INFECTION AND IMMUNITY, Jan. 1981, p. 445-452
0019-9567/81/010445-08$02.00/0

Vol. 31, No. 1

Pathogenicity of *Spiroplasma* sp. Strain SMCA in Syrian Hamsters: Clinical, Microbiological, and Histological Aspects

H. KIRCHHOFF,[1] T. KUWABARA,[2] AND M. F. BARILE[3]*

Institut fuer Mikrobiologie, Tieraerztliche Hochschule, 3 Hannover, West Germany[1]; National Eye Institute, National Institutes of Health,[2] and Mycoplasma Branch, Bureau of Biologics, Food and Drug Administration,[3] Bethesda, Maryland 20205

The intracerebral inoculation of newborn Syrian hamsters with pure cultures of *Spiroplasma* sp. strain SMCA caused severe, prolonged disease involving the central nervous system, culminating in death. The disease was characterized by spasms, muscular tremors, disturbances in motor control, inability to feed, dramatic loss of weight, and runting. The effect was dose related, with the largest numbers of viable spiroplasmas producing the highest incidence of disease and death in the shortest period of time. Severe hemorrhaging developed throughout the brain, liver, and spleen, and spiroplasmas were readily recovered from these organs, indicating that the agent disseminated from the initial site of infection to distant host tissues. Newborn animals were susceptible, but adults were resistant; these findings are similar to those reported for newborn mice and rats. Unlike mice and rats, hamsters did not develop cataracts visible to the unaided eye. The histopathological features of eye disease in hamsters were different from those in rats and were characterized by microophthalmia (especially in runted hamsters) and abnormal proliferation, disorientation, and disorganization of corneal, lens, and retinal tissues. The significance of these findings is discussed.

In 1964, Clark reported that newborn rats and mice inoculated intracerebrally with the suckling mouse cataract agent (SMCA), isolated initially from a pooled extract of rabbit ticks (*Haemaphylsalis leporispalustris*), produced a high incidence of bilateral cataract formation, with an occurrence of central nervous system disease and occasional death. The agent was filterable (2, 11), was grown in chicken embryos, could not be cultivated on standard broth and agar media (4), and was considered to be a "slow virus" (6). Electron microscopy studies suggested that SMCA might be a mycoplasma (15). Subsequently, the agent was grown on artificial media; it was shown to be a helical, cell wall-free procaryote belonging to the class Mollicutes (1) and related to the plant and insect mycoplasmas known as spiroplasmas (7, 14; J. G. Tully and R. F. Whitcomb, *in* M. P. Starr, H. Stolp, H. G. Trüper, A. Balow, and H. G. Schegel, ed., *The Prokaryotes*, in press). Koch's postulates were met when Tully et al. (12, 13) inoculated newborn rats intracerebrally with clone-purified broth cultures of SMCA and produced typical cataracts. The histopathological features of cataract disease were reported to be generalized endophthalmitis with early development of retinitis leading to posterior uveitis (9). The present report examines the effect of *Spiroplasma* sp. strain SMCA in newborn Syrian hamsters (H. Kirchhoff, T. Kuwabara, and M. F. Barile, Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **241**:196, 1978) and describes the clinical, microbiological, and histopathological features of the disease induced.

MATERIALS AND METHODS

Mycoplasmas. *Spiroplasma* sp. strain SMCA, triple filter cloned, passage 6, kindly supplied by J. G. Tully, Bethesda, Md., was grown in Vero monkey (*Cerocopithecus*) kidney cell cultures maintained at 36 ± 1°C on 4:1 (vol/vol) SP4 broth medium (13)-Eagle minimal essential medium. When cytopathic effects developed (within 5 to 7 days), the culture fluids were subcultured to additional Vero cell cultures. After the development of cytopathic effects on second passage, the culture fluids, hereafter denoted as stock culture, were harvested and stored at −70°C in vials until used for the study. Spiroplasmas were enumerated at the time of harvest by the broth dilution-extinction procedure (i.e., color-changing units [CCU]/ml) as follows: tenfold serial dilutions of the stock culture were made in SP4 broth medium containing glucose and phenol red, and the broth dilution cultures were incubated at 36 ± 1°C for 14 days. Because actively growing spiroplasmas break down glucose-producing acids, the broth cultures develop a color change indicating growth. The number of viable spiroplasmas was determined as the final culture dilution in which 1 CCU of growth per ml was observed. The undiluted stock culture contained 10^9 CCU/ml. *Spiroplasma* sp. strain SMCA grown in SP-4 medium alone was also used.

Animals. Pregnant Syrian hamsters (*Mesocricetus*

auratus) and Sprague-Dawley rats were received late in gestation from the Small Animal Section, National Institutes of Health, Bethesda, Md., and quarantined in our animal quarters until they delivered their litters. Each mother and her litter were kept together in a separate cage throughout the study.

Pathogenicity studies. Newborn (0- to 3-day-old) suckling hamsters and newborn rats were examined concurrently and were inoculated intracerebrally with 0.01 ml of undiluted stock culture or with one of a series of tenfold serial dilutions of stock culture. The culture medium used for maintenance of cell cultures was the diluent. Litters inoculated with diluent or with spent cell culture medium fluids (or both) served as the medium controls, and uninoculated litters served as normal controls. All animals in a given litter were treated as a group; i.e., they were all inoculated with the same material or were uninoculated controls.

Isolation studies. Hamsters were sacrificed, and tissues were removed by aseptic techniques and cultured for spiroplasmas. Fluid exudates expressed from these tissues (i.e., brain, liver, and spleen) were also examined directly for helical spiroplasmas by dark-field microscopy. For culture, small amounts (0.2 to 0.3 g) of tissue were inoculated into SP4 broth; the cultures were incubated at 36 ± 1°C and observed for growth periodically for 3 weeks. Broth cultures were also examined periodically for spiroplasmas by dark-field microscopy.

Histological studies. The enucleated eyes of ten uninoculated control and 22 experimentally infected hamsters with varying severity of disease were processed for histological examination as follows: eyes were fixed in 10% neutral Formalin, dehydrated in ethanol, embedded in glycol-methacrylate, cut into 3- to 5-μm sections, stained with hematoxylin-eosin or by the periodic acid-Shiff reaction, and examined by light microscopy.

RESULTS

Clinical aspects of disease in newborn hamsters. Three experiments were performed by using three separate vials of the same stock culture stored frozen at −70°C. A total of 26 pregnant mothers bearing 278 newborn hamsters were used in these studies; 179 newborn hamsters were inoculated with undiluted stock culture or one of a series of tenfold dilutions of stock culture; 67 were given cell culture fluid medium alone (medium control group), and 32 were uninoculated and served as normal controls. Animals were observed daily for 8 weeks for signs of disease. The lethal effect of the intracerebral inoculation of *Spiroplasma* sp. strain SMCA in newborn, suckling hamsters is summarized in Table 1. The effect was dose related, and the largest number of spiroplasmas produced the most severe disease and the highest incidence of death in the shortest period of time; e.g., the undiluted stock culture (containing 10^7 CCU of spiroplasmas per 0.01-ml inoculum) produced disease and death in 57 of 61

TABLE 1. *Intracerebral inoculation of newborn hamsters with various concentrations of Spiroplasma sp. strain SMCA*[a]

Inoculum (CCU/0.01 ml)	No. of litters examined	No. of animals dead/no. inoculated (%)	No. dead on day post-inoculation[b]																						Mean day of death
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	>25	
10^7	6	57/61 (93)	16	6	6	9	2	1	1	2	1		2	2	3	2		2		1						1			5.56
10^6	5	33/54 (61)	8	1			3		7				1	1		2	2	1	1		3		1		1		1		9.45
10^5	3	9/30 (30)	2			1			1											2			3						11.48
10^4	2	4/21 (19)				1			1		2																		7.25
10^3	1	2/13 (15)				1	1																						4.5
Medium	6	15/67 (22)	4	4			2	2	1									1		1									5.0
Uninoculated	3	6/32 (19)	5	1																									1.17

[a] *Spiroplasma* sp. strain SMCA (p6 in SP4 broth), obtained from J. G. Tully, Bethesda, Md., was subcultured twice to Vero cell cultures fed with SP4 broth (p10) and stored at −60°C until used. Data presented are a summation of three separate experiments using the same lot of culture (maintained frozen): 26 litters comprising a total of 278 newborn hamsters were used and inoculated with 0.01 ml of tenfold dilutions of viable organisms or with medium alone or were uninoculated controls. None of the inoculated newborn hamsters developed cataracts. Undiluted stock culture contained 10^9 CCU/ml, or 10^7 CCU/0.01 ml of inoculum.

[b] The majority of newborn hamsters dead on day 0 to 2 post-inoculation were cannibalized by the mother.

(93%) newborn hamsters inoculated, whereas only 33 of 54 (61%) and 9 of 30 (30%) of the animals inoculated with a 10^{-1} inoculum (10^6 CCU/0.01 ml) or a 10^{-2} inoculum (10^5 CCU/0.01 ml) died. The last dose contained the smallest number of spiroplasmas that caused death beyond that seen among the control groups. In litter groups inoculated with diluted culture, some of the animals developed a mild form of disease and survived. Similar results were obtained with *Spiroplasma* sp. strain SMCA cultures grown in the SP-4 medium alone, i.e., without the use of Vero cell cultures. Spent cell culture fluids provided results similar to those with fresh cell culture control fluids. The severe form of the disease was characterized by spasms, muscular tremors, disturbances in motor control, lameness, inability to feed, dramatic weight loss, runting, and death. Runting was a prominent feature of disease, and affected hamsters were frequently only one-half the weight of control animals at 21 days of age (Fig. 1). Unlike infected rats (Table 2), the infected hamsters did not produce clinically visible cataracts.

Early death was seen in 15 of 67 (22%) hamsters given medium alone and in 6 of 32 (19%) of the uninoculated controls. Most of the hamsters found dead early (0 to 2 days of age) were cannibalized by the mother. Cannibalism was not seen among rats in this study.

Clinical aspects of disease in newborn rats. The effect of the intracerebral inoculation of *Spiroplasma* sp. strain SMCA in newborn rats is shown in Table 2. None of the rats inoculated with medium alone and none of the uninoculated rats showed overt signs of disease, and none died. Death occurred in only 7 of 40 (18%) rats inoculated with the undiluted (10^7 CCU/0.01 ml) and in 2 of 22 (9%) rats inoculated with a 10^{-1} dilution (10^6 CCU/0.01 ml) of stock culture. However, most of these rats developed cataracts (Fig. 2b), and this disease was dose

TABLE 2. *Intracerebral inoculation of newborn rats with various concentrations of Spiroplasma sp. strain SMCA*[a]

Inoculum (CCU/0.01 ml)	No. of litters examined	No. of animals dead/no. inoculated (%)	No. of animals with cataracts/no. inoculated (%)
10^7	4	7/40 (18)	33/40 (83)
10^6	3	2/22 (9)	5/22 (23)
10^5	2	0/21 (0)	0/21 (0)
Medium	2	0/11 (0)	0/11 (0)
Uninoculated	2	0/13 (0)	0/13 (0)

[a] The culture of *Spiroplasm* sp. strain SMCA and the culture dilutions used were the same as those used for newborn hamsters; studies were done concurrently. Data presented are the summation of two separate experiments using 13 litters for a total of 111 newborn Sprague-Dawley rats (0 to 3 days old) inoculated intracerebrally with 0.01 ml of tenfold dilutions of viable organisms or medium control.

FIG. 1. *Runting was a prominent feature of the hamster disease. Note and compare the size of a runted animal to a normal animal at 21 days of age.*

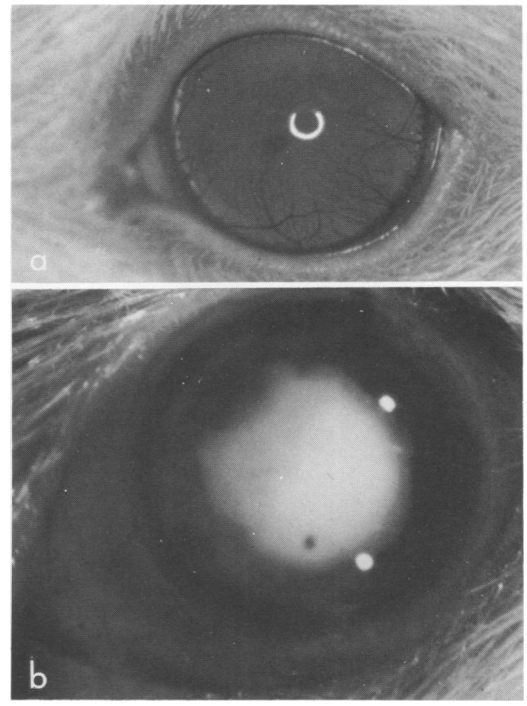


FIG. 2. *Clinically visible cataract formation (b) was a prominent feature of rat disease (×20). The normal rat eye (a) is shown for comparison (×12). Hamster eye disease was not visible to the unaided eye.*

dependent; i.e., cataracts developed in 33 of 40 (83%) and 5 of 22 (23%) rats inoculated with undiluted and 10^{-1}-diluted stock cultures, respectively. Thus, hamsters were more susceptible than rats to the lethal effects of *Spiroplasma* sp. strain SMCA, but surviving rats developed cataracts, whereas hamsters did not produce visible cataracts. As will be discussed later, the histopathological features of eye disease were also different in rats and hamsters.

Detection of *Spiroplasma* in diseased hamster tissues. The results of isolation of spiroplasmas from brain, liver, and spleen tissues of diseased hamsters are shown in Table 3. Most animals were examined during the terminal, moribund stages of disease. In addition to culture, spiroplasmas were readily detected by direct dark-field examination in fluids expressed from brain tissues of diseased hamsters; organisms were usually seen in microcolony formation (20 to 30 spiroplasmas per aggregated clump), with one or more clumps per high-powered (× 1,200) field. The broth culture procedure was more effective than direct dark-field examination of expressed fluids in detecting the presence of spiroplasmas in diseased tissues; cultures generally contained far greater numbers of organisms per microscopic field as well. Brain tissues from 27 of 30 (90%) hamsters inoculated with undiluted stock culture and a small sampling of brain tissues from hamsters inoculated with each serial dilution of the stock culture tested were positive (Table 3). In addition, 10 of 27 (37%) liver tissues and 4 of 9 (44%) spleen tissues from hamsters inoculated with the undiluted culture (and obtained during terminal stages of disease) were also culture positive. Thus, *Spiroplasma* sp. strain SMCA was isolated from brain tissue, the site of inoculation,

TABLE 3. *Isolation of Spiroplasma sp. strain SMCA from tissues of newborn hamsters inoculated intracerebrally with various concentrations of organisms*[a]

Inoculum (CCU/0.01 ml)	No. of SMCA-positive tissues/no. of hamsters examined (%)[b]		
	Brain	Liver	Spleen
10^7	27/30 (90)	10/27 (37)	4/9 (44)
10^6	3/5 (60)	0/5 (0)	0/5 (0)
Broth controls	0/10 (0)	0/10 (0)	0/10 (0)

[a] Tissues were obtained for examination from moribund animals at 7 to 21 days post-inoculation.

[b] In addition, one brain tissue each examined from hamsters inoculated with 10^5, 10^4, and 10^3 CCU/0.01 ml of inoculum was also culture positive; one liver and one spleen tissue each from these same animals were culture negative.

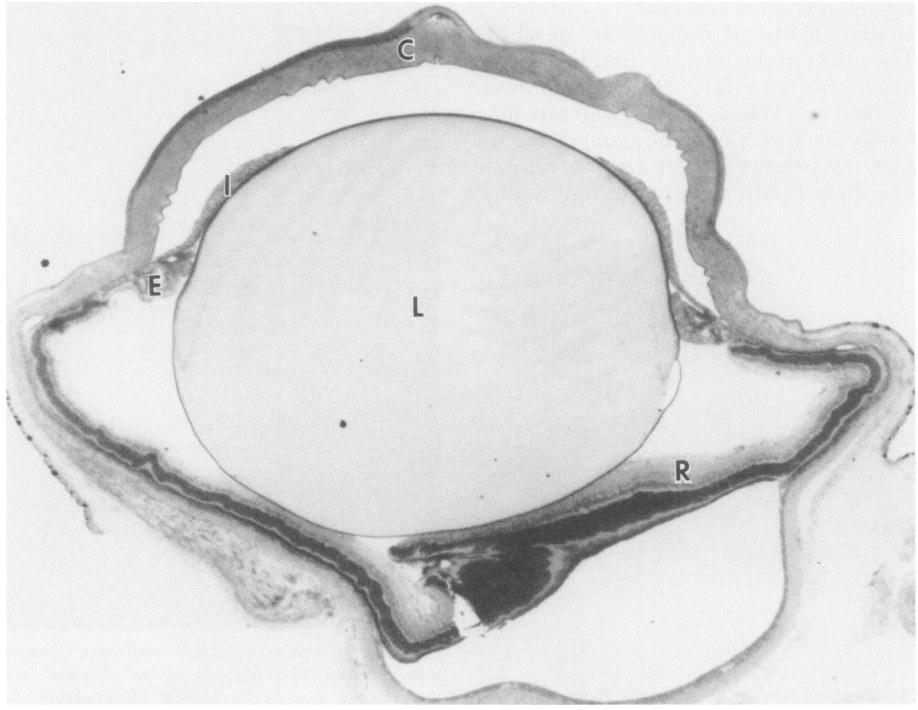


FIG. 3. *Section of whole eye from an uninoculated control hamster at 21 days of age showing intact cornea (C), iris (I), ciliary epithelium (E), lens (L), and retina (R) (×25).*

during the entire 21-day period of the study, and SMCA was readily recovered from liver and spleen tissues during the terminal stages of disease. Gross, severe hemorrhaging developed throughout the brain (including areas leading to the optic nerve), liver, and spleen. Spiroplasmas were not recovered from the normal tissues of control animals inoculated with broth medium alone (Table 3).

Histopathological aspects of hamster disease. The structure of normal eyes obtained from the uninoculated control hamsters at 21 days of age (Fig. 3, 4, and 5) was compared with the histopathological appearance of the diseased

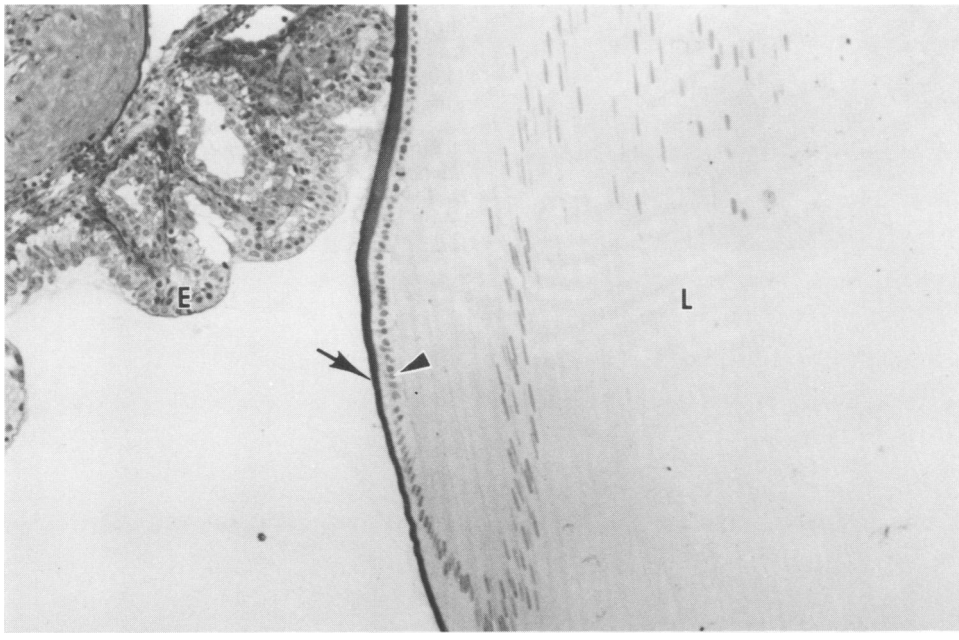


FIG. 4. *Enlarged regions of the ciliary epithelium (E) and lens (L) from Fig. 3 showing the normal thickness of the lens capsule (arrow) and a regular alignment of lens epithelial cells (arrowhead) (×150).*

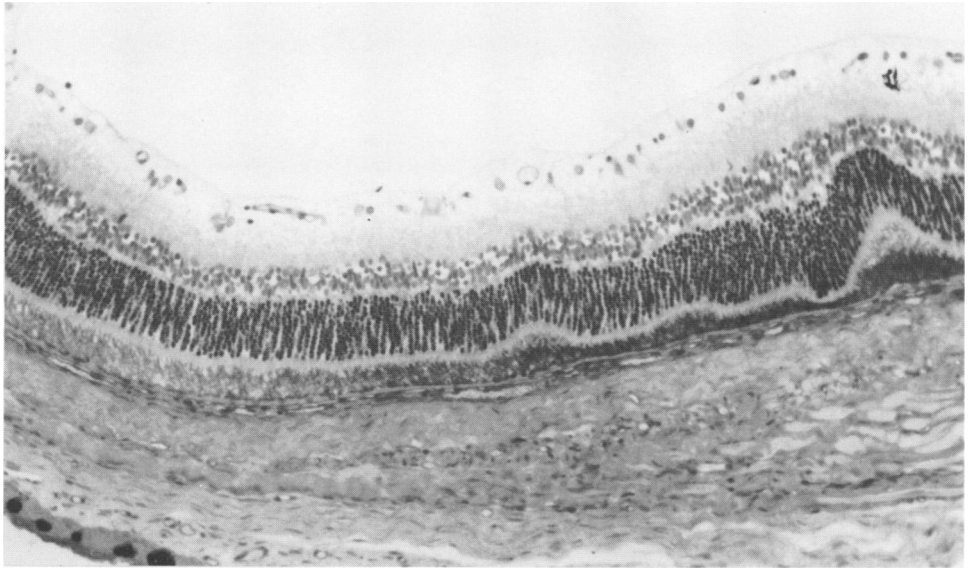
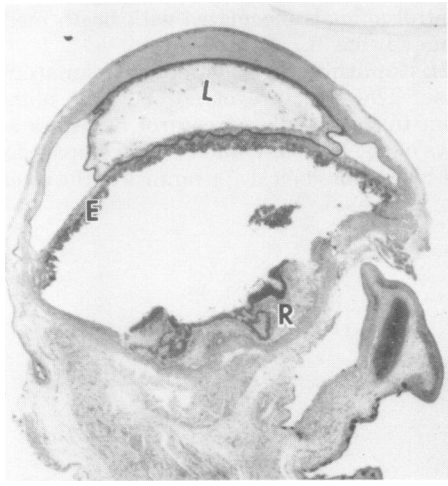


FIG. 5. *Enlarged region of the retina from Fig. 3 showing the normal alignment of retinal layers (×150).*



eyes at 21 days post-inoculation (Fig. 6, 7, and 8). Marked microophthalmia developed in the runted hamster (Fig. 6). Microophthalmia was accompanied by several pathological changes in the eye tissues, characterized by abnormal proliferation, disorganization, and disorientation of corneal, lens, and retinal cells. Note the excessive amounts of muscle tissue attached to the affected eye (Fig. 6). The lens fibers appeared liquified, and the lens epithelium showed marked abnormal proliferation and hyperplasia (Fig. 7). Aberrant, calcified undifferentiated cells and cell debris were seen within the lens capsule.

FIG. 6. *Section of whole eye from an inoculated hamster at 21 days of age demonstrating microophthalmia, irregularly shaped lens (L), proliferation of ciliary epithelium (E), and disoriented regions of the retina (R) (×25).*

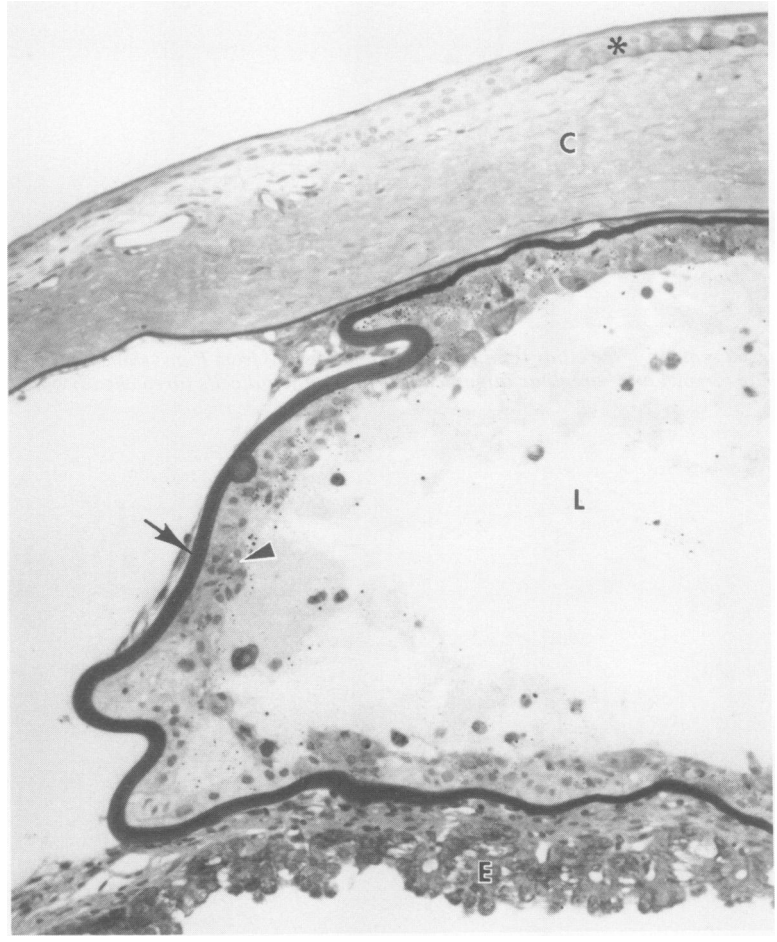


FIG. 7. *Enlarged regions of the cornea (C), lens (L), and ciliary epithelium (E) from Fig. 6, showing irregularities in corneal epithelium (asterisk), thickened lens capsule with irregular contour (arrow) and proliferated layers of lens epithelial cells (arrowhead) as well as many unidentified cell products and debris (×150).*

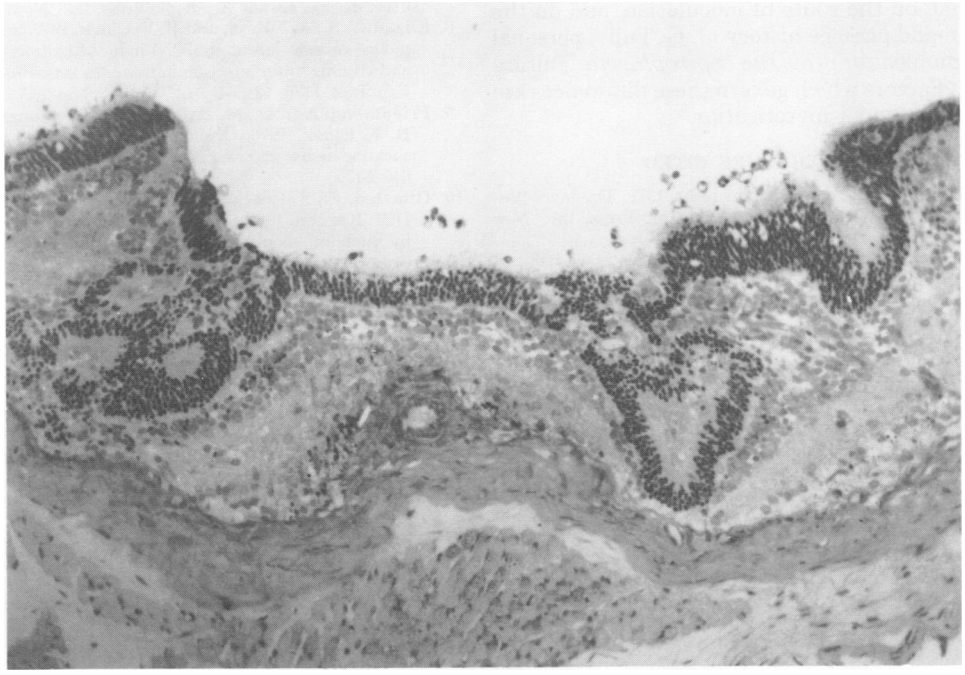


FIG. 8. *Enlarged region of retina from Fig. 6 showing irregular arrangement and a reversed alignment of retinal layers in some areas (×150).*

The lens capsule was thickened and had an irregular contour. Posterior to the lens, there was abnormal proliferation of ciliary tissues. The retinal tissues had undergone dysplasia, and the normal anatomical structures showed marked disorientation, exemplified by the development of abnormal rosette formation of neuronal cells (Fig. 8). There was also marked disorganization and disorientation of photoreceptor cells, which were proliferating in directions opposite to those normally observed in the healthy hamster eye.

DISCUSSION

Spiroplasma sp. strain SMCA, initially isolated from a pool of rabbit ticks, produces a high incidence of bilateral cataract formation in newborn mice (2, 3, 10), in rats and chickens (6), and in newborn rabbits (Kirchhoff, Heitmann, and Trautwein, Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **241**:257, 1978). This report shows that strain SMCA produced a different form of disease in newborn suckling hamsters; the disease was characterized primarily by central nervous system involvement, runting, and death. Unlike rats and other animals, hamsters did not produce visible cataracts, but developed microophthalmia and structurally aberrant lenses. The histological features of the hamster eye disease were characterized as abnormal proliferation, disorganization, and disorientation of lens, corneal, and retinal tissues. The histological features of the rat disease were primarily an endoopthalmitis with cataractous changes secondary to a generalized intraocular inflammation, i.e., early signs of retinitis leading to posterior uveitis followed by degenerative events with ultimate repair of damaged tissues (9). Only newborn animals (i.e., rats, mice, rabbits, chickens, guinea pigs [Kirchhoff, unpublished observation], and hamsters) are susceptible; adult animals are resistant (6). The route of inoculation is important; intracerebral inoculation produces disease, but all other routes of inoculation examined fail to produce disease (4–6). Spiroplasmas are readily recovered from brain, liver, and spleen tissues of diseased animals. Development of hydrocephalus has been reported in rats (8) and hamsters (6). Severe generalized hemorrhaging can develop throughout the affected animal.

The disease produced by strain SMCA in newborn hamsters is different from the disease produced in newborn rats, but it is similar to the disease produced by the GT-48 strain of *Spiroplasma* in newborn rats. Strain GT-48 was also isolated from rabbit ticks, is antigenically similar to strain SMCA, and produces a fatal, hemorrhagic, necrotizing encephalitis in newborn rats; it does not produce visible cataracts (6). Thus, the manifestations of SMCA disease are dependent on the species, strain (5), and age of the

animal, on the route of inoculation, and on the strain and passage history (J. G. Tully, personal communication) of the *Spiroplasma* culture used. Factors which govern these differences are currently under investigation.

LITERATURE CITED

1. **Barile, M. F., and S. Razin (ed.).** 1979. The mycoplasmas, vol. I. Cell biology. Academic Press, Inc., New York.
2. **Clark, H. F.** 1965. The suckling mouse cataract agent. J. Infect. Dis. **114**:476–487.
3. **Clark, H. F.** 1969. Rat cataract induced by suckling mouse cataract agent. Am. J. Ophthalmol. **68**:304–308.
4. **Clark, H. F.** 1974. The suckling mouse cataract agent (SMCA), a slow mycoplasma-like agent?, p. 307–322. *In* J. L. Melnick (ed.), Progress in medical virology, vol. 18. S. Karger, Basel.
5. **Clark, H. F., and D. T. Karzon.** 1969. Growth curve studies of the sucking mouse cataract agent in individual compartments of the eye. Proc. Soc. Exp. Biol. Med. **131**:693–696.
6. **Clark, H. F., and L. B. Rocke.** 1979. Spiroplasmas of tick origin and their pathogenicity, p. 155–174. *In* R. F. Whitcomb and J. F. Tully (ed.), The mycoplasmas, vol. III. Plant and insect mycoplasmas. Academic Press, Inc., New York.
7. **Davis, R. E.** 1979. Spiroplasmas: newly recognized arthropod borne pathogens, p. 415–484. *In* K. Maramorosch and K. F. Harris (ed.), Leafhopper vectors and plant disease agents. Academic Press, Inc., New York.
8. **Elizan, T. S., A. Fabiyi, and H. F. Clark.** 1972. Suckling mouse cataract agent (SMCA)-induced hydrocephalus and chronic brain infection in newborn rats. Proc. Soc. Exp. Biol. Med. **139**:51–55.
9. **Friedlaender, R. P., M. F. Barile, T. Kuwabara, and H. F. Clark.** 1976. Ocular pathology induced by the suckling mouse cataract agent. Invest. Ophthalmol. **15**: 640–647.
10. **Olmsted, E., S. Prasad, Y. Sheffer, H. F. Clark, and D. T. Karzon.** 1966. Ocular lesions induced in C57 mice by suckling mouse cataract agent (SMCA). Invest. Ophthalmol. **5**:413–420.
11. **Schwartz, J., and T. S. Elizan.** 1972. Further characterization of suckling mouse cataract agent (SMCA). A slow, persistent infection of the nervous system. Proc. Soc. Exp. Biol. Med. **141**:699–704.
12. **Tully, J. G., R. F. Whitcomb, H. F. Clark, and D. L. Williamson.** 1977. Pathogenic mycoplasma: cultivation and vertebrate pathogenicity of a new spiroplasma. Science **195**:892–894.
13. **Tully, J. G., R. F. Whitcomb, D. L. Williamson, and H. F. Clark.** 1976. Suckling mouse cataract agent is a helical wall-free prokaryote (spiroplasma) pathogenic for vertebrates. Nature (London) **259**:117–120.
14. **Whitcomb, R. F., and J. G. Tully (ed.).** 1979. The mycoplasmas, vol. III. Plant and insect mycoplasmas, p. 351. Academic Press, Inc., New York.
15. **Zeigel, R. F., and H. F. Clark.** 1974. Electron microscopy of the suckling mouse cataract agent: a noncultivable animal pathogen possibly related to mycoplasma. Infect. Immun. **9**:430–443.