament (10 to 25 μ). At its opposite pole was a rounded body, the sporoplasm, which became detached and floated away. This body is conventionally regarded as the infective component of the spore complex. These are the essential morphological characteristics of a microsporidian. They were illustrated in the earlier papers (3, 7, 10). Spores, but not their appendages, were also differentiated by Giemsa and Wayson strains.

Specific deaths were uncommon, less than 8%, and on occurrence were due to massive hemorrhage into the peritoneal cavity with blood red fluid. Survivors held for extended periods showed recovery with complete reabsorption of fluid and decreased spleen weights. They continued to harbor microsporidia in a latent state for at least a year as their spleens again produced an ascitic response on peritoneal passage. The transfer of spleen pools to susceptible weanlings is used throughout with all infected mice which failed to show ascites. It is referred to as the peritoneal test and termed positive in presence of fluid with microsporidia and negative in their absence.

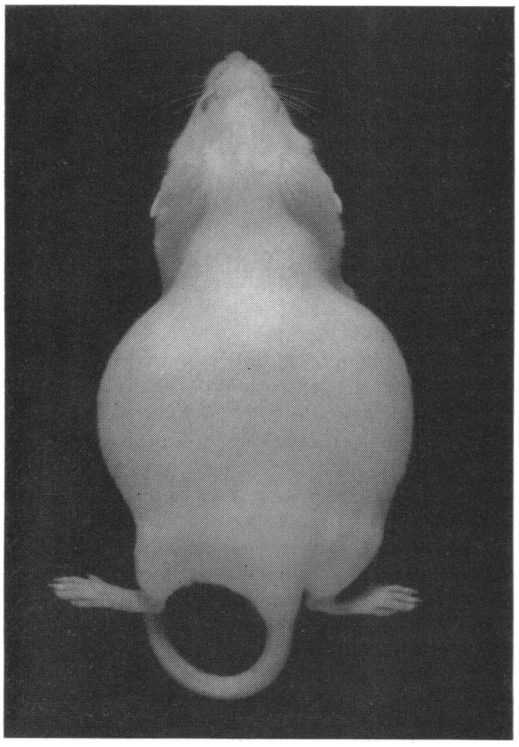

FIG. 1. *Abdominal distension in a Swiss mouse 3 weeks after intraperitoneal injection.*

Recovered mice showed evidence of resistance to reinfection, as earlier noted by Morris et al. (6). It was sufficient to prevent an ascitic response but failed to eliminate microsporidia from the spleen. In one experiment, five uninjected and five injected mice of the same age were held in separate cages for 113 days. At this time, all were normal in appearance and were challenged with active fluid. The controls showed abdominal distension with fluid, but the reinjected mice were outwardly normal and fluid-free at autopsy. Survival of the microsporidia was indicated, however, by positive peritoneal tests.

Heart blood removed from mice at the height of infection was tested with variable results. Five pools from different groups showed activity on injection, but the response was significantly reduced in comparison with that of ascitic fluid. Of 25 mice, 12 had both fluid and enlarged spleens, 4 had only enlarged spleens, and 9 were normal. Brain suspensions from mice of the passage series were also tested. Five pools were again used and all of them produced ascites with microsporidia on intraperitoneal injection.

Attention was then directed to the response of infant mice to intraperitoneal injection of active fluid. Three litters of eight to nine nurslings (1, 2, and 5 days old) were used. Their reaction differed from that of weanlings only in degree. Abdominal distension, volume of fluid, and size of spleen were proportionally reduced. Microsporidia were demonstrable in all ascitic fluids.

Transmission by other routes. Intracerebral injection with ascitic fluid was made in 30 weanlings (six groups of five). All survived and appeared normal when killed after 14 to 42 days. Ascitic fluid was absent at autopsy, but spleens were regularly enlarged and gave positive peritoneal tests. Heart blood removed after 32 and 33 days from the mice of two groups was also positive on injection, but that drawn after 37 days was negative. Four different pools of kidney, liver, and lungs were tested individually in groups of five and all were positive, as were three of four urine samples.

Intramuscular injection in 25 weanlings (five groups) was not attended by any local reaction. All showed enlarged spleens when killed after 4 to 5 weeks, but only one had an appreciable

amount of ascitic fluid. Eight had traces of fluid (less than 1 ml), and 16 were fluid-free.

Intranasal injection was followed by small pulmonary lesions in 16 of 20 mice killed after 4 to 5 weeks. All spleens were enlarged and positive on test, but only two mice had traces of fluid. Nasal passage of lung suspensions, made with two groups, was attended by a diminished number of lesions in the mice of the second transfer and none in the third.

Oral inoculation was made in three groups of five weanlings and in two litters of nine 5-day-old nurslings. None of the mice in either age group showed ascitic fluid when killed after 3 to 5 weeks. Their spleens showed variable enlargement and all gave positive peritoneal tests.

Contact transmission in weaned and infant mice was explored by exposing them to injected and fed cage mates. Three groups of five weanlings were placed in the same cage with two injected intraperitoneally and killed after 45, 64, and 110 days. The exposed mice were fluid-free and had normal sized spleens which were negative on transfer. The injected mice had recovered meanwhile and were also fluid-free. Their spleens varied in size, but all were positive on test. Contact between four uninjected and four injected nurslings was conducted with 1-, 2-, and 5-day-old litters. The results of three experiments with exposure periods of 21 to 47 days were negative. Four additional 5-day-old nurslings in contact for 103 days gave positive spleen tests as did their injected litter mates.

Three groups of five weanlings were each exposed to five others infected orally and killed after contact for 40, 52, and 93 days. The exposed mice were normal at autopsy and microsporidia-free. The spleens of their infected cage mates varied in size but were positive on transfer. Four 5-day-old nurslings from each of four litters were exposed to four orally inoculated litter mates for variable periods and then killed. The findings with those in contact for 31 and 37 days were negative, whereas those held for 26 and 42 days were positive. The spleens of the fed nurslings also gave positive peritoneal tests. The mother of the litter kept for 42 days was of particular interest as she had become infected by contact with her microsporidia-positive offspring. The other mothers were all normal when killed.

Tests for maternal transmission of microsporidia were conducted with four young breeders injected intraperitoneally with ascitic fluid prior to breeding. They had average sized litters of eight to nine infants which were reduced to six. The mothers and their offspring were killed 4 weeks after parturition. The 24 young mice, now weanlings, were normal at autopsy and microsporidia-free. The four mothers had enlarged spleens which gave positive peritoneal tests. The results of transmission by different routes are summarized in Table 1.

Gross pathology. Splenomegaly was the only visual finding which accompanied all routes of transmission. Enlargement of the liver with or without minute focal areas, enlargement of the mesenteric lymph node, necrotic foci in renal and genital fat, and membranous involvement of the spleen were seen sporadically after intraperitoneal injection.

Microscopic pathology. Lymphocytes and macrophages were the predominant cells in all loci, and leukocytes were rarely present. The cellular reaction was quite variable, however, and sometimes absent. After intraperitoneal injection, the lungs regularly showed lymphocytic cuffing around blood vessels and mononuclear cells in adjoining alveoli. The degree of reaction was intensified after intranasal injection with larger cuffs around blood vessels and also bronchi and more cells within alveoli (Fig. 2A). Some but not all kidneys had aggregates of deeply stained mononuclear cells in the vicinity of blood vessels (Fig. 2B). Livers showed similar cells distributed singly in sinusoids and as small islands which displaced the normal hepatic cells. There was little evidence of frank necrosis as in murine hepatitis. The lymphocytic nodules of the spleen were markedly enlarged and megakarocytes were rather numerous. Although brains were regularly infective on intraperitoneal injection, they showed surprisingly little reaction. After intracerebral injection, however, small groups of mononuclear cells appeared in areas where nerve cells would normally be found. There was also some cuffing of blood vessels. The meninges showed little or no involvement. The degree of cerebral reaction was by no means marked.

Sections and impression films of these organs were stained with hematoxylin-eosin and Giemsa and were examined at a magnification of 1,000. Spores of the microsporidian were found in only two instances. With the H and E stain, they were seen in one cell of a kidney section and one cell of a lung section. These findings were in marked contrast with those of Giemsa-stained films of ascitic fluid which regularly showed well-differentiated spores within macrophages.

DISCUSSION

After our report on the microsporidian-like nature of the murine organism (7), Lainson et al. (3) published evidence that *Encephalitozoon cuniculi* of rabbits and rats was also a microsporidian. Filament extrusion was accomplished by the use of hydrogen peroxide but not by pressure. They suggested that the correct name

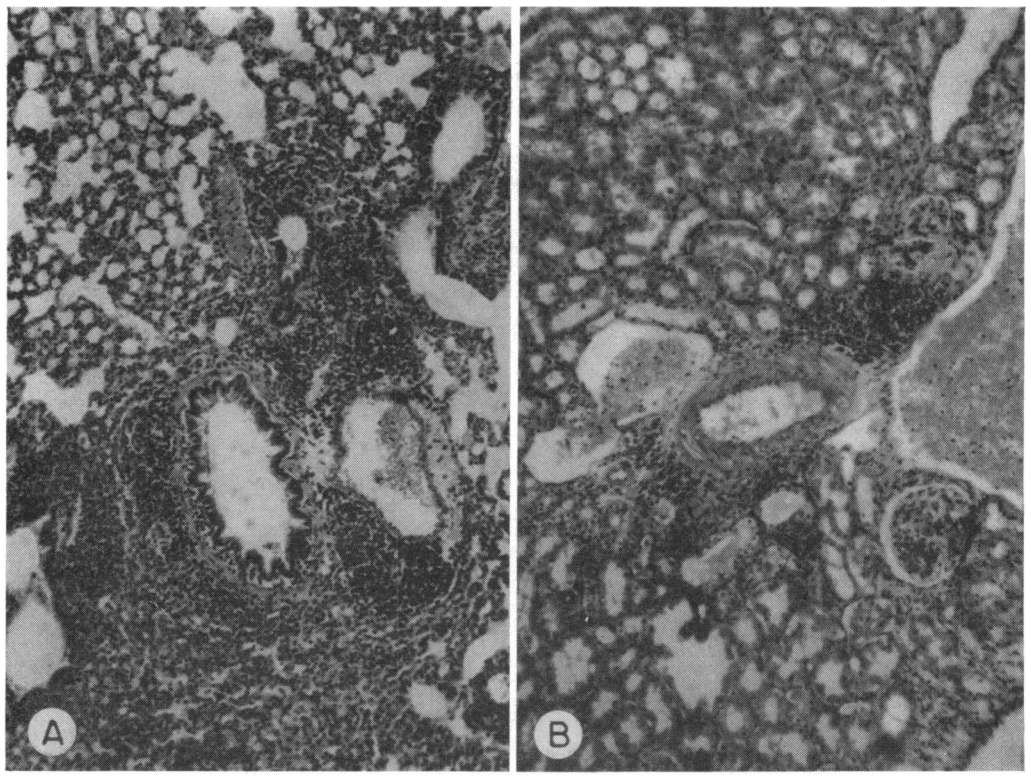

FIG. 2. *Sections stained with hematoxylin-eosin ×100. (A) Lungs with dark areas of cellular infiltration around vessels and within alveoli. (B) Kidney with similar areas near blood vessels.*

TABLE 1. *Summarized results of transmission tests in weaned and infant mice*

Age of mice	Route of transmission	Postmortem findings			Peritoneal test for microsporidia
		Abdominal distension	Ascitic fluid	Splenic enlargement	
Weaned	Intraperitoneal	+	+	+	+
	Intracerebral	−	−	+	+
	Intramuscular	±	±	+	+
	Intranasal	±	±	+	+
	Oral	−	−	+	+
	Contact*	−	−	−	−
Infant	Intraperitoneal	+	+	+	+
	Oral	−	−	+	+
	Contact intraperitoneal	1 group +, 3 groups −	−	Variable	+
			−	−	−
	Contact oral	2 groups +, 2 groups −	−	Variable	+
			−	−	−
	Contact maternal	4 groups −	−	−	−

* With the exception of one mother infected by her young.

of the protozoon should be *Nosema cuniculi*, whether found in rabbits, rats, mice, hamsters, or man. Meanwhile, Weiser had also allocated the murine protozoa to the genus *Nosema* but proposed three species names, one of them being *N. muris* (10).

The first published report of the rabbit protozoon was that by Wright and Craighead in 1922

(11), but Ten Broeck had seen it in rabbit kidney sections in 1918 (9). Levaditi et al. (4) introduced the name *E. cuniculi* in 1923 and later reviewed their work with it. They thought it might be a microsporidian but failed to show filament extrusion. The encephalitozoon was subsequently found under natural conditions in rats, mice, and once only in man. Matsubayashi et al. (5) in 1952 isolated it from cerebrospinal fluid, blood, and urine of a 9-year-old boy with cerebral symptoms. The most comprehensive study in mice was made in 1943 by Perrin (8) in conventionally reared Swiss albinos. A detailed investigation of the neuropathology in naturally infected mice from a supposedly SPF colony was reported by Innes et al. in 1962 (1).

Our transmission findings and those of Perrin were essentially similar. Both protozoa were readily established in Swiss mice by injection and were carried to the brains, kidneys, and other organs regardless of route. The maximal ascitic response occurred only after intraperitoneal injection. Our agent was also transmitted to both nurslings and weanlings by feeding.

The difference between the findings of others with the encephalitozoon and ours with the microsporidian was concerned with pathogenicity and spore distribution. There was general agreement that the encephalitozoon produced meningo encephalitis with destruction of nerve cells in both rabbits and mice. Paralysis was reported, however, only by Wright and Craighead in rabbits (11). Smith and Florence (9) stressed the plugging of kidney tubules in rabbits with interference in normal excretory function. Presence of spores in epithelial, nerve, and phagocytic cells was also a common finding in stained sections.

The microsporidian under study was seemingly more active than the encephalitozoon in the peritoneal cavity of the host but significantly less so in the brain and kidney. Spores were regularly demonstrable in ascitic fluids but were rarely found in organ films or sections. Our findings suggested the presence of a viable component other than the spore, implied earlier by the results of sedimentation tests (7). Filament extrusion was accomplished more readily with this microsporidian and the one described by Weiser (10) than with the encephalitozoon of Lainson et al. (3).

Mammalian microsporidia are obviously passed on from one generation to another in their natural hosts. The one studied by Lainson et al. (3) in mice was isolated from rats of a colony known to have been infected 8 years earlier. The present one has been recovered in the United States only from Swiss mice and is seemingly enzootic in a few colonies. It has been assumed that natural transmission is maintained by direct contact, placental transfer, or exposure to infected bedding. Our findings indicated, however, that the murine organism was not readily communicable under experimental conditions and failed to support the transplacental theory. These studies were carried out with SPF mice which were essentially free from ecto- and endoparasites. Most of the earlier work was done with animals from conventionally reared colonies which are often infested with pin worms and mites. Presence of these parasites might well affect communicability either indirectly by lowering the general resistance of the host or directly by actual carriage of microsporidia.

The early work with microsporidia was exhaustively treated by Kudo in his classic monograph (2). The types under discussion appear to be properly located in the genus *Nosema* (Balbini, 1882), but their species classification is still open to question. The present choice is between the single species of Lainson et al. (3) and the multiple ones of Weiser (10). Our findings tend to support the existence of a distinct murine type. It is doubtful, however, whether any of these proposals would meet the taxonomic rules for establishing a species. Regardless of species, it is important that all who work with laboratory rabbits, rats, and mice be aware of mammalian microsporidia and their significance as latent contaminants.

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