STUDIES IN RODENT POLIOMYELITIS

I. Further Experiments with the Murine Strain of SK Poliomyelitis Virus*

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In 1940, we reported the successful adaptation of the SK strain of human poliomyelitis virus from the *rhesus* monkey to white mice by way of intermediary passage through cotton rats (1). A close relationship between the adapted virus and monkey poliomyelitis virus was suggested by similarities in the symptomatology and pathology of the infection in monkeys and rodents as well as by the trend of serological cross neutralization tests. The SK murine virus, in some respects, resembled the Lansing mouse strain of poliomyelitis virus previously described by Armstrong (2) and the RMV and Philadelphia mouse strains of poliomyelitis virus which were later adapted by Toomey (3). It differed, however, from these other rodent strains in that it combined an extraordinary degree of virulence for mice with little or no pathogenicity for *rhesus* monkeys.

The fact that various murine strains of poliomyelitis virus exhibit divergent properties need cause neither surprise nor concern. What differences there are may readily be accounted for, provided one is prepared to regard the artificially induced transmission of poliomyelitis virus from its natural to an unnatural host not as a simple, straight transfer of virus but as a phenomenon of biological adaptation. In this process different virus strains may lose old and gain new properties in proportion to the extent of the occurring transformation. Thus, with virus only partially adapted, residual virulence for the original host would be retained and blended with low virulence for the new host into a bivalent agent of dual pathogenicity; if, on the other hand, the adaptive process progresses to an ideal end point, the original virulence may conceivably be lost in a total exchange against the newly acquired virulence, leaving a strictly monovalent pathogenic agent.

The SK murine virus, since the time of its isolation, has been maintained in two parallel lines of propagation, *i.e.* by serial transfer in tissue culture and by intracerebral passage in mice. During more than 200 consecutive passages *in vitro* the tissue culture virus has preserved its original potency for mice and,

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at no time, has given rise to paralysis in animals other than mice or cotton rats. The mouse passage virus, on the other hand, during a similar number of animal passages, has gradually increased in virulence for mice from an initial titer of about 1:1 million to a maximum titer of about 1:1 billion dilution activity, as determined by intracerebral tests. When reexamining murine virus of later mouse passages for pathogenicity in other laboratory animals it was found that this virus, after having been transmitted through 70 mouse passages, was capable of producing flaccid paralysis in guinea pigs (4). A detailed description of this guinea pig paralysis will be found in another paper of the current series of communications (5). The present paper offers new data concerning certain properties of murine virus obtained from remote mouse passages which have hitherto not been reported. These data include: first, a reexamination of the pathogenicity of murine virus for *rhesus* monkeys and rabbits; second, an appraisal of age as a factor conditioning natural resistance of mice to infection and reinfection with murine virus; third, an investigation of seasonal fluctuations in the susceptibility of mice to murine infection; and fourth, further studies of the murine virus by means of immunological methods.

Pathogenicity of Murine Virus from Remote Mouse Passage for Monkeys and Rabbits

(*a*) *Pathogenicity for Rhesus Monkeys.*—It will be recalled that murine virus, in its earlier passages, had failed to bring about paralysis in *rhesus* monkeys. However, the available data indicated that the virus, especially when introduced by intracerebral injection, was not altogether harmless for monkeys even though it fell short of producing typical poliomyelitis.

After transmission through 70 mouse passages, the murine virus gave signs of more definite activity in the central nervous system of infected monkeys. Thus, of a group of 13 monkeys injected intracerebrally with brain or cord of paralyzed mice, 1 monkey developed extensive pareses and 5 monkeys passed through a well defined encephalitic syndrome consisting of high fever, coarse tremor, and tonic-clonic convulsions, followed by extreme prostration which proved fatal in two instances. The described symptoms, together with the pathological examination of the two fatal cases, suggest that the infectious agent is capable of localizing in the monkey brain with the production of an acute polioencephalitis. Yet none of these animals can be said to have developed "classical" poliomyelitis if this diagnosis be limited to a disease characterized by spinal localization of the virus with subsequent production of flaccid paralysis. Moreover, the observed process differed from true poliomyelitis in that transmission to new monkeys failed, whereas transfers to mice were positive; furthermore, monkeys which were permitted to survive failed to resist subsequent reinfection, 4 weeks later, with SK monkey poliomyelitis virus.

Additional data concerning the pathogenic potentialities of mouse virus for

rhesus monkeys are available from experiments in which *rhesus* monkeys received a series of repeated injections of large doses of mouse virus by the intravenous route, extending over a period of 5 days. These experiments will be reported in detail elsewhere. Suffice it to state here that in a group of 14 monkeys thus treated there were 2 animals which presented partial or complete flaccid paralysis, strictly limited to the right leg, on the 5th day when the last dose of murine virus was injected. Upon intracerebral reinfection with poliomyelitis monkey virus (0.5 cc. 1:10 dilution of RMV strain) both animals developed renewed paralysis. However, whereas paralysis in one monkey was generalized, involving the arms as well as the legs, the other animal became paralyzed only in the arms, the healthy leg remaining unaffected.

At the time these observations were made it was thought conceivable that repeated intravenous injections of massive doses of virus might serve as a means to transmit poliomyelitis virus directly from the monkey to mice and guinea pigs. Several experiments, however, in which this method was used in an effort to acclimatize the RMV and Aycock strains of monkey poliomyelitis virus to mice and guinea pigs led to negative results.

(*b*) *Pathogenicity for Rabbits.*—One of the outstanding characteristics of the murine virus, at the time of its isolation, was its complete lack of pathogenicity for rabbits. It therefore seemed of interest to reexamine murine virus from remote mouse passages for its capacity to induce paralysis in rabbits.

Five rabbits (1500 gm. weight) were injected intracerebrally with 0.4 cc. of a 10 per cent mouse brain suspension (70th to 100th mouse passage). During a period of careful observation, extending over 3 weeks, none of these animals showed any signs of disease.

The experience of the foregoing experiments may be summarized by saying that prolonged passage of murine virus through mice apparently is associated with an appreciable change in biological properties. This change manifests itself chiefly in an increased potency for mice and, at the same time, in a broader range of virulence for other rodent hosts.[1] The original lack of pathogenicity for rabbits, on the other hand, remained entirely unaffected. As far as its pathogenic power for *rhesus* monkeys is concerned, continued mouse passage seems to have brought about a better ability of murine virus to localize in the central nervous system, particularly the brain, of monkeys following intracerebral injection. The fact that repeated peripheral introduction of massive doses of virus may cause, on occasion, typical flaccid paralysis must also be recognized. The murine virus, therefore, although incapable of producing regularly typical poliomyelitis, must still be regarded as an infectious agent with limited pathogenic properties for the *rhesus* monkey.

[1] It may be mentioned in this connection that murine virus harvested from remote mouse passages produced flaccid paralysis in 7 of 12 albino rats.

Age Resistance of Mice to Infection with Murine Virus

The influence of age on the susceptibility of mice to infection with various neurotropic viruses has been made the subject of recent extensive investigations. The sum total of this work indicates that there develops in growing mice a well marked resistance to peripheral infection which can clearly be demonstrated with such viruses as equine encephalomyelitis (6), vesicular stomatitis (7), and rabies (8); in no case could any difference be detected between young and old mice in their susceptibility to intracerebral injection of virus. It was therefore suggested that the resistance acquired with age in animals which had experienced no previous immunizing contact with the infectious agent is caused by the development and perfection of physiological barriers which serve to arrest centripetal progression of the virus from a peripheral point of injection. The virus of "spontaneous mouse encephalomyelitis" appears to be quite different in this respect since an increased resistance with age is demonstrable in mice irrespective of whether virus is introduced by the intracerebral or the intranasal route (9). This difference is probably due to the fact that the protective mechanism against Theiler's virus represents a specific form of latent immunity which is acquired by previous contact with the particular virus.

Owing to the absence of susceptible small laboratory animals it has heretofore been impossible to study age resistance to poliomyelitis virus by suitable experimental methods; it was therefore decided to investigate this problem in mice infected with murine virus. Swiss mice, representing three different age groups, according to weight, received graded doses of murine virus by intracerebral injection or by various peripheral routes, including intraperitoneal, intranasal, and oral injections. The incidence of paralysis and the average incubation period are given in Table I.

It will be seen from Table I that no appreciable difference exists in the response of the three age groups of mice to intracerebral injection of murine virus as measured by the incidence of paralysis and the average length of the incubation period. The disease thus produced was fatal with remarkable uniformity in all animals. However, when the results of peripheral infection of virus are compared between the oldest group of mice and the two younger groups a marked difference in susceptibility becomes readily apparent. Thus, whereas the incidence of paralysis among the youngest and the intermediate groups, throughout the entire range of virus dilutions, remained at 100 per cent, or close to 100 per cent, for all three peripheral routes (intraperitoneal, intranasal, feeding), the percentage of paralyzed mice in the oldest age group dropped sharply with each successive dilution of virus; in fact, almost half of all older mice survived peripheral infection with the smallest amounts of virus without showing any symptoms of the disease. The above data therefore indicate that the central nervous system of old mice remains fully suscepti-

ble to infection with murine virus but that the advance in age is associated with the development of a relative resistance to peripheral infection which seems to operate best when virus is introduced by the nasal route.

For the second part of this experiment it seemed of interest to determine whether old mice, which had survived infection by various peripheral routes, possessed any increased resistance towards peripheral reinfection with doses of virus large enough to paralyze all normal control animals of similar age. Accordingly, a total of 42 old mice surviving from primary infection by either intraperitoneal, nasal, or gastrointestinal routes, were reinfected intraperitoneally with 0.1 cc. of 10^{-2} or 10^{-3} dilution of murine virus; these amounts of virus, in our experience, have never failed to paralyze old mice (see Table I). In this group of 42 reinfected old mice, only 2 mice developed paralysis, whereas 40 mice remained free from any symptoms of disease. The results of this experiment, therefore, indicate that symptomless murine infection induced in old mice, by subeffective doses of virus, is followed by the development of a strong immunity which protects against peripheral reinfection with the same virus.

TABLE I

Infection with Murine Virus by Various Routes in Mice of Different Age Groups

Route of infection	Virus dilution	12–15 gm. mice			20–25 gm. mice			30 gm. (and over) mice		
		No. of mice	Paralysis	Incubation period	No. of mice	Paralysis	Incubation period	No. of mice	Paralysis	Incubation period
			per cent	*days*		*per cent*	*days*		*per cent*	*days*
Intracerebral	10^{-5}	70	100	2–3	30	100	2–3	42	100	2–4
	10^{-6}	402*	100	2–3	30	100	2–4	36	100	2–5
	10^{-7}	16	100	2–3	30	100	2–4	47	100	2–5
	10^{-8}	16	100	3–4	10	100	3–4	39	92.7	3–5
Intraperitoneal	10^{-2}	23	100	3–4	811*	100	3–4	62	100	4
	10^{-3}	56	100	3–4	196*	100	3–5	65	98.4	4–7
	10^{-4}	59	100	3–4	85	100	3–5	179*	74.4	4–7
	10^{-5}	12	100	3–4	67	100	4–6	25	68	5–7
	10^{-6}	10	100	4–5	30	100	4–6	25	60	5–7
Intranasal	10^{-2}	12	100	3–5	10	100	3–6	25	48	4–7
	10^{-3}	8	100	3–5	10	100	4–7	21	66.7	5–7
	10^{-4}	12	92	4–6	10	70	4–7	15	40	5–7
Feeding (by gavage)	10^{-2}	16	81.2	3–5	10	90	4–6	15	60	4–7

* Unusually large numbers of mice were available in these instances, since the brains of these animals were used for the preparation of immune sera, as described later in this paper.

Seasonal Fluctuations in Susceptibility of Mice to Infection with Murine Virus

As mentioned before, the murine virus, since its isolation, has been maintained by unbroken serial passage from mouse to mouse. These passages were carried out as routine, at the rate of two or three transfers each week, in Swiss mice, weighing from 12 to 15 gm. As a rule, groups of 6 mice were used for each passage, all animals receiving 0.03 cc. of a 10^{-6} dilution of virus (mouse brain suspension) by intracerebral injection. Records which extend over a considerable period of time are therefore available for a continuous study of the infectivity of murine virus under standard conditions of infection. The results obtained during the entire year of 1941 have been brought together in Chart 1.

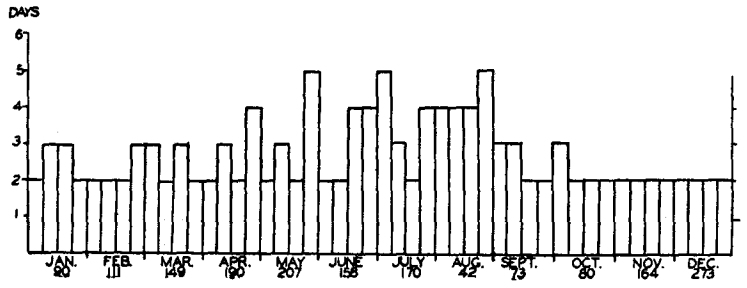

CHART 1. Average incubation period throughout the year 1941 of Swiss mice (12 to 15 gm.) following intracerebral infection with a constant dose of 0.03 cc. of murine virus in a dilution of 10^{-6}. Each column represents the average incubation period observed in 2 to 3 mouse passages carried out within periods of 6 to 9 days. The figures under each month indicate the total number of mice used during that period.

The data assembled in Chart 1 indicate that the disease was uniformly fatal in all mice, there being no survivors among a total of 1707 animals following injection with the chosen test dose of virus. It will be observed, however, that the average incubation period in mice injected at certain times of the year is subject to marked variation. Thus, whereas the incubation period from January to April and, again, from October to December was regularly between 2 and 3 days, somewhat longer incubation periods were noted between May and September, the most definite prolongations (4 to 5 days) occurring during the summer months of June, July, and August. The reasons for this phenomenon are unknown. To mention only one point, it is not certain whether the observed fluctuations should be ascribed to cyclic variations in virulence of the virus or to seasonal changes in susceptibility of the host, although the latter seems more likely.

Immunological Studies with the Murine Virus

(*a*) *Serological Tests.*—Previous experiments (1) had shown that inactivation of murine virus, in its early mouse passages, may be obtained by *in vitro* contact with various antipoliomyelitis sera. The degree of neutralization demonstrable in these tests was only slight in the case of several convalescent monkey sera, but marked neutralization occurred with one poliomyelitis hyperimmune horse serum. Conversely, neutralization tests carried out with antimurine sera prepared in rabbits or monkeys indicated that some of these sera possessed the power to inactivate the homologous (SK) or related (Aycock) strains of monkey poliomyelitis virus while no neutralizing power could generally be demonstrated against the RMV virus. To be precise, of a total of 24 antimurine sera tested against SK or Aycock virus, inactivation of either virus occurred with exactly one-half, *i.e.* 12 sera, whereas the remaining 12 sera showed no virucidal power. Neutralization was therefore demonstrable, but far from regular in these tests.

In view of the irregular results obtained heretofore it was decided to reinvestigate the serological analysis of SK murine virus with new antisera. The following immune sera were tested in mice for neutralizing activity against murine virus: hyperimmune antimurine horse sera,[2] hyperimmune antimurine and anticavian rabbit sera,[2] sera obtained from monkeys convalescing from infection with SK or Aycock poliomyelitis virus, hyperimmune antipoliomyelitis horse sera,[3] hyperimmune anti lymphocytic-choriomeningitis rabbit serum,[4] and normal mouse, rabbit, horse, and monkey sera. The hyperimmune antimurine horse sera were also tested for neutralizing activity against the RMV and the Aycock strains of poliomyelitis virus in monkeys and a hyperimmune antimurine rabbit serum for neutralizing activity against the WE strain[4] of lymphocytic choriomeningitis virus in mice. Finally, hyperimmune antimurine horse serum and hyperimmune antipoliomyelitis horse serum were tested in mice for neutralizing power against the GD VII strain[5] of Theiler's "spontaneous mouse encephalomyelitis" virus.

The preparation of the hyperimmune antimurine sera will be briefly outlined here.

[2] Acknowledgment is gratefully made to Lederle Laboratories for preparation of these sera and for financial support in connection with this phase of the work.

[3] These sera were obtained through the courtesy of Dr. J. A. Toomey from the City Hospital, Cleveland.

[4] This serum and virus were obtained through the courtesy of Dr. J. E. Smadel from The Rockefeller Institute.

[5] This virus was obtained through the courtesy of Dr. Max Theiler from The Rockefeller Foundation.

Three horses (A2050, A2051, A2052) were subjected to the following course of immunization with murine virus: 4 series of injections were administered over a period of 6 months (Feb., 1941, to July, 1941[6]), each series consisting of 4 to 9 injections given at intervals of 2 to 4 days during a 2 to 3 week period. The material used for immunization consisted of a mixture of tissue culture murine virus with 10 per cent mouse brain suspension prepared from paralyzed mice, in a ratio of 3:1 or 2:1. For the first series of injections formolized antigen (0.2 per cent) was used; subsequently untreated antigen was employed. The former was injected in 100 to 200 cc. doses, the latter in doses increasing from 20 cc. to 250 cc. Injections were made by the intravenous, intraperitoneal, and subcutaneous routes, or by combined routes, as was deemed most judicious at the time of each injection. One horse (A2050) died with indefinite symptoms after 16 injections had been given; two horses (A2051, A2052[7]) lived through the entire immunizing period and received approximately 27 injections. After each series of immunizing injections the horses were bled and their sera tested for neutralizing activity.

In addition, three groups of rabbits, each group consisting of 15 animals, were immunized as follows: group A with cavian virus (10 per cent brain and cord suspension from paralyzed guinea pigs), group B with murine virus (10 per cent mouse brain suspensions from paralyzed mice), and group C with tissue culture fluid containing murine virus. The rabbits in groups A and B received an average of 10 to 12 injections, by the intravenous and intraperitoneal routes, in 2 to 3 series of injections. For the first series formolized antigen (0.2 per cent) was injected in 5 to 20 cc. doses, subsequent injections of untreated antigen were given in doses increasing from 2 cc. to 30 cc. Many animals in these two groups died from shock during immunization. The rabbits in group C were treated similarly, except that larger doses of antigen were used, the animals receiving an average of 12 to 18 injections (10 to 50 cc. in the first, 5 to 100 cc. in the second and third series of injections). This material was well tolerated and there were no losses in this group. All surviving animals were bled after each series of injections, the sera pooled according to origin and tested for neutralizing activity.

The results obtained with the various immune sera used for this serological study are brought together in Tables II to VIII. An examination of the data presented shows that the immunization of horses with murine virus led to the development in the serum of a very high virucidal titer against this virus (Tables II and III). Similarly, antisera produced by the immunization of rabbits with either murine or cavian virus were capable of inactivating large

[6] During this time these animals also received, as routine preventative measures, two injections of equine encephalomyelitis chick vaccine and tetanus toxoid.

[7] It should be mentioned that one horse (A2052), 9 days after termination of the last series of murine virus injections, showed stumbling of the front legs. One week later progressive flaccid paralysis of the posterior quarters was noted. The next day this paralysis was complete and the animal was destroyed. No virus pathogenic for mice, guinea pigs, or monkeys could be recovered from the brain or cord. The cause of the paralysis in this horse was unknown.

amounts of murine virus. On the other hand, a hyperimmune serum against the virus of lymphocytic choriomeningitis was as completely devoid of neu-

TABLE II

Titration in Mice of Successive Trial Bleedings Obtained from Horses under Immunization with Murine Virus against a Constant Test Dose (10^{-3}) of Murine Virus

Serum	Virus dilution	Serum dilutions						
		Undiluted	1:10	1:20	1:50	1:100	1:500	1:1000
Horse A2050								
1st trial bleeding	10^{-3}	0/3	2/3	3/3	2/3	3/3		
2nd " "	10^{-3}	0/3	0/3	2/3	2/3	3/3		
3rd " "	10^{-3}	0/3	0/3	0/3	1/3	1/3		
Horse A2051								
1st trial bleeding	10^{-3}	1/3	2/3	3/3	3/3	3/3		
2nd " "	10^{-3}	0/3	0/3	1/3	0/3	1/3		
3rd " "	10^{-3}	0/6	0/3	0/3	0/6	0/6	0/3	
4th " "	10^{-3}	1/3			0/3	0/3	1/3	
Horse A2052								
1st trial bleeding	10^{-3}	0/3	1/3	1/3	3/3	3/3		
2nd " "	10^{-3}	0/3	1/3	0/3	2/3	1/3		
3rd " "	10^{-3}	1/6	0/3	0/3	0/6	1/6	0/3	
4th " "	10^{-3}	0/3			0/3	0/3	0/3	
5th " "	10^{-3}	0/7	1/4		2/7	1/7	3/7	4/4
Normal horse serum 1	10^{-3}	3/3						
" " "	10^{-4}	3/3						
" " "	10^{-5}	2/3						
" " "	10^{-6}	1/3						
Normal horse serum 2	10^{-3}	3/3						
" " "	10^{-4}	3/3						
" " "	10^{-5}	1/3						
" " "	10^{-6}	1/3						

Numerator = number of mice paralyzed. Denominator = number of mice injected.

Technique.—In preparing serum-virus mixtures, 0.5 cc. of virus dilution was added to 0.5 cc. of undiluted or diluted serum; after incubation for 1 hour at 37°C., 0.2 cc. of each mixture was injected intraperitoneally into each of 3 or more mice. The mice were observed routinely over a period of 10 to 14 days; following injection of non-neutralizing mixtures paralysis appeared regularly within 3 to 5 days. The same technique was used in all subsequent tests unless otherwise noted.

tralizing power against murine virus as was normal rabbit serum (Table IV). Conversely, lymphocytic choriomeningitis virus was not inactivated by anti-murine serum (Table V). Neutralization tests with Aycock and SK monkey

poliomyelitis-convalescent sera gave irregular results; thus, one each of two Aycock and two SK sera neutralized large amounts of murine virus, but the other Aycock and SK sera had little or no demonstrable virucidal power

TABLE III

Neutralization in Mice of Variable Doses of Murine Virus by a Constant Amount of Undiluted Hyperimmune Antimurine Horse Serum

Undiluted serum, trial bleeding after 2 series of injections	Virus dilutions					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Immune horse serum A2050	2/3	0/3	0/3	1/3	0/3	0/3
" " " A2051	1/3	0/3	0/3	0/3	0/3	0/3
" " " A2052	0/3	1/3	0/3	0/3	0/3	0/3
Normal horse serum 1		3/3	3/3	3/3	2/3	0/3
" " " 2		3/3	3/3	2/3	0/3	0/3
" " " 3		3/3	3/3	3/3	1/3	0/3
" " " 4		3/3	3/3	3/3	1/3	0/3
" " " 5 (phenolized*)		3/3	3/3	3/3	1/3	0/3
" " " 6		3/3	3/3	3/3	3/3	
" " " 7 (tricresolized*)		4/4	4/4	4/4	4/4	

Numerator = number of mice paralyzed. Denominator = number of mice injected.

* Serum 5 represents serum 4 to which sufficient phenol has been added to make a final concentration of 0.5 per cent; serum 7 represents serum 6 to which sufficient tricresol has been added to make a final concentration of 0.3 per cent.

TABLE IV

Neutralization Tests in Mice with Murine Virus against Hyperimmune Anti Murine-, Anti Cavian-, and Anti Lymphocytic-Choriomeningitis Virus Rabbit Sera

Undiluted serum	Virus dilutions				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Antimurine rabbit serum	0/3	1/3	0/3	0/3	
Anticavian rabbit serum	0/3	0/3	0/3	0/3	
Anti lymphocytic-choriomeningitis rabbit serum	6/6	6/6	5/6	4/6	
Normal rabbit serum		5/6	4/6	2/6	1/6

Numerator = number of mice paralyzed. Denominator = number of mice injected.

(Table VI). The irregularity of these results renders their interpretation difficult, particularly since none of these sera had been tested for their ability to neutralize corresponding strains of poliomyelitis virus in the monkey. It will also be observed that the second Aycock serum, when run against an early passage of murine virus, failed to give clear-cut neutralization (Table VI). More reliable results were obtained with a potent hyperimmune poliomyelitis

horse serum (pseudoglobulin fraction)[8] which proved capable, in repeated tests, of inactivating comparatively large amounts of murine virus; the unrefined serum, on the other hand, possessed little or no neutralizing power (Table VII). Normal sera from various species of animals showed no neutralizing effect on murine virus, including sera obtained from normal adult mice and cotton rats (Table VII). By contrast, the same samples of adult normal mouse serum brought about complete inactivation of Theiler's virus (Table VIII). As far as

TABLE V

Neutralization Tests in Mice with Lymphocytic Choriomeningitis Virus against Hyperimmune Anti Lymphocytic-Choriomeningitis Serum and Hyperimmune Antimurine Rabbit Serum

Serum	Virus dilutions	Days								
		6th	7th	8th	9th	10th	11th	12th	13th	14th
Hyperimmune anti lymphocytic-choriomeningitis rabbit serum	10^{-2}	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —
	10^{-3}	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —
	10^{-4}	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —
Hyperimmune antimurine rabbit serum	10^{-2}	− + ±	− ++ ±	− D ±	+ +	+ +	+ +	D +	D	
	10^{-3}	− + +	− D ++	− D	±	±	±	+	D	
	10^{-4}	± + +	++ ++ +	D D ++	D					

− = no symptoms. ++ = marked symptoms.
± = beginning illness. D = dead.
\+ = ill.

Technique.—0.5 cc. of undiluted serum was combined with 0.5 cc. of virus dilution to give the final dilutions indicated in the table. Mixtures were held for 1 hour at 37°C. and 2 hours at room temperature. 0.03 cc. of each mixture was injected intracerebrally into each of 3 mice.

the neutralization of Theiler's virus by antimurine horse serum is concerned, it appears that this virus was definitely neutralized by antimurine horse serum

[8] According to information received from Dr. Toomey, this particular serum contained 0.5 per cent phenol. Marked neutralization had previously been obtained with the pseudoglobulin fraction of another antipoliomyelitis horse serum (1). As far as could be ascertained at that time the latter serum was free from any preservative; it has since become heavily contaminated, thus precluding its further use. The question has been raised (10) whether the presence in serum of a preservative, such as phenol in proper concentration, might serve to bring about spurious inactivation of murine virus by chemical action. To answer this question samples of normal horse serum were phenolized (0.5 per cent concentration) or tricresolized (0.3 per cent concentration) and compared for virucidal action with the untreated samples. The results of this experiment are given in Table III. They show that the addition of such preservatives had in no way altered the inability of normal serum to inactivate murine virus. Intracerebral injection of such serum-virus mixtures into mice likewise failed to result in virus inactivation.

up to a titer of 1:50 serum dilution, as contrasted with a titer of approximately 1:500 serum dilution against a comparable dose of murine virus. It is further evident that some neutralization of Theiler's virus occurred with potent antipoliomyelitis horse serum though such neutralization was in no way comparable with the marked neutralizing action of the same serum on SK murine virus. As far as the neutralization of monkey poliomyelitis virus is concerned, it was found that neither antimurine horse sera nor antimurine or anticavian rabbit sera possessed any demonstrable inactivating power against the SK, Aycock, or RMV virus as determined by intracerebral tests in mon-

TABLE VI

Neutralization Tests in Mice with Murine Virus of Remote or Early Mouse Passages against Poliomyelitis-Convalescent Monkey Sera

Undiluted serum	Murine virus used	Virus dilutions						
		10^{-2}	10^{-3}	10^{-4}	$10^{-4.7}$	10^{-5}	$10^{-5.3}$	10^{-6}
Aycock convalescent serum 1.....	179th–223rd	0/3	0/3	0/3		0/3		
" " " 2.....	mouse passage	3/3	6/6	6/6		4/6		0/3
SK convalescent serum 1.....	" "	0/3	0/3	0/3		0/3		
" " " 2.....		6/6	6/6	6/6		6/6		
Normal monkey serum 1.....	" "		3/3	3/3		3/3		3/3
" " " 2.....			3/3	3/3		3/3		3/3
Aycock convalescent serum 2.....	16th mouse passage		12/12	12/12	9/12	13/18	6/18	0/6
Normal monkey serum 3.....	" "		6/6	5/6	5/6	5/9	6/9	2/6

keys. This statement is based on results obtained with a total of 32 monkeys which received immune serum-virus mixtures and 14 accompanying control animals, none of which escaped paralysis.

Since all neutralization tests against murine virus in mice were done by the intraperitoneal route, it is pertinent to mention here that wide discrepancies were found to exist between intraperitoneal and intracerebral injection of similar virus-serum mixtures. Thus, it has been impossible to demonstrate neutralizing activity of antimurine sera against either SK murine virus or against Theiler's virus in mice by the latter route. The reasons for this discrepancy are not yet clear and the problem is undergoing further investigation.

In summary, it may therefore be stated that no serological relationship was demonstrated between SK murine virus and the virus of lymphocytic choriomeningitis. That some serological relationship exists between SK murine

TABLE VII

Neutralization Tests in Mice with Murine Virus against Hyperimmune Antipoliomyelitis Horse Sera

Undiluted serum*	Virus dilutions				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
.096464-G	3/3	2/3	1/3	0/3	
.097607-A	1/3	1/3	0/3	0/3	
Normal horse serum		3/3	2/3	0/3	0/3
.096464-G	3/3	3/3	3/3	3/3	
.097607-A	2/3	1/3	0/3	0/3	
Normal monkey serum		3/3	3/3	3/3	3/3
.096464-G	3/3	3/3	2/3	0/3	
.097607-A	3/3	0/3	0/3	0/3	
Normal monkey serum		3/3	3/3	3/3	3/3
.097607-A	3/3	0/3	0/3	0/3	
Normal horse serum		3/3	3/3	2/3	0/3
Adult normal mouse serum	3/3	3/3	3/3	3/3	
.097607-A	3/3	0/3	0/3	0/3	
Normal horse serum		3/3	3/3	1/3	1/3
Adult normal mouse serum	3/3	3/3	2/3	2/3	
Adult normal cotton rat serum	3/3	3/3	3/3	3/3	3/3

Numerator = number of mice paralyzed. Denominator = number of mice injected.

* These sera were obtained through the courtesy of Dr. Toomey. Serum .097607-A is the pseudoglobulin fraction of .096464-G.

TABLE VIII

Neutralization Tests in Mice with Theiler's Virus against Hyperimmune Antimurine Horse Serum, Hyperimmune Antipoliomyelitis Horse Serum, and Adult Normal Mouse Serum

Serum	Virus dilution	Serum dilutions			
		Undiluted	1:10	1:50	1:100
Antimurine horse serum	10^{-1}	4/28	0/8	2/8	6/8
Antipoliomyelitis horse serum (.097607-A)	10^{-1}	9/16			
Normal adult mouse serum	10^{-1}	2/20			
Normal horse serum	10^{-1}	25/28			

Numerator = number of mice paralyzed. Denominator = number of mice injected.

Technique.—2.0 cc. of a 10^{-1} dilution of Theiler-infected mouse brains (strain GD VII) were added to 2.0 cc. of serum; after incubation for 1 hour at 37°C., 0.4 cc. of each mixture was injected intraperitoneally into the designated number of mice.

virus and Theiler's virus of spontaneous mouse encephalomyelitis is indicated by the ability of antimurine serum and of potent antipoliomyelitis horse serum to neutralize Theiler's virus to varying extent. On the other hand, that SK murine and Theiler's virus represent entirely distinct infectious agents, in so far as their origin is concerned, is amply demonstrated by the differential action of normal adult mouse serum on the two viruses. With respect to any antigenic relation that might exist between SK murine virus and authentic poliomyelitis monkey passage virus, it would appear that poliomyelitic immune sera are capable of bringing about a certain degree of inactivation of murine virus which ranges from weak neutralization, in the case of some convalescent sera, to complete neutralization with one hyperimmune serum. These results are therefore very similar to those which had previously been reported (1). Nor do they necessarily conflict with Toomey's (11) inability to demonstrate neutralization in cotton rats between SK murine virus and poliomyelitis immune sera since the amounts of virus used were far in excess of those which, in our experience with mice, proved neutralizable by the same sera. New and conflicting data, however, come from the fact that hyperimmune antimurine horse and rabbit sera failed to show any virucidal action against SK, RMV, or Aycock poliomyelitis virus in monkeys. However, in view of the fact that antimurine sera are not even capable of inactivating murine virus in mice injected intracerebrally with serum-virus mixtures, it may be that no critical significance can be attached at this time to the failure of antimurine sera to neutralize poliomyelitis virus in monkeys by routine intracerebral tests.

Cross Immunity Tests.—It had previously been found that mice from a colony immune to Theiler's virus, when infected intracerebrally or intraperitoneally with murine virus, developed paralysis in the same manner as did normal control mice. In view of the significance of this observation, if correct, it was decided to repeat the experiment with a more virulent strain of Theiler's virus.

A group of 75 young mice were inoculated intraperitoneally with 0.1 cc. of a 1:10 dilution of mouse brain suspension prepared from mice paralyzed by Theiler's strain GD VII. Within 11 days, 45 mice had developed paralysis and subsequently died of the disease. Six weeks later the remaining 30 survivors, divided into three groups of 10 animals each, were reinfected intracerebrally with the same strain of Theiler's virus (0.03 cc. 10^{-2}), murine SK virus (0.03 cc. 10^{-5}), or the Lansing strain of murine virus (0.03 cc. 10^{-1}), respectively. At the same time 30 normal mice, divided into three groups, were injected intracerebrally with similar doses of the three viruses for control purposes.

The result of this experiment may be briefly summarized by saying that all Theiler-immune mice developed paralysis upon injection with any of the three viruses used for reinfection. But, whereas the disease produced by either SK or Lansing murine virus differed in no significant way from that observed in

normal controls, paralysis produced by Theiler's virus was considerably delayed in its incubation period (6 to 7 days) as compared with normal control mice (2 to 4 days).

Another experiment was performed with Theiler-immune mice in which reinfection was carried out by the intraperitoneal rather than the intracerebral route.

Thus, among a total of 13 mice which had survived 3 successive injections of Theiler's virus (0.1 cc. 10^{-1} or 10^{-2} intraperitoneally), 7 showed no symptoms whatsoever upon intraperitoneal reinfection with Theiler's virus (0.1 cc. 10^{-1} intraperitoneally) whereas the other 6 all became paralyzed when reinfected with SK murine virus (0.1 cc. 10^{-4} intraperitoneally). Of 7 normal control mice which received the same dose of Theiler's virus 5 succumbed to the disease and all of 7 controls injected with SK murine virus developed paralysis.

It may therefore be concluded from these two experiments that previous infection with Theiler's virus had left the surviving mice with a relative or absolute resistance to reinfection with the same virus but that no cross protection whatsoever had been induced against reinfection with the two strains of murine poliomyelitis virus.

DISCUSSION

Sufficient time seems to have elapsed since the transfer of SK poliomyelitis virus from the monkey to rodent hosts to attempt a critical appraisal and, if possible, some coordination of the data obtained so far in the course of this investigation. Most important among the many unsettled issues is, of course, the question of the nature of the murine virus. Is this virus true poliomyelitis virus in mice, possibly a mutant or variant of the SK monkey strain but otherwise embodying its essential properties, or are we dealing with another neurotropic virus, either well established or new, which was introduced by accident as a contaminant during the passages from monkeys to cotton rats and white mice? This question may be legitimately posed because SK murine virus differs not only characteristically from SK monkey virus, but shows also certain discrepancies when compared with other mouse-adapted strains of monkey poliomyelitis virus, such as Armstrong's Lansing strain or the RMV and Philadelphia strains of Toomey.

The existing differences revolve around the degree of pathogenicity for monkeys, the level of virulence in rodents (cotton rats, albino rats, mice, and guinea pigs), and the extent of neutralization *in vitro* by poliomyelitis immune sera. In general, the Armstrong (Lansing) and Toomey (Philadelphia, RMV) murine strains approximate more closely, on all counts, the basic properties of monkey poliomyelitis virus because they combine definite monkey pathogenicity with low rodent virulence and are neutralized *in vitro* by convalescent

human and monkey sera. Most of these distinguishing features, however, are only relative. Thus, murine as well as cavian SK virus will, on occasion, produce flaccid paralysis in *rhesus* monkeys, the virulence of cavian virus in guinea pigs is not appreciably higher than the virulence level of poliomyelitis virus in monkeys, and specific neutralization *in vitro* of both, cavian and murine virus, is demonstrable with antipoliomyelitis hyperimmune horse sera. It is further significant that Toomey's (12) recent experience with the adaptation of SK monkey virus has been similar to ours in that established rodent strains of this virus do not transfer back to the monkey. On the other hand, the initial monkey pathogenicity of the Lansing strain of murine poliomyelitis virus is apparently lost with long continued mouse passages (13). The actual significance of the existing differences, as a measure of similarity or dissimilarity, is therefore probably more apparent than real. Certainly none of these rodent strains stand sufficiently apart with regard to any of the above mentioned criteria to warrant an assumption that they represent totally unrelated virus entities.

As far as the possibility of confusion with any of the known viruses is concerned, the chances for a mistaken identity appear exceedingly slight. While lymphocytic choriomeningitis virus may occur as a stray virus in stocks of normal rodents, the symptomatology and pathology of either SK murine or SK cavian infection are incompatible with the picture of lymphocytic choriomeningitis. Additional evidence to the contrary comes from the serological data and the dissimilar particle size of the two respective viruses (14). With regard to Theiler's virus of so called "spontaneous mouse encephalomyelitis" the necessity for a careful differential diagnosis becomes more pressing, since the symptomatology, pathology, and epidemiology of that disease, as well as the morphology of its virus, are closely akin to simian and human poliomyelitis. In view of the circumstances it is only possible to single out certain fundamental characteristics in the behavior in mice of Theiler's virus and of SK murine virus which would tend to show whether the two viruses are or are not identical. Since mice are commonly exposed, early in life, to Theiler's virus by virtue of carrying the infectious agent in feces a herd immunity rapidly develops which is sufficiently strong to leave only young animals uniformly susceptible to infection. Even in young mice intracerebral injection of virus succeeds better than peripheral injection, presumably because nerve tissue is slow in participating in the subclinical specific immunity. The local strength of the protective mechanism is also indicated by the inability to infect mice with Theiler's virus from the gastrointestinal route. While older animals can be infected, particularly with certain Theiler passage strains of unusually high virulence, massive doses are usually required to paralyze old mice with any degree of regularity, even when the virus is injected directly into the central nervous system. By comparison, SK murine virus behaves in mice like

any virus which is introduced on virgin soil. Thus, SK murine virus will infect young mice uniformly to a high titer, not only by intracerebral inoculation but by any peripheral route that one may choose. Old mice, when infected peripherally with SK murine virus, exhibit the same type of nonspecific age resistance observed with other neurotropic viruses upon propagation in previously unexposed herds or animals; again, in contrast to Theiler's virus, there is no loss of cerebral susceptibility to murine virus with age. As far as the immunological evidence is concerned, antimurine horse serum and, to a lesser extent, concentrated antipoliomyelitis horse serum are found to bring about some inactivation of Theiler's virus, although such inactivation is distinctly inferior to that observed with SK murine virus. While some serological relationship between murine SK and Theiler's virus is therefore undeniable, the fact that normal adult mouse serum has no effect whatsoever on SK murine virus, whereas the same serum completely inactivates Theiler's virus, is of crucial significance in establishing the different origin of the two viruses in question. Some essential difference in the antigenic pattern of the two viruses is furthermore indicated by the results of cross immunity tests which show that Theiler-immune mice remain fully susceptible to reinfection with murine SK virus. To the criteria mentioned above must be added the divergent response that certain other animals exhibit to infection with the two viral agents. Thus far the guinea pig and the monkey have proven insusceptible to Theiler's virus. SK murine virus, on the other hand, establishes itself in both monkeys and guinea pigs and is capable of producing paralysis, irregularly in monkeys, and regularly in guinea pigs. The weight of the evidence is therefore in favor of regarding the murine strain of SK virus as a virus etiologically distinct from the known strains of Theiler's virus, even though the overlapping serological immune reactions suggest some genetic relationship between the two infectious agents and, indeed, with monkey poliomyelitis virus itself.

The possibility that the SK murine virus originated as a hitherto unknown virus in cotton rats is strongly contraindicated by the fact that normal adult cotton rat serum is without effect on this virus; in addition, the behavior of SK virus in cotton rats, in all essential respects, is similar to that in mice.

Since both SK murine and SK cavian virus were obtained as the result of continuous passage of SK poliomyelitis virus from the monkey to cotton rats and thence to white mice and guinea pigs, it remains to review the evidence in favor of regarding these rodent viruses as true descendants of poliomyelitis virus. To begin with, in its basic characteristics, such as symptomatology, pathology, and epidemiology, the disease in guinea pigs (5), and to a lesser extent in mice, has much in common with the disease in man or monkey. Moreover, the particle size of cultured murine virus, as determined by ultrafiltration, is similar to that of poliomyelitis virus (14). On the other hand, identification

by serological methods cannot be considered as fully established. While murine virus and cavian virus are both definitely neutralized *in vitro* by concentrated antipoliomyelitis horse sera, unconcentrated antipoliomyelitis horse serum or poliomyelitis-convalescent monkey sera show none or irregular neutralizing power. Likewise, immunization of horses with murine virus leads to the development of a high virucidal titer against both rodent viruses with no demonstrable neutralizing activity against three strains of monkey poliomyelitis virus. These observations therefore indicate the existence of certain quantitative and perhaps qualitative immunochemical differences between monkey poliomyelitis and SK rodent virus which cannot be satisfactorily explained at present.

In summarizing the available data it would seem more rational, at this time, to place the SK murine virus in line with the other rodent strains of poliomyelitis virus and to accept all of them as variants of true poliomyelitis virus rather than to seize upon minor individual differences in an attempt to accord to the SK murine virus an autonomous status as a new virus. While the latter hypothesis is tenable, in principle, one would have to insist on positive corroborative evidence to that effect in order to justify taking such a position.

CONCLUSIONS

1. SK murine virus maintained over more than 200 serial mouse passages increased in virulence for mice from an initial intracerebral titer of about 1:1 million to a maximum titer of not less than 1:1 billion dilution activity.

2. Following intracerebral injection with murine virus of remote mouse passages, 5 of 13 *rhesus* monkeys developed a characteristic encephalitic syndrome. Repeated intravenous injection of massive doses of virus caused localized flaccid paralysis in 2 of 14 monkeys.

3. Intracerebral injection of graded doses of murine virus into mice of different age groups caused fatal paralysis in young and old animals alike. Infection with small doses of virus by peripheral routes, while uniformly fatal to young mice, was followed by survival of almost half of the old mice.

4. The incubation period of the disease in young mice infected intracerebrally with a standard dose of murine virus, when studied throughout the period of 1 year, was found considerably lengthened during the summer months.

5. Cross neutralization tests furnished no evidence for any serological relationship between SK murine virus and lymphocytic choriomeningitis virus. Theiler's virus was found to be neutralizable by antimurine horse serum and, to a lesser extent, by concentrated antipoliomyelitis horse serum; however, such inactivation, in both cases, was distinctly inferior to that occurring with SK murine virus. On the other hand, no neutralization whatsoever was obtained between SK murine virus and normal adult mouse serum, whereas the same serum completely neutralized Theiler's virus. Mice surviving

infection with Theiler's virus, though acquiring immunity to this virus, remained fully susceptible to reinfection with SK murine virus.

6. Neutralization tests with SK murine virus against poliomyelitis-convalescent monkey sera gave irregular results, but neutralization of murine virus occurred regularly with a hyperimmune antipoliomyelitis horse serum. Hyperimmune antimurine horse and rabbit sera, on the other hand, failed to inactivate three strains of monkey poliomyelitis virus (SK, RMV, Aycock) by intracerebral tests in monkeys. The same sera inactivated murine virus in mice by intraperitoneal, but not by intracerebral injection of virus-serum mixtures.

7. The identity of SK murine virus and its relation to other rodent strains of poliomyelitis virus is discussed on the basis of the available data.

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