THE SUSCEPTIBILITY OF THE HAMSTER TO MOUSE ENCEPHALOMYELITIS VIRUS

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Only the mouse has been shown to be susceptible to the original (1) strains of mouse encephalomyelitis. The FA and GDVII (2) strains have a wider host range although they, too, have been found to occur naturally only in the mouse. When they have been detected in other species the circumstances suggested infection from mice (3). In our experience virus has never been recovered from normal hamsters nor has hamster serum reacted with Theiler viruses. Indeed it is frequently used in this laboratory as a negative control. On the other hand, in experiments reported some years ago (4) indirect evidence was secured of infection of hamsters without manifest signs and this has now been investigated. It has been learned that unmodified strains do cause inapparent infections and that strains experimentally manipulated may become fully pathogenic, capable of inducing both paralysis and encephalitis.

EXPERIMENTAL

Methods.—The virus used in the present experiments, strain 4727, was isolated from the intestinal contents of a mouse from the laboratory-bred colony in 1945 and has been extensively used in mouse experiments. There has been no change in the signs of infection it induces, which are those Theiler originally described (1), although the mouse infectivity titer has increased. The estimated median effective dose of mouse brain is now approximately 10^{-5}. A stock 5 per cent mouse brain suspension of virus in 0.85 per cent salt solution containing 10 per cent beef infusion broth has been employed in neutralization tests, the suspensions being customarily stored at −70°C. until used. All the suspensions have been cultured aerobically by streaking blood-agar plates. The neutralization tests have been performed by mixing equal parts of twofold dilutions of the virus and serum and incubating the mixtures for 3 hours at room temperature. The inoculations have been intracerebral. The use of twofold dilutions and the moving averages method (5) for estimating the median effective dose have been used to analyze the results of tests.

The hamsters (*Mesocricetus aureatus*) were from the laboratory colony. The mice were albinos of the Albany standard strain. The hamsters were observed for 30 days, the mice for 35 days except for neutralization and titration tests for which 28 and 21 day periods have been adopted.

Survival of Virus in Suckling Hamsters.—The initial experiments were made to determine whether virus survives in the brains of immature hamsters inoculated intracerebrally (Table I). Two litters were used, one 4 and the other 9 days old. Each animal was inoculated with a 20 per cent suspension of mouse

brain virus. Thereafter, at intervals of 2 or more days, a single hamster was killed and its brain removed and tested for the presence of virus by the inoculation of 10 to 12 gm. mice. The first litter was sampled on five occasions between the 3rd and 14th days. Virus was present up to and including the 10th day. The second litter was sampled from the 3rd to the 30th days and virus found as late as the 14th day. None of the hamsters exhibited any signs of illness.

Serial Passage in Hamsters.—The next experiment was planned to determine whether the pathogenicity of the virus could be increased by serial, blind passages in suckling hamsters (6). Animals were inoculated as before, sacrificing each generation, one or more litters, on the 4th or 5th day. Parts of the brain and cord were preserved for histologic examination; the remainder

TABLE I

Survival of Virus and Occurrence of Lesions in Suckling Hamsters Inoculated with Mouse Encephalomyelitis Virus

Days	Response of test mice		Lesions of CNS of hamsters
	Litter 1	Litter 2	
3	9/10	10/10	None
5	9/9	11/11	Acute encephalomyelitis
7	10/10	10/10	None
10	8/10	1/8	Scanty meningitis
14	0/10	2/8	Scanty meningitis
22		0/9	None
30		0/10	Scanty meningitis

The denominator indicates the number of mice inoculated with hamster brain suspensions, the numerator, the number paralyzed or dead.

was suspended in four parts of broth-saline and inoculated into the next generation of hamsters. Each inoculum was also tested in groups of young mice. Nine passages were made. In none of the 103 hamsters was paralysis seen. Virus was present throughout five generations. All the test mice inoculated with hamster brain of the first four generations were paralyzed. Brains of the last four generations induced paralysis in but one of the forty test mice. The progressive loss of infectivity was paralleled by the disappearance of lesions which were present in the second and third generations but not after the fifth passage (Table II).

These results confirmed the previous experiment and supplemented it by showing that strain 4727 was capable of causing lesions in suckling hamsters. The method failed to adapt the virus to hamsters possibly because infectivity titer was not maintained.

Alternate Hamster-Mouse Passage.—Since preliminary experiments had

indicated that infectivity of the hamster passage virus for mice could be maintained by alternating the species we next undertook to alternate mouse and hamster passage, testing for the presence of virus and lesions as before.

The hamsters were litters between 4 and 11 days of age and the mice, usually ten in number, were 10 to 12 gm. albinos of the Albany standard strain. The inoculum was uniformly 0.03 ml. of a 20 per cent brain suspension. Some of the suspensions were prepared and injected immediately upon harvesting the brains and others after storage in 50 per cent glycerol. The hamsters were sacrificed 5 to 7 days following inoculation during the first three generations and when paralyzed in the subsequent generations. The mouse brains were harvested from the first mice of each group to succumb.

TABLE II

Blind Passage of Encephalomyelitis Virus in Immature Hamsters

Passage	Response of hamsters	Mouse infectivity of hamster brains	Lesions in hamsters	
			CNS	Spinal muscles
1	0/15	9/9	Acute encephalomyelitis	+
2	0/15	11/11	Acute encephalomyelitis	+
3	0/6	—	None	+
4	0/14	9/9	Scanty meningitis	
5	0/12	3/9	None	+
6	0/17	0/10	None	
7	0/10	1/10	None	
8	0/14	0/10	None	
9	—	0/10	None	

The denominator indicates the number of hamsters or mice inoculated, the numerator, the number paralyzed or dead.

All the mice became paralyzed. The uniformly high infectivity of the hamster brains was verified by titrations in mice (Table III). No signs of infection were observed in the first two hamster generations but the third passage seemed to introduce a change. One of the two litters that had been inoculated grew poorly and a number of the animals died. The brain chosen for passage proved to have a slightly lower mouse titer but six of eight immature hamsters inoculated with it nevertheless became paralyzed. This was the first appearance of paralysis. It occurred regularly in each of the six subsequent generations. In the fifth and sixth hamster passages, all the animals exhibited symptoms of encephalitis as well. This appeared on the 5th post-injection day and was followed in a third of the animals by paralysis. The non-paralyzed survivors were stunted.

Thus there were two obvious reasons for suspecting that the virus had been modified, the development of paralysis and subsequently and independently the appearance of encephalitis. Further evidence of alteration was secured

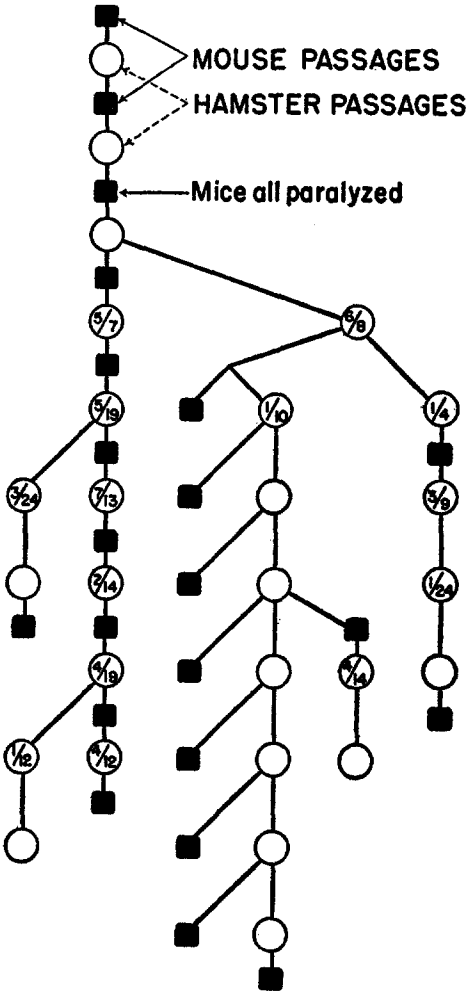

FIG. 1. Modification of mouse encephalomyelitis virus by alternating mouse-hamster passages. The numerators show the number of test hamsters that were paralyzed. The empty circles indicate silent infection. Note the rapid loss of pathogenicity following serial hamster transfers and its restoration by a single mouse passage. Note also the persistence of mouse infectivity throughout nine serial hamster passages.

by serially transferring the virus in hamsters. Whereas the original strain died out after four or five hamster transfers the modified virus persisted indefinitely. Fig. 1 illustrates some of the tests, including the successful transmission of the agent through nine hamster transfers. It may be noted that six of these were without signs of disease but that paralysis was again induced

by a single mouse passage. The restoration of pathogenicity by a single mouse passage occurred whenever tried, on all of four different occasions.

Table III lists the results of tests for infectivity titer of mouse and hamster brain suspensions from various stages of the experiment. Infectivity for mice remained high throughout five serial hamster passages. Brains of the test mice of the fifth hamster generation induced paralysis in four of fourteen young hamsters. The sixth serial passage in hamsters, completely without

TABLE III

Mouse-Infectivity Titer of Strain 4727 before and after Mouse Passage, Alternating Mouse-Hamster, and Serial Hamster Passages

	Hamster brain dilutions tested											
	1/1000	1/2000	1/4000	1/8000	1/16,000	1/32,000	1/64,000	1/128,000	1/256,000	1/512,000	1/1,024,000	$m(10^5)$*
At beginning of experiment	12/12	10/12	9/12	12/12	6/12	9/12	4/12	—	—	—	—	2.9
After 5 mouse passages	8/8	8/8	8/8	7/8	7/8	8/8	8/8	8/8	3/8	2/8	—	0.4
After 9 mouse passages	8/8	8/8	8/8	7/8	7/8	3/8	6/8	4/8	1/8	0/8	2/8	1.1
After 4 hamster mouse passages	6/6	6/6	6/6	5/6	4/6	6/6	6/6	4/6	2/6	—	—	0.5
After 9 hamster mouse passages	6/8	6/8	7/8	4/8	0/8	2/8	1/8	1/8	3/8	0/8	—	14.04
After 5 hamster passages of the hamster-adapted strain	8/8	5/8	3/8	4/8	1/8	1/8	0/8	0/8	—	—	—	25.0

The denominator indicates the number of test mice inoculated with the particular dilution, the numerator, the number paralyzed or dead.

* Median effective dose (5).

signs of disease, nevertheless induced a sharp antibody response (Table IV), the serum activity equalling that seen in mice hyperimmunized with encephalomyelitis virus.

It was thus evident that alternate mouse-hamster passages had modified the virus of mouse encephalomyelitis. The modified virus differed from the parent virus in its pathogenicity for the hamster, a greater ability to maintain itself throughout serial hamster transfers, and a kind of residual pathogenicity which could be activated by a single mouse passage.

Signs of Encephalitis in Hamsters.—In our experience with the OT strain of mouse encephalomyelitis, encephalitic signs have been rarely seen. With

strain 4727, mice develop only flaccid paralysis, usually of the hind legs. The shortest incubation period is 6, rarely 5, days with additional animals becoming paralyzed in subsequent weeks. The longest incubation period we have observed was 44 days.

A comparison was made of the incubation periods in 114 paralyzed mice and 38 paralyzed hamsters inoculated with the suspensions used in the alternating hamster-mouse passages (Fig. 2). The incubation period of the mice varied

TABLE IV

Development of Serum Antibodies in Hamsters Inoculated with Hamster-Adapted Mouse Encephalomyelitis Virus without Obvious Response

Serum	Response of test mice to indicated virus dilutions plus serum or broth salt											$m(10^8)$
	1/250	1/500	1/1000	1/2000	1/4000	1/8000	1/16,000	1/32,000	1/64,000	1/128,000	1/256,000	
Sera of inoculated hamsters (30 days)*			3/8	5/8	3/8	2/8	1/8	1/8	—			100
Sera of normal hamsters			8/8	8/8	7/8	4/8	6/8	3/8	—			5.3
Broth salt			8/8	7/8	6/8	6/8	5/8	2/8	1/8			5.5
Sera of inoculated hamsters (30 days)‡	8/8	7/8	4/8	2/8	3/8	0/8	0/8	0/8	0/8	0/8	—	84.14
Convalescent hamster sera (30 days)§	7/8	3/8	2/7	1/8	4/8	2/8	0/8	0/8	0/8	0/8	—	193.2
Normal hamster sera	—	8/8	7/7	6/8	6/8	3/8	2/7	0/8	0/8	1/8	—	14.26
Broth salt	—	—	8/8	4/8	4/8	5/8	3/8	2/8	1/8	0/8	1/8	12.50

The denominator indicates the number of hamsters or mice inoculated, the numerator the number paralyzed or dead.

* The sixth serial hamster passage of modified virus.

‡ The third serial hamster passage of modified virus.

§ The sixth alternate mouse-hamster passage of modified virus.

from 5 to 18 days with a mean of 9.1 days. The hamsters were paralyzed between the 5th and 9th days with a mean of 6.1 days. Of the thirty-eight paralyzed hamsters, twenty-eight were paralyzed in one or both hind legs and six in the fore legs, roughly the same as occurs in mice.

The lesions in mice are essentially the same as those described by Olitsky and Schlesinger (7) but the lesions in hamsters differ greatly in degree. The reaction in the brain and brain stem resembles that seen in mice but the punctate, destructive lesions of the anterior horns of the spinal cords of mice have rarely been duplicated in hamsters despite the frank, flaccid paralysis which has been so common. On the other hand, changes have been regularly found

in the striated muscles about the vertebral column. The muscle lesions consist of hyaline degeneration shortly followed by intensive regeneration of young muscle cells and the appearance of endothelial phagocytes. Such lesions have been found in all the hamsters in which virus was demonstrated whether paralyzed or not, as well as in paralyzed mice. Similar lesions have been noted in hamsters inoculated with MM virus and in mice infected with Theiler viruses by Pappenheimer (8) whose preparations we have examined.

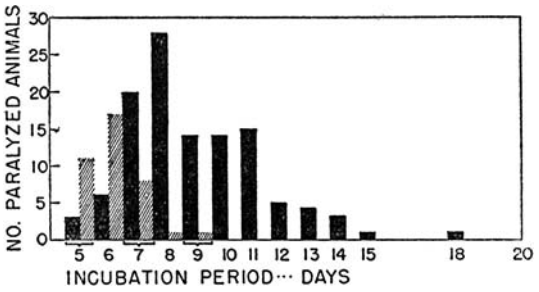

FIG. 2. Incubation periods of paralysis in mice (solid columns) and hamsters (shaded columns) inoculated with hamster-adapted mouse encephalomyelitis virus.

TABLE V

Neutralization of Hamster-Adapted Virus by Normal and Immunized Mouse Sera

Serum	Response of test mice							$m(10^8)$
	1/1000	1/2000	1/4000	1/8000	1/16,000	1/32,000	1/64,000	
Normal mouse 10/7/47	6/8	3/7	1/8	0/8	0/8	—	—	59.98
GDVII mouse 3/11/47	5/8	2/8	2/8	0/8	0/8	—	—	75.86
FA mouse 5/27/47	4/8	6/8	0/8	1/8	0/8	—	—	79.43
Broth salt	—	—	6/8	7/8	7/8	2/8	2/7	3.5

They are similar to those recently reported in mice inoculated with a newly isolated and unidentified agent (9). Histologic studies of the muscle lesions and the related nerves are underway.

Identification of the Virus.—The strain of virus used in these experiments is regularly neutralized by adult mouse serum, the titer of which increases with the age of the animals. The hamster-passaged strain induces similar antibodies (Table V). Moreover, the behavior and anatomical response of mice inoculated with either the parent or hamster-passaged strain is unchanged. These observations lead us to believe that the virus in hamsters and mice is the same.

DISCUSSION

Baker has recently called attention to the ease with which certain viruses may be adapted to resistant hosts by alternately passing them through a susceptible species (10), adapting rinderpest and hog cholera to the rabbit in this way. Koprowski, James, and Cox have also used the method and refer to earlier studies (11). The present results are a further successful application of this principle. The principle is doubtless useful in the manipulation of viruses and one is tempted to speculate on its occurrence and epidemiologic importance in nature.

A particularly revealing observation was made by Coffey while studying the characteristics of vaccinia during repeated transfers through chicken embryo medium (12). Coffey noted that the titer of her strain of vaccinia diminished rapidly during serial tissue culture transfers but could be promptly restored by two rabbit passages. Following such passages the loss of titer in tissue culture transfers decreased more slowly suggesting that the rabbit passages had altered the virus. This seems to be of the same nature as the changes noted in our experiments in which the adapted strain has been repeatedly restored to its initial pathogenicity by a single mouse passage, that pathogenicity being thereafter slowly lost by transfer in hamsters.

Our results also have something in common with those of Hirst (13). Hirst followed the mouse pathogenicity and egg infectivity of a strain of influenza virus while adapting it to mouse lung. Infectivity titers were uniformly high throughout, but pathogenicity titers slowly increased from generation to generation. In the case of mouse encephalomyelitis, mouse infectivity has likewise been relatively constant throughout the hamster generations but pathogenicity developed only after several transfers. Hirst suggested that several generations were necessary for the adapted, pathogenic strain to outgrow the parent, non-pathogenic strain.

Reference should also be made to the studies of Jungeblut, Feiner, and Sanders (14) who worked with Columbia SK virus. The adaptation of this agent to mice was effected through blind cotton rat passages. Its further adaptation to guinea pigs appeared to depend on the mouse passages used for transfer. Thus the third to twelfth mouse generations were not infective for guinea pigs while the seventieth to 162d generations were. This pattern was repeated in later generations and suggested fluctuations in the virulence of the agent so far as guinea pigs were concerned. Whether cyclic variations played a part in our experiments cannot be determined.

It is also of interest that hamster-adapted OT mouse encephalomyelitis virus is capable of inducing encephalitic signs which are rarely seen in mice inoculated with the OT type of virus but are so characteristic of the FA strain. The results suggest that the capacity to induce flaccid paralysis and encephalitis is inherent in both strains, and that these signs represent rather superficial

differences. Our experience with MM virus in hamsters supports this view since central nervous system inoculations of MM virus characteristically induce encephalitis, while the same suspensions cause flaccid paralysis if given intraperitoneally (4) or by gastric intubation.

Sanders observed (15) a change in the Lansing strain of poliomyelitis virus after repeated hamster passages. The titer of the virus rose rapidly when the agent was transferred to suckling hamsters. Adult and infant hamster strains were identical but were not neutralized by pooled human sera and were but irregularly neutralized by convalescent poliomyelitis monkey serum, all of which did neutralize the original mouse-adapted virus. It appeared "not impossible that our hamster virus represents a poliomyelitis-like agent of hamsters." The possibility cannot be dismissed. It seems an unlikely explanation of our own results since our hamster colony has been so carefully watched and tested for latent infections of this kind and because the virus did not change its behavior in the mouse. The neutralization tests are unfortunately incapable of distinguishing between the OT and FA strains. Sanders' results may possibly be explained as a change in antigenic pattern comparable to those produced by Hirst in influenza viruses. Whether such changes occur in mouse encephalomyelitis viruses is not known but relationship between them and poliomyelitis virus has been reported (16).

The short incubation period in hamsters may be due to species or to a difference in the immune status of the host, since the Albany mice are almost uniformly infected with the OT strain of encephalomyelitis virus and develop humoral antibodies while laboratory-bred hamsters are uninfected. If this is the correct explanation, the observations would be comparable to those of Schaeffer and Muckenfuss (17) who observed a prolongation of the incubation period in monkey poliomyelitis when the virus was mixed with immune serum. The mean incubation period following inoculation with virus–non-immune-serum mixtures was 8.3 days and with virus–immune-serum mixtures, 14.3 days.

CONCLUSIONS

The OT strain of mouse encephalomyelitis virus induces an inapparent infection in suckling hamsters associated with lesions of the central nervous system and skeletal muscles. The virus increases in pathogenicity after alternating mouse-hamster transfers and then induces both paralysis and encephalitis. Pathogenicity is lost through serial hamster passages but is restored by a single mouse transfer.

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