Some Properties of the Scrapie Agent and Its Behavior in Mice. (28225)

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Scrapie, a progressive degenerative disease of the central nervous system of adult sheep (1), can be induced serially in sheep and goats by intracerebral or subcutaneous inoculation of suspensions of brain from scrapie-affected animals. The incubation period is surprisingly long, 4 to 36 months. As judged by the occurrence of clinical disease, British breeds of sheep vary widely in susceptibility to the transmissible agent. The common breeds of dairy goats, on the other hand, seem more uniformly susceptible. The transmissible agent is reputed to withstand boiling for hours and exposure to 0.35% formalin for days. This combination of properties is most unusual for an infectious agent. Indeed, these generally-accepted attributes have given rise to much speculation about whether the scrapie agent represents a hitherto unknown type of transmissible agent. The recent demonstration(2) that the scrapie agent can be passed intracerebrally in mice has provided an opportunity to study characteristics of the agent in another biologic system. Preliminary observations on some properties of the agent and its behavior in mice are given here.

Materials and methods. The scrapie agent was obtained from Dr. R. L. Chandler, Agricultural Research Council Field Station, Compton, Berkshire, England, as frozen mouse brain representing the second mouse brain passage after 8 serial intracerebral passages in goats. Male and female Swiss mice of the Rocky Mountain Laboratory stock were used, generally when 21-27 days old. This stock has been a random-bred closed colony for the past 25 years. Suspensions of tissues were prepared in a 0.85% solution of NaCl containing 10% normal rabbit serum. Seitz EK pads were used for preparing filtrates. For intracerebral injections, 0.03 ml of material was used; 0.05 ml was used for all other routes. The occurrence of a typical pattern of fatal progressive disease in inoculated mice was taken as evidence of the presence of scrapie agent in the inoculum. As determined by microscopic examination of tissues from representative mice, this clinical pattern was regularly accompanied by characteristic degenerative changes in the central nervous system. In our experience with the stock of mice used, clinical disease always became manifest within less than 12 months after inoculation. Hence, an observation period of 12 months seemed adequate. Endpoints of titrations were calculated by the method of Reed and Muench(3).

Results. Routes of inoculation. The disease was induced by intracerebral, intraperitoneal, intranasal, and subcutaneous injections of infected mouse brain suspensions. Moreover, the disease was observed in litters born to female mice inoculated intracerebrally, but the route of infection in these litters has not been determined.

Incubation period. In 4 different titrations of brain suspensions in mice inoculated intracerebrally, the earliest signs of illness were seen 97 to 123 days in mice that received the 10^{-1} dilution and 149 to 169 days in those that received the 10^{-6} dilution, the highest tested.

When a small amount of scrapie agent was used, incubation periods of up to 315 days were observed. The incubation period was longer in mice inoculated by peripheral routes than in mice inoculated intracerebrally. Representative incubation periods are shown in Tables I and II. The rather regular increase in average incubation period and in average interval between inoculation and death that accompanied serial dilution of a tissue suspension suggests that either of these values can be used in estimating the amount of scrapie agent in the inoculum.

Clinical observation. Onset of clinical disease was characterized largely by such vague signs as a roughened coat and slight changes

TABLE I. Incubation Period and Time of Death in Mice Inoculated Intracerebrally with Scrapie Agent.

Dilu. of mouse brain suspension	No. of mice*	Incubation period		Time of death	
		1st mouse	Last mouse	1st mouse	Last mouse
		(days)	(days)	(days after inoculation)	
10^{-1}	6/6	111	118	137	145
		avg = 113		avg = 144	
10^{-2}	6/6	112	127	138	150
		avg = 119		avg = 145	
10^{-3}	4/4	128	132	152	169
		avg = 131		avg = 160	
10^{-4}	6/6	132	134	160	176
		avg = 134		avg = 168	
10^{-5}	6/6	137	155	165	194
		avg = 142		avg = 180	
10^{-6}	6/6	155	160	183	215
		avg = 157		avg = 201	

* Each dilution was injected into 6 21-day-old mice; those that died before any illness appeared in the group were excluded.

Numerator represents No. of affected mice; denominator, No. of mice in experiment.

in demeanor. A few mice were hyperexcitable; they would jump out of a shallow examining pan at the slightest stimulus. Others were reluctant to move or seemed drowsy much of the time. However, periods of somnolence alternated with periods of activity manifested either by continued circling or by aimless wandering. Typically, the tail was arched over the back or was held at unnatural angles; it had a peculiar "plastic" quality and would temporarily remain in an abnormal position when so placed. A low waddling gait accompanied by atrophy of muscles of the hindquarters appeared early in the course of the disease. When an affected mouse was held up by its tail, the hind limbs were clasped together over the abdomen rather than extended laterally from the body as in a normal mouse. As the disease progressed, incoordination of gait became more pronounced; mice then had great difficulty walking and would frequently fall on their sides. Eventually, mice usually remained recumbent, even while eating. Kyphosis was prominent. In advanced stages of the disease, priapism was often observed. As judged by persistent wetting of the perineum, urinary incontinence apparently supervened in many mice. Terminally, they became extremely emaciated, were deeply somnolent most of the time, and were unable to walk, apparently because of incoordination and pronounced weakness. Actual paralysis of the limbs was not seen. Once the characteristic disturbances in gait appeared, affected mice did not recover.

The duration of clinical disease in individual mice was not recorded, but the first death in groups of 6 to 20 inoculated mice usually occurred 21 to 36 days after onset of detectable illness in the group.

Histopathologic changes. Thus far, significant morphologic changes have been found only in the central nervous system. When the disease was fully manifest, the characteristic lesion consisted of variable degeneration of nerve cells, prominent astrocytosis, and spongy alteration of the neuroparenchyma. As seen in sections stained with buffered

TABLE II. Titration of Scrapie Agent in Tissues of Scrapie-Affected Mice.

Dilu.	Brain*		Spleen*		Thymus*		Liver*	
	No. of mice†	Avg incubation period	No. of mice†	Avg incubation period	No. of mice†	Avg incubation period	No. of mice†	Avg incubation period
		(days)		(days)		(days)		(days)
10^{-1}	19/19	117	6/6	122	5/5	126	2/6	229
10^{-2}	6/6	117	6/6	120	6/6	143	1/5	315
10^{-3}	6/6	127	6/6	156	5/5	155		
10^{-4}	6/6	159	6/6	179	5/5	180		
10^{-5}	5/5	169	5/6	212	3/6	187		
10^{-6}	5/5	197	2/6	231	0/6			
The LD_{50}	$10^{-6.5}$ or less		$10^{-5.64}$		$10^{-5.0}$		$10^{-0.88}$ or greater	

* Pooled from 4 mice inoculated intracerebrally.

† The 10^{-1} dilution was injected intracerebrally into 20 21-day-old mice. Each of the other dilutions was injected intracerebrally into 6 21-day-old mice; those that died before any illness appeared in the group were excluded.

Numerator represents No. of affected mice; denominator, No. of mice in experiment.

azure-eosinate, some neurons were shrunken and deeply basophilic; others appeared as irregularly frayed remnants (Fig. 1). Discrete vacuoles were present in the perikarya of a

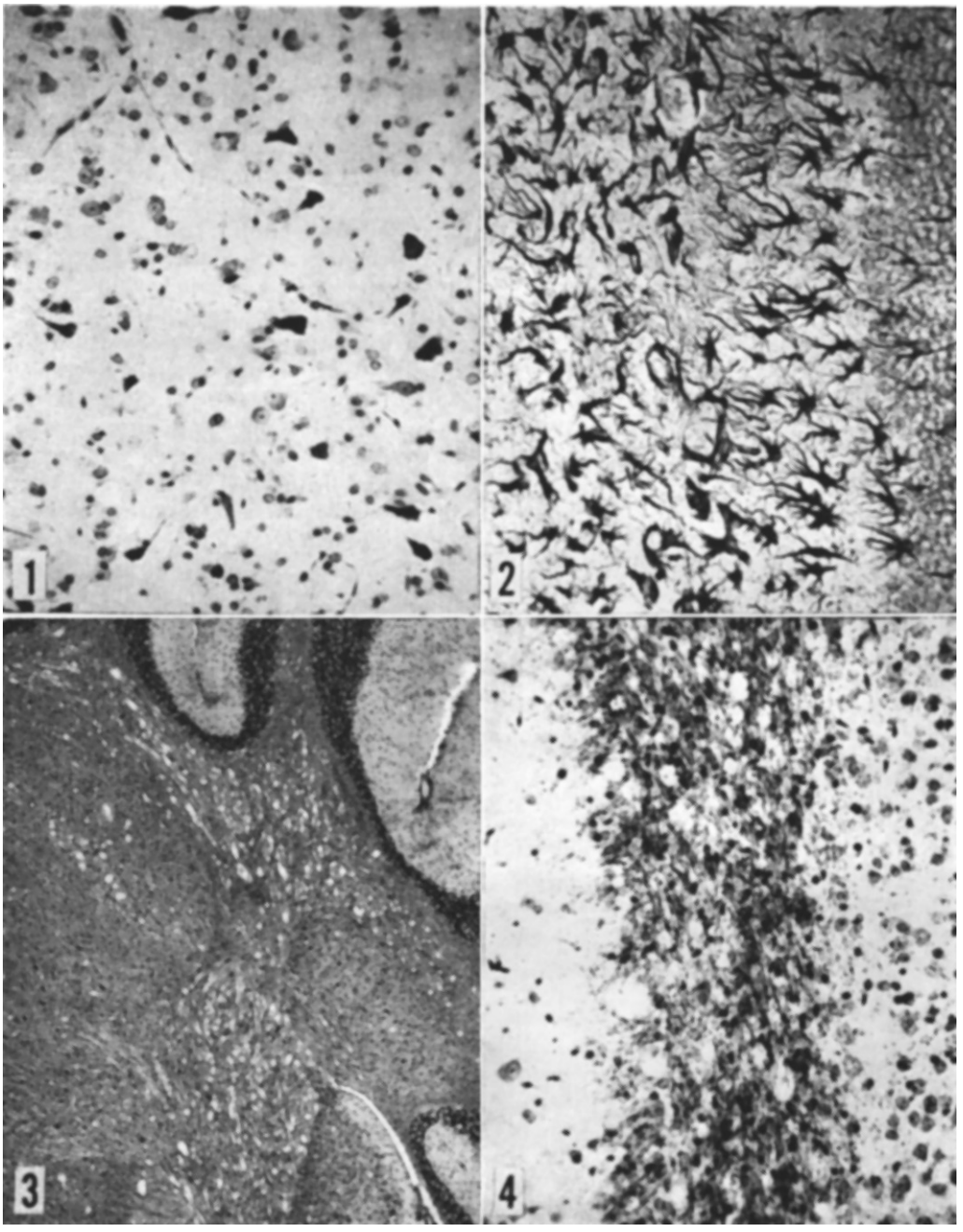

FIG. 1. Medulla oblongata, scrapie-affected mouse. Typical neuronal degeneration and astrocytosis. Azure-eosinate. × 180.

FIG. 2. Hippocampus, scrapie-affected mouse. Numerous enlarged astrocytes as seen early in disease. Cajal's gold sublimate. × 180.

FIG. 3. Cerebellum, scrapie-affected mouse. Typical spongy alteration of the white matter. Luxol fast blue-PAS. × 50.

FIG. 4. Cerebrum, scrapie-affected mouse. Partial destruction of white matter sometimes seen in terminal phase of the disease. Luxol fast blue-cresylect violet. × 180.

few nerve cells. Swelling, chromatolysis, and coagulative necrosis of neurons were seldom evident. Typically, neuronal degeneration was most apparent in the brain stem and diencephalon. However, the extent of the change varied considerably; often it was not particularly impressive, even though clinical disease was overt.

By contrast, hypertrophy and proliferation of astrocytes was much more conspicuous, particularly early in the disease. In sections impregnated by Cajal's gold sublimate method, numerous enlarged astrocytes were seen in the gray matter of the spinal cord, brain stem, diencephalon, and hippocampus (Fig. 2). Such cells were present also in the contiguous white matter of the cerebral hemispheres, the internal capsules, and corpus callosum.

The cellular changes were regularly accompanied by alterations in the gray matter that varied from faint reticulation to patches of sharply outlined circular holes. Characteristically, such sponginess was especially prominent also in the cerebellar brachia and in white matter of the cerebellum and spinal cord (Fig. 3). In the terminal phase of the disease, partial destruction of myelin was sometimes observed in the corpus callosum, white matter of the cerebral hemispheres, and cerebral peduncles (Fig. 4). At no time were inflammatory changes seen in either the neuroparenchyma or the leptomeninx.

Distribution of agent. Serial dilutions through 10^{-6} were made from separate pools of brain, spleen, thymus, liver, and kidneys from 4 mice that became typically affected with the disease 4 months after they were inoculated intracerebrally. The greatest amount of agent was found in the brain; all mice inoculated with dilutions of this tissue became fatally infected. By comparison, the spleen and thymus contained smaller amounts; average incubation periods were longer and not all mice inoculated with dilutions of these tissues became affected with the disease. The liver contained the least amount of agent. It was not detected in the kidneys; all mice inoculated with dilutions of this tissue have remained healthy during 11 months of observation (Table II).

Serial dilutions through 10^{-5} were prepared from separate suspensions of the frontal lobes, diencephalon and midbrain, cerebellum, cerebral cortex, and spinal cord of a typically affected mouse. Each dilution was injected intracerebrally into 6 mice. All mice became typically affected and died. Furthermore, the length of the incubation periods and intervals between inoculation and death did not indicate significant difference in the amount of agent in the various parts examined.

Size of agent. A Seitz filtrate of a 10^{-3} suspension of infected mouse brain was passed serially through gradacol membranes (Carl Schleicher and Schuell Co.) of the following average pore size: 400 mμ, 250 mμ, 150 mμ, 100 mμ, 30-100 mμ and 20-35 mμ. By intracerebral inoculation of mice, scrapie agent was detected in filtrates from the first 4 membranes. Thus, the size of the agent appears to be of the order of 50 mμ. The same results were obtained when this experiment was repeated.

Effect of heat. A Seitz filtrate of a 10^{-1} suspension of infected mouse brain was divided into 6 aliquots kept at the following temperatures for 30 minutes: 56°C, 60°C, 80°C, 95.5°C and 1°C. Mice inoculated with the aliquot exposed to 95.5°C have not become ill during 11 months of observation, though all mice inoculated with aliquots exposed to the other temperatures have died; the last mouse died 7½ months after inoculation (Table III).

TABLE III. Effect of Heat on Scrapie Agent in Seitz Filtrate of 10^{-1} Mouse Brain Suspension.

Temp for 30 min (°C)	No. of mice*	Average incubation period (days)	Avg time of death (days after inoculation)
56	11/11	170	209
60	15/15	174	203
80	13/13	170	206
95.5 (Boiling water)	0/15	No illness after 11 mo observation	No deaths after 11 mo observation
1 Control	11/11	162	184

* Each aliquot was injected intracerebrally into 15 24-27-day-old mice; those that died before any illness appeared in the group were excluded.

Numerator represents No. of affected mice; denominator, No. of mice in experiment.

TABLE IV. Effect of Ether on Scrapie Agent in Seitz Filtrate of Mouse Brain Suspension.

Dilu.	Ether treated			Untreated		
	No. of mice*	Avg incubation period	Avg time of death†	No. of mice*	Avg incubation period	Avg time of death†
		(days)	(days)		(days)	(days)
10^{-1}	13/13	183	225	12/12	168	191
10^{-2}	12/15	199	236	12/12	174	211
10^{-3}	3/13	242	263	10/13	185	226

* Each dilution was injected intracerebrally into 15 21-26-day-old mice; those that died before any illness appeared in the group were excluded.

† Days after inoculation.

Numerator represents No. of affected mice; denominator, No. of mice in experiment.

Effect of ether. From a Seitz filtrate of a 10^{-1} suspension of infected mouse brain, 10^{-2} and 10^{-3} dilutions were made. The 3 dilutions were shaken with ethyl ether and then allowed to stand in a refrigerator overnight. In mice inoculated with the ether treated suspensions, average incubation periods and average intervals between inoculation and death were longer than in mice that received the untreated suspensions. Moreover, in the group that received the 10^{-3} dilution treated with ether, significantly fewer mice became affected than in the corresponding group that received untreated material (Table IV).

Discussion and summary. The agent isolated by Chandler in mice inoculated intracerebrally with brain tissue from goats affected with experimental scrapie can be passed serially in mice by this route. In the Rocky Mountain Laboratory stock of Swiss mice it causes a relentlessly progressive, and invariably fatal, disease of the central nervous system, usually after an incubation period of 4 to 5 months. The disease is characterized clinically by incoordination of gait, muscular atrophy, and drowsiness and neuropathologically by prominent astrocytosis, degeneration of neurons, and spongy alteration of the neuroparenchyma.

That the agent causing this disease in mice is the transmissible agent of scrapie may be questioned. At present, identification of the scrapie agent can be made only by observing its pathogenic capacity in susceptible sheep and goats. In these hosts it induces a distinctive clinicopathologic picture whose essential features also characterize the infection in mice. Thus, the long incubation period, the slowly progressive course of the disease, and the degenerative changes in the central nervous system all are consistent with the view that the agent under study is the transmissible agent of scrapie. Moreover, first passage mouse brain has induced scrapie in goats inoculated intracerebrally(4). At this laboratory, goats inoculated intracerebrally or subcutaneously with a 10^{-1} suspension of fourth passage mouse brain have been observed for too brief a period (5 months) to permit any conclusion about the ability of this inoculum to cause typical caprine scrapie.

The high concentrations of the agent in the brain, spinal cord, spleen, and thymus of affected mice make it appear unlikely that a toxic substance is being passed. That the agent passes through a gradacol membrane of 100 mμ average pore diameter, but not one of 30-100 mμ, suggests it is a medium-sized virus. Previous reports that the scrapie agent withstands 99.5°C were not confirmed when a Seitz filtrate of a 10^{-1} suspension of infected mouse brain was used. Yet, because the agent withstood 80°C for 30 minutes, it is unusually resistant to heat. In view of the results given in Table IV, the agent is somewhat sensitive to ether.

These preliminary findings suggest that the scrapie agent is not a member of a unique group of pathogens but is a medium-sized virus whose resistance to heat is analogous to that of serum hepatitis virus. However, the prolonged incubation period, the protracted course of the disease, and the absence of inflammatory changes in brain and spinal cord comprise a pathologic entity hitherto unknown among viral infections of the central nervous system.

ADDENDUM. Results of experiments now in progress indicate that the method used to test the heat stability of scrapie virus is critical. For example, illness typical of scrapie has appeared in mice inoculated with 10^{-1} and 10^{-2} dilutions of an aliquot of scrapie virus suspension, prepared in 25% meat infusion broth and 5% normal rabbit serum in saline, that was passed serially through millipore membranes of 1200, 800, and 650 mμ average pore diameter and then exposed to 95.5°C for 30 minutes. Yet, mice inoculated with an unfiltered aliquot of this suspension that was similarly exposed to 95.5°C are still healthy, though disease has appeared in mice inoculated with dilutions through 10^{-5} of portions exposed to lower temperatures.

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Received January 28, 1963. P.S.E.B.M., 1963, v112.

Infection of Human Volunteers with a Reovirus of Bovine Origin. (28226)

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The reoviruses which have been recovered from lower animals are, with certain possible exceptions, indistinguishable by *in vitro* tests from those which have been recovered from man(1). Moreover, it has been demonstrated experimentally that strains of human origin are able to infect a variety of animals including cattle(2). This report describes the experimental infection of human volunteers with a strain of reovirus type 1 recovered from a naturally infected calf.

Materials and methods. Clinical Procedures. The 3 volunteers used in this study were adult male negro inmates from a federal correctional institution. Selection was based on willingness to participate and apparent good health. Prior to administration of the inoculum, each volunteer received a complete history and physical examination. Laboratory tests included urine analysis, routine blood count, chemical tests of liver function, chest x-ray, an electrocardiogram, and bacteriologic examination of nose, throat and rectum. These laboratory studies were repeated at weekly intervals after inoculation for 2 or 3 weeks.

The volunteers were isolated in a single room for approximately 2 weeks. Oral temperature, pulse and respiratory rates were determined 4 times daily. Once daily physical examination was performed by a team of 2 physicians and this, with a record of any complaints of illness, was recorded before leaving the room. No antibiotics or aspirin were given to the volunteers.

Inoculum. The inoculum was obtained from a bovine anal specimen containing reovirus type 1. The material used was the second passage in primary human embryonic kidney. Maintenance medium consisted of Eagle's basal medium(3) which contained 250 units of penicillin and 250 μg of streptomycin per ml. Following a single cycle of freezing and thawing, fluid from cultures was pooled, centrifuged at 3000 rpm for 15 minutes, filtered through a millipore filter of an 800 mμ pore diameter and frozen at –60°C until used. Aliquots of the inoculum were safety-tested as previously described(4).

The inoculum was instilled by pipette into the nasal cavity (0.5 ml into each nostril). Immediately following administration of the virus a sample of the inoculum was titered in primary human embryonic kidney cultures. The end-point which was determined after a 19-day incubation period by the method of Reed and Muench(5) was $10^{-8.5}$ $TCID_{50}$ per ml.