Further Modification of the Mouse Adapted Type III Poliomyelitis Virus. (20466)

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Recently, Li and Habel(1) reported the adaptation of poliomyelitis Type III (Leon) virus to mice, employing the intraspinal route (2). This has been confirmed by Casals *et al.*(3). The virus was pathogenic only after intraspinal inoculation and could be recovered from the spinal cord, but not from the brain. Although the incubation period was relatively short, usually 4-10 days, it was inconstant. In addition, the low virulence of the virus limited its usefulness in laboratory and field studies. For this reason attempts were made to increase its virulence for mice by a number of procedures, including the use of cortisone, varying host ages and strains, and exposure of mice to cold before and after inoculation. None of these was successful. With consecutive mouse passage, however, despite some fluctuation, the virulence gradually increased. This was particularly obvious after 69 passages, when the incubation period was sharply reduced.

Materials and methods. The mouse adapted Type III (Leon) virus, originally reported by Li and Habel(1), was brought to this laboratory in its 54th passage. Consecutive passage in mice was then made by the intraspinal route with 10% or 20% cord suspension derived from paralyzed mice and inoculated into 4- to 6-week-old CFW mice.

Hyperimmune serum was prepared in monkeys with adjuvants following the procedure of Ward *et al.*(4) against Type I (Brunhilde), Type II (Lansing), and Type III (Leon) monkey propagated stock viruses. The technic for tissue culture utilized in this study was described previously(5) and the technic of neutralization tests is described elsewhere(6).

Experimental. A number of methods were employed in an effort to increase the virulence of the virus, but it appears that this occurred spontaneously with consecutive mouse passages, and notably after the 69th passage. Two obvious changes in the behavior of the virus took place. These were an increase of the titer of the virus in the mouse cord and a shortening and fixing of the incubation period (Tables I and II).

TABLE I. Comparison of Titration of Earlier and Later Passage Mouse-Adapted Leon Virus.

Passage	Dilution of virus 10^{-1}	10^{-2}	10^{-3}	10^{-4}
Pool of mouse passage 20, 21, 22	4/8*	2/17	0/8	
Pool of mouse passage 72, 73, 74	11/12	6/11	5/11	0/12

* No. mice paralyzed/No. inoculated.

In the early passages, the inoculation of a 20% mouse cord suspension did not paralyze all the mice. When such a suspension was mixed with serum and .02 ml of the mixture was inoculated intraspinally into mice, the PD_{50} was less than one log. It was not suitable, therefore, for neutralization tests. In the later passages, the titer increase to $10^{-2.5}$ to $10^{-3.0}$ so that the inoculation of a mixture of 20% virus suspension and serum represents 30-100 PD_{50}, a dose more adequate for reliable and reproducible results. In the early passages the incubation period was not constant and varied from 4 to 10 days or longer, while in the later passages the incubation period not only was shortened but also became more constant. An occasional shortening of incubation period to 2 days was first observed on the 69th passage.

In carrying out neutralization tests on about 110 human serum specimens with this virus, entirely clear-cut results were obtained (6). Out of 587 mice inoculated in these tests, 435 became paralyzed and of these more than 80% were paralyzed within 7 days after inoculation. This characteristic of the modified virus enhances its usefulness in laboratory and field studies.

Besides the 2 main changes noted, the virus can now occasionally infect mice by the intra

TABLE II. Comparison of Incubation Period of Earlier and Later Passage Mouse-Adapted Leon Virus.

Passage	Suspension, %	Paralysis	Incubation period	Avg incub. period (days)
55	20	17/22*	3^2†, 4^2, 5^4, 6^4, 8^4, 9^1	5.8
56	20	13/18	4^4, 5^4, 6^2, 7^1, 10^2	5.7
69	10	7/9	2^1, 3^2, 4^1, 5^1, 6^1, 8^1	4.1
70	10	11/11	2^1, 3^8, 4^7	3.5
74	10	8/8	3^8	3.0

* No. mice paralyzed/No. inoculated. † 2 mice paralyzed on 3rd day.

TABLE III. Neutralization Tests with Modified Mouse-Adapted Leon Virus against Monkey Immune Sera.

	Serum of monkey immune to types							
	I Brunhilde		II Lansing		III Monkey Leon		Normal monkey serum	
	Undil.	1/10	Undil.	1/10	Undil.	1/10	Undil.	1/10
Paralysis*	8/9	9/10	10/10	9/9	0/10	3/10	9/10	9/9

* No. mice paralyzed/No. inoculated, observed 11 days.

cerebral route. This was also recently observed by Habel(7). The serologic specificity of the virus has not been altered. It is still specifically neutralized by Leon immune serum but not by other antipoliomyelitic sera (Table III).

The original Leon virus had a high degree of virulence for the monkey. With passage through mice the virulence appears to be decreasing. A 20% mouse cord suspension of the 73rd passage was inoculated into 2 adult rhesus monkeys by the intracerebral route. One of them became paralyzed on the 14th day, showing typical histopathological lesions in the cord. The other remained asymptomatic for an observation period of over 45 days when it was sacrificed and showed no histopathologic lesions in the CNS. Virus from mouse cord in the 73rd mouse passage was cultivated in monkey testis tissue culture. After 6 serial passages in this tissue culture, the tissue culture fluid was inoculated intracerebrally as well as intranasally to 2 rhesus monkeys. One remained well for 30 days; the other showed slight weakness of the left leg in the second week after inoculation but recovered rapidly. Histopathologic examination of this monkey, sacrificed on the 30th day after inoculation, revealed the typical lesions of poliomyelitis in recovery phase.

A rather interesting observation involving virulence was made in the course of study with this virus. When the virus from infected mouse cord suspension was transferred to monkey testicular tissue culture, cellular degeneration occurred in 4-9 days. However, when the pooled fluid harvested successively within a period of 3 weeks or ground tissue fragments from such infected cultures were inoculated into mice intraspinally, the virulence was lost entirely or was greatly reduced (Table IV). This occurrence is in distinct contrast with the behavior of the mouse-adapted Type I (Mahoney) virus which when grown in monkey testicular tissue culture infects mice quite readily(8). Further studies with respect to the virulence of this virus are in progress.

Summary. A further modification of the mouse-adapted Leon virus is described. With successive mouse passages the virulence for

TABLE IV. Virulence Loss of Mouse-Adapted Leon Virus after One Tissue Culture Passage.

Mouse passage No.	Inoculated into:		Paralysis	Avg incubation period
P55	mice		6/10	7.5
P55	t.c.*	mice	1/9	7.0
P56	t.c.*	"	0/10	0
P72	mice		6/8	3
P72	t.c.†	"	2/8	7
P73	t.c.‡	"	0/10	0
P79	mice		64/72	3
P79	t.c.*	"	4/40	5.2

* Undiluted fluid of tissue culture inoculated to mice.
† Tissue fragments inoculated to mice.
‡ Six consecutive passages in tissue culture.

mice has spontaneously increased and the incubation period has become shorter and more constant. Passage from mice to tissue culture results in loss or marked reduction of virulence for mice. This modified virus can be employed quite satisfactorily for serum neutralization tests and makes available an additional field and laboratory tool for poliomyelitis research.

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Received June 29, 1953. P.S.E.B.M., 1953, v83.

Effect of Cold and Restraint on Blood and Liver Non-Protein Sulfhydryl Compounds. (20467)

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Beck and Linkenheimer(1) have reported a drop in concentration of liver non-protein sulfhydryl (glutathione) in mice exposed to cold and indicated that this fall in concentration was not due to the fall in body temperature which occurred(2). In the present studies 2 groups of rats (restricted and unrestricted) were exposed to cold. Liver and blood ergothioneine (ESH), glutathione (GSH), and total non-protein sulfhydryl (TSH) concentrations were compared with values for control animals.

Methods and materials. Healthy adult Sprague-Dawley rats were used: The 21 males (250-300 g) and 21 females (180-250 g) were divided into 3 groups each: 7 controls exposed at 22°C, 7 unrestricted exposed at 0°C, and 7 restricted exposed at 0°C. The control rats were housed in regular laboratory cages until time of sacrifice while the latter 2 groups were placed, for periods varying between 2 and 4 hours, in a refrigerator set to maintain a temperature of 0°C ± 2°C. The duration of exposure was determined by the time necessary for the rectal temperatures of the restricted animals to fall to between 15 and 25°C. Restraint was produced by means of a loose fitting wire mesh cylinder. All the animals were stunned with a blow on the head, blood obtained with a cardiac puncture and liver excised immediately and frozen with dry ice. ESH was determined by a modified method of Hunter(3); GSH by a modification of the method of Grunert and Phillips(4) and TSH by amperometric titration using a modification of the method of Benesch and Benesch(5). Details of the modifications will be published(6).

Results. As shown in Table I, no significant change in GSH or TSH concentration in the blood of rats was produced by restraint or cold. There was a slight drop in ESH. Similar treatment produced no change in ESH concentrations of the liver. However, there was a significant drop in the concentration of GSH and TSH in the livers of the restricted animals as compared to the control animals and to those which were maintained unrestricted in the cold. The fall in concentration of both GSH and TSH of the liver was more marked in the females than in the males under the conditions of the present experiment.

Discussion. The data showing a decrease in liver TSH in animals maintained in the cold are in agreement with the work of Beck and Linkenheimer(1,2). They measured only