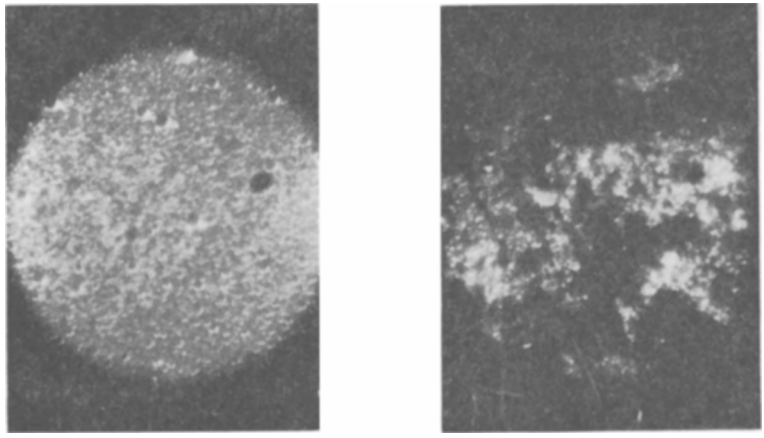
Examples of

A negative reaction

A positive reaction



Protein adsorbed on oil emulsion and normal rabbit's serum

Protein adsorbed on oil emulsion and immune rabbit's serum

FIG. 1.

Streptococcus pyogenes upon droplets of olive oil in emulsion, and that such droplets react with appropriate antiserum in much the same manner as an agglutinogen.

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Further Studies of the Agent in Intestines of Normal Mice Which Induces Encephalomyelitis.

PETER K. OLITSKY.*

From the Laboratories of The Rockefeller Institute for Medical Research, New York City.

It has been shown[1] that normal mice, as well as those affected by spontaneous encephalomyelitis (Theiler's disease—now commonly called "poliomyelitis of mice"[2, 3]), harbor in their intestinal contents an active agent which, after cerebral transfer to normal mice, induces

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1 Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 434.

2 Theiler, M., *Science*, 1934, **80**, 122; *J. Exp. Med.*, 1937, **65**, 705.

3 Iguchi, M., *Kitasato Arch. Exp. Med.*, 1939, **16**, 56.

the characteristic signs of spontaneous encephalomyelitis. It was demonstrated[1] that cross-active immunity exists between the mouse-passaged experimental disease, originally derived from spontaneous encephalomyelitis, and the malady brought about by the active agent found in the intestines of normal mice.[1, 4] In view of this and further evidence already brought to light, it would seem that the latter is a strain of Theiler's virus, or is closely related thereto. It should be added that since that time no definite instance of the spontaneous, paralytic disease has been noted, although more than 4000 normal mice or those injected intracerebrally with a wide variety of different infective agents have been under observation during this period.

In the first paper[1] reference was made to certain properties and epidemiological factors of the transmissible agent occurring normally in the intestinal tract of mice. The present communication will summarize the results of additional findings on the characteristics of this intestinal incitant of encephalomyelitis.

Methods. The methods employed were the same as those already reported.[1] In all experiments 8 to 10% of intestinal contents ground in broth and filtered through "V" Berkefeld candles was prepared and of the filtrate 0.03 cc was injected intracerebrally† into each of a selected number of 30-day-old mice.[1] The encephalomyelitis which developed was determined as specific by transfer of suspensions of bacteria-free cerebral tissues to fresh mice, or the presence of characteristic pathological changes resembling those of Theiler's disease, or by both methods.

Throughout the past year and at different periods within that time, we have succeeded in finding evidence of the presence of the active material in the intestinal tract of every normal mouse or groups of mice (in experiments with pooled material) examined for this purpose providing the animals were young adults. The results summarized in Table I serve to demonstrate this finding. Thus, in 18 different experiments, the intestinal contents of 60 mice, filtrates of which[1] were injected intracerebrally into a total of 130 fresh mice, were found to be characteristically active in every trial. It would, therefore, appear that normal mice of a certain age (Rockefeller Institute strain of albinos were employed throughout this study) harbor this agent invariably in their intestinal contents.

In 2 recent experiments, all of 8 control mice used in each test developed the experimental affection after receiving, intracerebrally,

[4] Theiler, M., and Gard, S., personal communication.

† Full ether-anesthesia was employed in operations on mice.

TABLE I.
Age at Which Virus Can Be Detected in Intestinal Contents of Normal Mice.

Age	Experiment	No. of mice used as source	Contents injected cerebrally: No. of mice	Contents injected cerebrally: No. showing encephalomyelitis	Remarks
Fetus	A	6	8	0	Mothers of A and B groups pregnant about 16 days when fetuses removed. Virus in mothers' intestinal contents
"	B	8	8	0	
4 hr		4	8	0	
24 "	A	5	8	0	Mothers of A and B groups, virus in intestinal contents
" "	B	5	8	0	
2 days		3	8	0	
5 "	A	5	8	0	Mothers of A and B groups, no virus in pooled intestinal contents; their age, 6 months or older (see below for record of older mice)
" "	B	5	8	0	
" "	C	4	10	0	
12 "	A	5	8	0	Mothers of A and B groups, virus in intestinal contents
" "	B	5	8	0	
20 "	A	4	8	0	
" "	B	4	8	7	
" "	C	4	10	0	
" "	D	4	16	1	
25 "	A	4	8	7	
" "	B	4	8	1	
30 "		60	130	123	Summary of 18 different experiments (usually pooled 3 mice for each) and all positive
6 months or older	A	3	8	2	All females injected
	B	3	8	2	" males "
	C	2	4	2	
	D	2	8	3	
	E	2	7	0	
	F	2	8	5	

filtered intestinal contents which had been derived from normal mice. On the other hand, only 3 of 8 mice, in both tests, given suspensions of the corresponding intestinal walls (7-8% concentration) exhibited encephalomyelitis. The walls in the first trial were washed in tap water for 2 minutes and in the second, for 1 hour in 4 changes of saline solution (80 cc for each washing). We have thus confirmed an earlier observation on the activity of intestinal walls.[4] Further work is in progress to define more exactly whether suspensions of walls are disease-producing because of the adherence to the sides of the contents of the lumen, or because the active agent is located within the walls themselves.

Age in Relation to Presence of the Intestinal Agent. It will be

seen from Table I that the active agent is not demonstrable in the intestines of fetal mice, nor at later periods of life, until some time after the 12th day of age—and the negative results occur even when mothers harbor it in their own intestinal contents. Thereafter, on the 20th day of age, it is detectable irregularly, until the 30th day, when it is invariably found. In old mice, on the other hand, it is still recoverable but again irregularly.

Normal mice, therefore, apparently harbor the active agent in their intestinal contents beginning at some short period after weaning. Experiments thus far made indicate that mothers' milk contains no substance that neutralizes the effects of the agent (equal parts of fresh milk, expressed on the 5th day of nursing, plus a suspension of 5% active brain, induced disease in all of 8 mice injected intracerebrally with the mixture kept at room temperature for 30 minutes—a similar response having been obtained in control mice in which broth replaced the milk). In addition, the milk by itself (20% in broth) failed to reveal the presence of any specific activity. It was shown previously[1] that feeding active brain-material failed to induce disease and in a recent experiment, the same food serving as rations for the mice after weaning, when mixed and suspended in tap water and filtered, the filtrate then being inoculated intracerebrally into 30-day-old mice, likewise did not bring about the experimental malady. Further work is being considered for elucidation of the problem which is: How does the active agent get into the intestines, and why does it become detectable shortly after the suckling period is over?

It is of interest that older mice, *i. e.*, those 6 months of age or older, apparently harbor less of the intestinal agent than do young adults. This circumstance may be correlated with the finding of Theiler[2] that old mice are resistant to intracerebral inoculation of his virus. In our hands, 1:10 dilutions of brains of mice having encephalomyelitis, induced by cerebral injection of the intestinal agent, brought down with disease 10 of 10 30-day-old mice; 7 of 9 4-months-old, and 6 of 10 6-months-old ones. Whether this is due to prolonged carriage of the agent in the intestines, giving rise to an immune response, or to some other factor, is yet to be determined.

It was also shown that pregnant mice, artificially infected in the same way as the old animals just mentioned, developed encephalomyelitis 1, 5, and 12 days after the births of litters. The litter-siblings (total 15 young) were tested for the presence of the intestinal agent at birth and at 2, 5, and 20 days of age. The intestinal contents of 3 or 4 mice were pooled in each age group. Only the 20-day-old animals

were positive. Since in this series no such agent was found until the young reached 20 days of age (Table I), it would appear that they were not affected by the presence of the active agent in their mothers during pregnancy, either as a result of artificial, intracerebral inoculation, or, as already mentioned, of their harboring it naturally in their intestines.

Absence of Agent in Brains of Normal Mice. The brains of 32 normal mice, the intestinal contents of which yielded the active agent, failed to exhibit any specific effect on intracerebral passage to 52 normal mice. In other words, while the encephalomyelitis-producing agent can be found invariably in the intestinal tract of normal mice of a certain age, their brains remain free from it.

Conclusions. Certain additional characteristics have been described of the active agent which is present in the intestines of normal mice and which can induce encephalomyelitis indistinguishable from that of the spontaneous Theiler's disease. Among other findings, it was ascertained that, irrespective of whether mothers harbor virus (by means of artificial, cerebral inoculation or naturally in their intestinal contents), the incitant is not detectable in the intestines of their young from the fetal stage to 12 days of age, but it is demonstrable at the age of 20 days or older. Moreover, at 30 days of age, practically every mouse that has come under observation has yielded specifically active intestinal contents. By some mechanism not as yet understood, old mice harbor less of the agent than do young adults. Finally, in the latter age-group, while the mice carry regularly the active substance in their intestines, it is not at all recoverable from their brains.

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Sleep Induced by Subthalamic Lesions with the Hypothalamus Intact.

H. R. MILLER AND E. A. SPIEGEL.

From the Department of Experimental Neurology, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia, Pa., and Montefiore Hospital, New York City.

The sleep-producing effect of hypothalamic lesions has been convincingly demonstrated (Ranson[1]). There are, however, still many unsolved problems which bear upon the central mechanism of the

1 Ranson, S. W., *Arch. Neur. and Psych.*, 1936, **35**, 1175; 1939, **41**, 1.