

STUDIES IN RODENT POLIOMYELITIS

III. EXPERIMENTAL POLIOMYELITIS IN GUINEA PIGS PRODUCED WITH THE MURINE STRAIN OF SK POLIOMYELITIS VIRUS*

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In earlier attempts to transmit the murine strain of SK poliomyelitis virus from mice to guinea pigs active virus could be recovered from intracerebrally injected guinea pigs up to 96 hours after injection (1). Meanwhile other observations served to indicate that some virus multiplication occurred in tissue cultures prepared with embryonic guinea pig brain. For the above reasons we ventured to predict "the possibility of training the infectious agent to produce ultimately disease in that rodent as well." It is therefore of interest to record that renewed efforts, beginning with the 70th mouse passage, to transmit the murine virus from paralyzed mice to guinea pigs resulted in the occurrence of frank paralysis in the latter animal (2). The present communication sets forth in detail the various experimental aspects of this guinea pig paralysis.

The data presented deal with the adaptation of the murine virus from mice to guinea pigs and describe the symptomatology of the disease thus produced. There are also included certain facts concerning the properties of the guinea pig virus and its manifestations in this host. In addition, the immunity mechanism, which develops in the guinea pig as the result of this infection, was studied. Finally, the behavior of the guinea pig virus in *rhesus* monkeys was made the subject of careful investigation.

Transfer of Murine Virus from Mice to Guinea Pigs and Establishment of a Fixed Strain of Cavian Virus in Guinea Pigs

The murine virus was maintained by unbroken serial transfer from mouse to mouse during the entire course of this investigation. At various points of this continuous line of mouse passages transmission to guinea pigs was attempted by injecting the latter animals with a standard dose of 0.1 cc. of 10 per cent brain suspensions prepared from paralyzed mice. As stated before, virus obtained from several early mouse passages (3rd to 12th mouse passage) proved ineffective in bringing about paralysis in guinea pigs. However, numerous transfers

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of murine virus between the 70th and 162nd mouse passage produced extensive paralysis in almost all of the injected guinea pigs. Paralysis also followed the injection of virus mouse brain after filtration through Berkefeld N or W candles, whereas control injections of normal mouse brain never caused any paralytic symptoms in guinea pigs.

With continued mouse passages a gradual loss in infectivity of murine virus for guinea pigs was noted; this first became apparent at the 168th mouse passage. In subsequent mouse passages the murine virus virtually never produced paralysis in guinea pigs. This failure to paralyze guinea pigs was a constant characteristic of all further attempts to transfer virus from mouse to guinea pig between the 173rd and 214th mouse passage. Beginning with the 230th mouse passage, however, transfer of murine virus again brought on occasional paralysis in the injected guinea pigs. In subsequent passages (233rd to 272nd) the incidence of paralysis reached again the approximate level of the original infectivity. Obviously, it is impossible to say at present whether the murine virus underwent periodic changes in its virulence for guinea pigs, or whether the phenomenon represents cyclic fluctuations in susceptibility of this animal, possibly of a seasonal character. The relevant data are given in Table I and are graphically presented in Chart 1.

Efforts were made to obtain from paralyzed guinea pigs a passage virus by means of serial transmission from guinea pig to guinea pig. On two such occasions a marked diminution in virulence occurred, both lines of propagation coming to an abrupt end at the 5th or 6th passages, respectively. A third attempt, however, led to complete adaptation and eventual fixation of the virus in guinea pigs by approximately the 15th consecutive passage. At the present time, this cavian strain of SK murine virus is running in its 52nd generation, and, on routine intracerebral transfer, (0.1 cc. 10 per cent brain-cord suspension) paralyzes nearly all injected guinea pigs after an incubation period of from 3 to 6 days. Cavian virus also regularly transfers back to mice with the production of paralysis.

Murine virus maintained in tissue culture has consistently failed to produce paralysis in guinea pigs. Thus, tissue cultures of murine virus of the 119th, 121st, and 149th *in vitro* subculture produced no symptoms whatsoever upon combined intracerebral and intraperitoneal injection of massive doses into guinea pigs. An attempt to enhance the activity of the culture virus by mixing it with normal mouse brain was not successful.

The above observations suggested some difference in the biological character of murine culture virus as compared with mouse passage virus. Further experiments were therefore planned to elucidate this point. Murine culture virus originating from the 125th *in vitro* passage was passed serially through mice. Virus obtained from the 3rd mouse passage still proved ineffective in paralyzing guinea pigs. However, when virus in the 15th consecutive mouse passage was transferred to guinea pigs, 1 of 4 injected animals developed paralysis after an

incubation period of 16 days. The outcome of this experiment leaves the inference that murine virus, originating from a culture strain, is intrinsically capable of producing paralysis in guinea pigs, but that some adaptation in the

TABLE I
Transmission of SK Murine Virus to Guinea Pigs

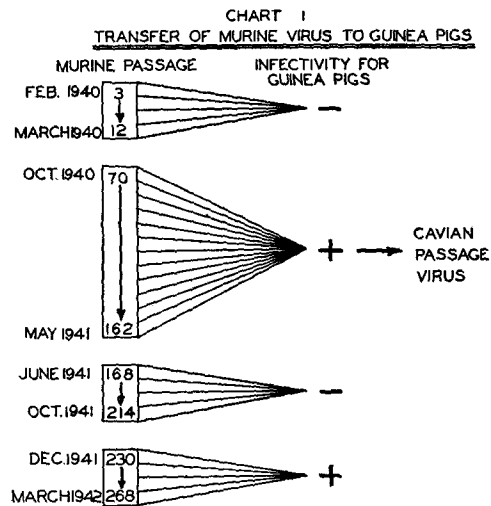
Mouse passage	Date	No. of guinea pigs injected intracerebrally	Result		Remarks
			Paralysis	No paralysis	
3rd	2/28/40	2	0	2	Virus recovered from brain, cord, and spleen 96 hrs. after infection
4th, 5th, 12th	2/28/40	2	0	2	
8th	3/18/40	2	0	2	
70th	10/13/40	1	1	0	
72nd	10/23/40	2	2	0	Carried in guinea pigs for 5 passages, then lost
73rd	10/25/40	1	1	0	
75th	10/30/40	1	1	0	
84th	11/22/40	6	5	1	Carried in guinea pigs for 6 passages, then lost
91st	12/ 6/40	2	1	1	
103rd	1/ 7/41	6	6	0	
110th	1/24/41	5	5	0	Now in 52nd passage in guinea pigs
128th	3/ 7/41	2	2	0	
137th	3/26/41	1	1	0	
141st	4/ 4/41	2	2	0	
158th	5/17/41	2	2	0	
162nd	5/26/41	1	1	0	
168th	6/12/41	4	2	2	
173rd	6/27/41	2	0	2	
196th	9/16/41	2	0	2	
200th	9/25/41	4	1	3	
214th	10/31/41	3	0	3	
230th	12/11/41	4	1	3	
233rd	12/20/41	4	3	1	
245th	1/10/42	8	5	3	
257th	2/26/42	4	3	1	
268th	3/5/42	4	3	1	
272nd	4/18/42	3	3	0	

living host is necessary before the virus acquires full pathogenicity for guinea pigs.

Symptomatology of Guinea Pig Paralysis

Following the injection of guinea pigs with murine or cavian passage virus, several days elapse before the onset of paralysis. In earlier cavian passages this

incubation period has been as long as from 2 to 3 weeks; once the virus is fixed in the guinea pig, as the result of prolonged passage, the disease develops with marked regularity between the 3rd and 6th day. No symptoms are noted, as a rule, during the incubation period. Observations extending over more than 175 infected guinea pigs indicate that some animals may show a preparalytic fever of 104 to 105°F. which drops with the onset of paralysis. This febrile reaction, when present, seems well enough defined, but individual variations are such as to preclude any pathognomonic fever curve. Paralysis sets in abruptly, often overnight, and is complete within a few hours, characteristically involving the hind legs more often than the front legs. This condition in a majority of animals progresses to prostration with a terminal fatal issue. Occasionally cavian virus may produce a syndrome wherein infected animals, after a some-



what longer incubation period, show salivation and become marasmic, but exhibit no distinct flaccid paralysis. In two instances animals showing such a syndrome were sacrificed and blood as well as suspensions of spleen, brain, and cord were inoculated intracerebrally or intraperitoneally into mice and guinea pigs. Blood and spleen produced no symptoms whatsoever, whereas all mice and guinea pigs injected with brain or cord developed typical flaccid paralysis in from 3 to 4 days. The conclusion seems justified, therefore, that the described aberrant syndrome was not caused by the activity of any possible viral contaminant, but that the encephalitic symptoms should be ascribed to an unusual persistence and localization of the cavian virus in the brain of infected guinea pigs. This conclusion is further supported by careful pathological examination of the central nervous system of such guinea pigs which disclosed the presence of a polioencephalitis, together with characteristic lesions in the

anterior horn of the spinal cord. A detailed report of the pathology of the guinea pig paralysis will be found in another paper of this series of communications (4).

Routes of Infection in Guinea Pigs

During the two periods that murine virus could be successfully transmitted from mouse to guinea pig the incidence of paralysis in the latter animal was

TABLE II
Incidence of Paralysis in Guinea Pigs Following Injection with Murine Virus (70th to 168th Mouse Passage) or Cavian Virus (3rd and 21st Guinea Pig Passage) by Different Routes

Route of injection	Virus	Dose	No. of guinea pigs	Result	
				Paralysis	No paralysis
Intracerebral	Murine*	0.1	36	32	4
	Cavian†	0.1	7	7	0
Intravenous	Murine	1.0	3	2	1
	Cavian	1.0	3	2	1
Intraperitoneal	Murine	2.0	6	4	2
	Cavian	1.0-2.0	6	0	6
Subcutaneous	Murine	2.0	3	1	2
	Cavian	1.0-2.0	6	0	6
Intranasal	Murine	1.0	5	0	5
	Cavian	0.2-1.0	6	0	6
Oral	Murine	2.0§	58	0	58
	Cavian	1.0-2.0§	5	0	5

* 10 per cent mouse brain suspension.

† 10 per cent guinea pig brain and cord suspension.

§ On 3 consecutive or alternate days.

uniformly high, with an average infectivity of 90 and 75 per cent, respectively. Paralysis was also obtained by intravenous, intraperitoneal, or subcutaneous injection, although the last route proved definitely the least effective. Intranasal instillation or oral administration of murine virus have consistently failed to evoke any unmistakable signs of paralysis (Table II).

When virus is employed which has been passed through guinea pigs for several generations paralysis can readily be produced by intracerebral injection but not by intraperitoneal, subcutaneous, intranasal, or oral administration. However, cavian fixed virus of the 21st generation was capable of inducing

paralysis upon intravenous as well as intracerebral inoculation; the same material when injected into guinea pigs by the intraperitoneal, subcutaneous, nasal, or oral route again failed to cause any paralytic symptoms (Table II).

The inability of murine virus to infect guinea pigs by oral administration stands in marked contrast to the results obtained in mice. This problem was therefore made the subject of further study. It was first thought that the ingestion of certain other substances along with the virus might render the latter infectious from the gastrointestinal tract. Thus, in one experiment, feeding of murine virus was followed by feeding of egg yolk, gastric mucin, *coli* bacteriophage, or of a suspension of triturated mouse intestine. Attempts were also made to bring about changes in the condition of the animal which might facilitate invasion of the infectious agent. One series of guinea pigs, prior to oral administration of murine virus, was therefore injected with sublethal doses of diphtheria toxin, while another series had previously been maintained on a scorbutic diet. Suffice it to say that none of these methods were of any avail in rendering the murine virus infectious for guinea pigs by the gastrointestinal route.

Titration of Virus

Titration of the infectivity of murine virus (141st to 144th mouse passage) in guinea pigs showed that dilutions of mouse brain suspension up to 1:500 were effective in producing paralysis following intracerebral injection. On the other hand, cavian virus of the 13th guinea pig passage seemed to possess much less potency since brain and cord suspensions, injected intracerebrally, failed to produce paralysis in dilutions above 1:10. A more recent titration of cavian virus in its 38th guinea pig passage, however, indicates a substantial increase in virulence in that brain and cord dilutions up to 1:500 proved infective.

Titration of the infectivity of cavian virus (brain and cord suspensions of the 25th and 31st guinea pig passages) for mice revealed that guinea pig passage apparently reduces the mouse virulence of the virus, titration end points in mice being 10^{-3} , as determined by intracerebral test. When transferred further to new mice, however, the virus proved again infective to a titer of 10^{-7} . Inasmuch as this titer almost reached the virulence level of mouse passage virus (10^{-8} by the intracerebral route), passage through mice seems to cause a rapid return of the cavian virus to its original murine character.

Distribution of Virus in Guinea Pigs

Pathological observations (2, 4) had left little question that the lesions in paralyzed guinea pigs are strictly confined to the central nervous system, particularly the anterior horn cell in the spinal cord. The marked neurotropism of the virus in this animal is further substantiated by the results of experiments in which the distribution of murine or cavian passage virus was studied in intra-

cerebrally infected guinea pigs. For this purpose nervous and non-nervous tissues of infected guinea pigs, at the height of paralysis, were transferred to mice. Mice were used in these tests because they were known to react to small doses of virus with great regularity. It was found that the transfer to mice of brain and cord from paralyzed guinea pigs always resulted in typical paralysis after a short incubation period (2 to 4 days), indicating the presence of large amounts of virus in such tissues. By contrast, transfer of blood, spleen, or liver was negative in nearly all instances (Table III). The technique employed throughout these experiments consisted in the intracerebral or intraperitoneal

TABLE III
Distribution of Virus in Guinea Pigs Following Administration of Murine or Cavian Virus

Route of infection	Tissues examined for virus content									
	Brain and cord		Spleen		Liver		Blood		Feces	
	No. of animals examined	Presence of virus	No. of animals examined	Presence of virus	No. of animals examined	Presence of virus	No. of animals examined	Presence of virus	No. of animals examined	Presence of virus
Intracerebral*										
Murine.....	7	+	2	±	1	—	1	—	5‡	—
Cavian.....	21	+	8	—	6	—	6	—		
Oral§										
Murine.....	28	—					10	—	45	±
Cavian.....	1	—							6‡	—

+ = virus present in all instances; ± = virus present irregularly; — = virus not present.

* Guinea pigs examined were sacrificed during incubation period or at height of paralysis.

‡ One pooled sample tested.

§ Guinea pigs examined were sacrificed at intervals of 1 to 14 days after oral administration of virus.

|| Nine pooled samples tested. Three samples were positive, 6 were negative.

injection of mice with 0.03 cc. or 0.1 cc. of 10 per cent suspensions of the various tissues; blood was either injected intraperitoneally (0.5 to 1 cc.), freshly drawn, or intracerebrally (0.03 cc.) and intraperitoneally after laking or heparinization. These findings stand in sharp contrast to analogous observations in mice which had revealed a systemic distribution of the virus in that animal, at least during certain phases of the infectious process.

The possibility of a centrifugal spread of the virus from the infected central nervous system was next examined by testing the feces of paralyzed guinea pigs for virus content. In these experiments, 10 per cent saline suspensions of feces were filtered through a N Berkefeld candle and then injected intraperitoneally into mice in amounts of 0.2 to 0.5 cc. In several tests it proved impossible to

recover any virus from the feces of guinea pigs paralyzed by intracerebral injection of either murine or cavian virus. However, active virus could be recovered, on three occasions, from the feces of guinea pigs following oral administration of murine virus. None of these animals had shown any paralytic symptoms and transfer of brain and cord to mice indicated that their central nervous system was demonstrably free from virus. Virus has not been encountered, in repeated tests, in the feces of normal guinea pigs.

Cultivation of Cavian Virus in Vitro

Repeated attempts to grow cavian virus in tissue culture were unsuccessful until virus of the 15th guinea pig passage was inoculated into embryonic mouse brain tissue culture. This type of tissue culture and the technique of subculturing are fully described in another paper of this series of communications (5). When tested intracerebrally in guinea pigs, undiluted tissue culture fluid of the 12th *in vitro* subculture paralyzed 1 of 2 animals, whereas a similar inoculation of the 30th subculture gave negative results. Both subcultures, however, proved infectious for mice, the latter to a titer of 10^{-5} by the intracerebral route. These preliminary experiments suggest that cavian virus can be grown in tissue culture but that optimum conditions for its propagation, with full maintenance of guinea pig virulence, have as yet not been obtained. Possibly embryonic guinea pig brain rather than mouse brain may make a better substrate for *in vitro* cultivation of this virus.

Serological Tests with the Cavian Virus

Neutralization tests were carried out in order to study the nature of the cavian virus with the help of serological methods. The object of this work was, first, to establish, if possible, the identity between murine and cavian virus, and, secondly, to determine whether cavian virus was antigenically related to authentic poliomyelitis virus. These tests therefore consisted of: (1) experiments in which immune rabbit sera prepared against cavian or murine passage virus were examined for their ability to inactivate the homologous and heterologous viruses in mice and guinea pigs; (2) experiments in which guinea pig convalescent serum, monkey SK and Aycock convalescent sera, and anti-poliomyelitis horse sera¹ were tested for neutralizing power against cavian virus in guinea pigs. Similar tests were carried out, for control purpose, with a hyperimmune rabbit serum against the virus of lymphocytic choriomeningitis² and with normal sera from rabbits, guinea pigs, monkeys, and horses.

It was clearly shown that anticavian rabbit immune serum possesses strong

¹ These sera were obtained through the courtesy of Dr. J. A. Toomey from the City Hospital, Cleveland.

² This serum was obtained through the courtesy of Dr. J. E. Smadel from Dr. Rivers' laboratory.

virucidal power against murine virus in mice since the serum neutralized the maximum dose of virus used, *i.e.* 10^{-1} . These tests were run by intraperitoneal injection of mice with virus-serum mixtures, as described in another paper (3). The reverse is also true, namely that cavian virus in guinea pigs is completely

TABLE IV
Neutralization in Vitro of Cavian Virus in Guinea Pigs by Various Antisera

Serum	Result
Anticavian rabbit serum.....	0/3
Antimurine rabbit serum 1st test.....	0/3
2nd test.....	0/3
3rd test.....	1/4
Antilymphocytic choriomeningitis rabbit serum 1st test.....	2/3
2nd test.....	3/4
Normal rabbit serum 1st test.....	3/3
2nd test.....	3/4
Guinea pig convalescent serum.....	0/3
Normal guinea pig serum 1st test.....	2/3
2nd test.....	3/4
SK monkey convalescent serum 1st test.....	0/3
2nd test.....	3/4
Aycock monkey convalescent serum 1st test.....	2/3
2nd test.....	0/3
3rd test.....	4/4
Normal monkey serum 1st test.....	3/3
2nd test.....	4/4
Antipoliomyelitis horse serum	
Untreated (.096464-G).....	7/7
Concentrated fraction (.097607-A).....	0/7
Normal horse serum.....	6/6

Numerator = number of guinea pigs with symptoms or paralysis. Denominator = number of guinea pigs injected.

Technique.—0.5 cc. of 20 per cent guinea pig brain and cord suspension was mixed with 0.5 cc. of undiluted serum; after incubation for 1 hour at 37°C., 0.1 cc. of each mixture was injected intracerebrally into several guinea pigs.

inactivated by antimurine rabbit immune serum (Table IV). This neutralization is as marked as that occurring with homologous anticavian rabbit immune serum or with serum obtained from convalescent paralyzed guinea pigs. Thus, the serological identity of murine and cavian virus can be said to be firmly established. The results with the various monkey convalescent sera are irregular and difficult of interpretation, especially in view of the fact that parallel neu-

tralization tests with the same sera against their corresponding strains of monkey poliomyelitis virus had not been carried out. However, one of two potent anti poliomyelitis horse sera (concentrated fraction) gave complete neutralization in repeated tests; the same serum had previously neutralized murine virus in mice (3). In contrast herewith, neutralization of cavian virus was not obtained with anti lymphocytic-choriomeningitis rabbit immune serum nor with any of the several normal sera.

Immunity Phenomena of the Disease in Guinea Pigs

During the course of this investigation there were available for further study a number of guinea pigs which had survived infection by various routes with either murine or cavian passage virus without showing any paralytic symptoms. These guinea pigs were in no way convalescent animals but had remained completely free from any objective signs of disease. Three to 6 weeks after their first injection these symptomless guinea pigs were reinfected intracerebrally either with potent murine or cavian passage virus. The results are listed in Table V.

Upon considering the data as a whole it will be gathered that of a total of 70 animals which had failed to show symptoms following a first inoculation with either murine or cavian virus, 46 were solidly immune to reinfection with the same viruses whereas 24 proved susceptible; all 33 accompanying control animals succumbed to the disease. The described protection occurred irrespective of whether the guinea pigs had previously received cavian virus or murine virus. It will be noted, however, that guinea pigs which had received the initial virus injection intracerebrally were better protected than those which had previously been injected by the nasal or oral route.³

The above observations indicate that murine or cavian virus, when causing an inapparent infection in guinea pigs, is capable of leaving the animal in a state of immunity which is of considerable proportions. The possibility must be considered that this resistance may be, in some way, connected with persistence of the virus. For active virus, as determined by transfer to mice, could be recovered, on one occasion, from symptomless guinea pigs injected intracerebrally with cavian passage virus as late as 3 weeks following such injection.

It became of interest to investigate further the mechanism of this "latent immunity" by determining to what extent the tissues or the serum of resistant guinea pigs possessed the power to inactivate virus. The first experiment included a group of 8 guinea pigs, none of which had shown any symptoms fol-

³ It was found that the injection of massive doses of Theiler's virus in guinea pigs fails to produce any clinical signs of disease, even though the virus may be recovered as late as 72 hours after intracerebral injection from the central nervous system of such animals. It is of interest to note in this connection that such symptomlessly infected guinea pigs proved fully susceptible to reinfection with cavian virus.

TABLE V
*Reinfection with Murine or Cavian Virus of Guinea Pigs Giving a Previous History of
 "Symptomless Infection"*

Experiments	Previous history of guinea pigs	No. of guinea pigs	Virus used for reinfection	Results	
				Paralysis	No paralysis
Experiment 1 1/7/41	Murine virus I.C.	1	Murine virus I.C.	0	1
	Cavian " "	9	" " "	3	6
	Normal controls	6	" " "	6	0
Experiment 2 1/24/41	Murine virus I.C.	1	" " "	0	1
	Cavian " "	5	" " "	3	2
	Normal controls	5	" " "	5	0
Experiment 3 3/7/41	Murine virus I.P.	1	" " "	0	1
	" " S.C.	1	" " "	0	1
	" " orally	6	" " "	3	3
	Normal controls	2	" " "	2	0
Experiment 4 4/4/41	Murine virus orally	6	" " "	2	4
	Cavian " I.C.	2	" " "	0	2
	Normal controls	2	" " "	2	0
Experiment 5 5/17/41	Cavian virus I.C.	3	" " "	1	2
	Normal controls	2	" " "	2	0
Experiment 6 9/25/41	Murine virus I.C.	4*	Cavian virus I.C.	1	3
	Cavian " "	7	" " "	1	6
	" " I.P.	2	" " "	1	1
	" " S.C.	3	" " "	1	2
	" " I.N.	3	" " "	3	0
	" " orally	2	" " "	2	0
	Normal controls	5	" " "	5	0
Experiment 7 10/8/41	Murine virus I.C.	5*	" " "	0	5
	Normal controls	4	" " "	4	0
Experiment 8 11/21/41	Murine virus I.C.	3*	" " "	0	3
	Normal controls	4	" " "	4	0
Experiment 9 2/10/42	Murine virus I.C.	3	" " "	0	3
	Cavian " orally	3	" " "	3	0
	Normal controls	3	" " "	3	0

I.C. = intracerebrally. S.C. = subcutaneously.

I.P. = intraperitoneally. I.N. = intranasally.

* These guinea pigs had received murine virus during the time that negative transfers were obtained from mouse to guinea pig.

lowing injection with murine virus, and another group of 7 guinea pigs which were convalescing from an attack of murine paralysis; a third group of 4 normal guinea pigs was added for control purpose. All 19 guinea pigs received an intracerebral dose of 0.1 cc. of a 10 per cent suspension of potent murine virus. At intervals varying from 24 to 72 hours these animals were sacrificed and their brain and cord transferred to mice. No virus, or at best only traces of virus, were present in the brain or cord of either symptomless or convalescent animals, whereas active virus could regularly be recovered at 48 to 72 hour intervals from the nervous tissue of infected normal guinea pigs. The results of these tests suggest, first, that the central nervous system of symptomless guinea pigs is capable of disposing of virus in a highly effective manner and, second, that this disposal is analogous to the mechanism which operates in convalescent animals. The protection, therefore, appears to be essentially the same, irrespective of whether the guinea pig had or had not had paralysis before.

In a second experiment the sera of latently immune guinea pigs were examined for their power to inactivate murine virus *in vitro*. These sera were obtained from various groups of symptomless guinea pigs, *i.e.* guinea pigs which had been fed murine virus, or guinea pigs which had received either murine or cavian virus intracerebrally without developing paralysis. A total of 11 sera, pooled in three lots according to origin, was thus examined; normal guinea pig sera were included for control purposes. The results of these neutralization tests showed that the pooled sera of latently immune guinea pigs, without exception, were strongly virucidal for murine virus as determined by intraperitoneal tests in mice, inasmuch as they neutralized doses of virus as high as 10^{-2} . By contrast, no virucidal power could be detected in normal control sera which failed to neutralize virus dilutions up to 10^{-5} .

When the two experiments are taken together there can be little doubt that guinea pigs rendered latently immune to virus by symptomless infection are endowed with a well developed protective mechanism, which can be demonstrated, on the one hand, by the power of nerve tissue to dispose of virus *in vivo*, and, on the other, by the ability of serum to inactivate the infectious agent *in vitro*. While no unequivocal distinction can be made between cellular or humoral factors involved, the nature of the clearing mechanism invites further study and clarification. What seems to be of particular interest, however, even at this early stage of the problem, is the fact that neutralizing antibodies were readily found in the sera of guinea pigs following oral administration of the virus, even though no active virus could be discovered in the central nervous system of such animals.

Non-Specific Age Resistance of Guinea Pigs to Infection with Murine Virus

The next experiments deal with the question whether non-specific protection against cavian virus can be demonstrated in guinea pigs as the result of increased resistance with age. As is well known, many neurotropic viruses,

when injected by peripheral routes into adult animals, encounter physiological barriers which impede their further travel to the central nervous system. As far as poliomyelitis is concerned, this question has not yet been put to a satisfactory experimental trial because small laboratory animals susceptible to this virus have heretofore not been available. The opportunity was therefore seized to investigate this problem in guinea pigs.

A group of 33 guinea pigs, weighing 600 gm. or more, received murine virus by various routes, 8 intracerebrally, 10 intravenously, 10 interperitoneally, and 5 subcutaneously. The incidence of paralysis among these animals, following intravenous or intraperitoneal injection, did not differ materially from that observed in young guinea pigs. There was a suggestion, however, that the older animals were slightly more resistant to subcutaneous and, perhaps, even to intracerebral inoculation since by the former route only 3 of 8 came down with paralysis and by the latter none of 5 animals. Admittedly, the number of guinea pigs used was too small and the experiment not sufficiently complete to yield conclusive evidence. However, it is interesting in this connection to mention that neutralizing substances for this virus are not present in the sera of normal adult guinea pigs (5 sera tested) in contrast with the sera of symptomless infected animals which regularly contain such antibodies. The problem of age resistance in the guinea pig obviously requires further study, with consideration of the pathogenesis of the disease following different routes of injection, and the use of graded infecting doses, such as was done for murine virus in mice (3).

Pathogenicity of Cavian Virus for Rhesus Monkeys

The monkey pathogenicity of the guinea pig virus was studied by inoculating a number of *rhesus* monkeys with the brain or cord of guinea pigs paralyzed by passage virus; the usual dose was 1 cc. of 10 per cent suspensions injected intracerebrally. Such transfers from guinea pig to monkey were carried out with every consecutive passage during the first two lines of virus propagation in guinea pigs, and with the 8th and 21st passage of the third line. The results are given in Table VI.

It will be seen that of a total of 35 monkeys which had received guinea pig passage virus intracerebrally, 26 failed to respond with any manifest symptoms of disease, save for a transient fever and occasional awkwardness in movements. Intracerebral reinfection with SK poliomyelitis virus which paralyzed all of 12 accompanying normal controls failed to produce paralysis in 5 of 19 such symptomless monkeys. There remain 9 monkeys in which the injection of cavian virus was followed by various signs of definite involvement of the central nervous system. Thus, 5 animals developed a characteristic encephalitic syndrome consisting of coarse tremor, convulsions and facial palsy, 3 showed a paresis of one or more extremities, and 1, on the 8th day after infection, presented a complete flaccid paralysis of the left leg. The clinical picture ob-

served in this latter animal was that of "classical" poliomyelitis, the train of symptoms progressing from a preparalytic fever to paresis and, thence, to frank paralysis; moreover, pathological examination of the central nervous system, upon sacrifice on the 10th day of the disease, revealed typical unilateral poliomyelitic lesions in the lumbar level of the spinal cord.

The available data may be summarized by saying that cavian passage virus, in principle, is no more pathogenic for *rhesus* monkeys than is murine passage virus. In other words, a single intracerebral injection of virus usually causes

TABLE VI
Transfer of Guinea Pig Passage Virus to Rhesus Monkeys

Line of propagation in guinea pigs	Guinea pig passage	No. of monkeys injected intracerebrally with guinea pig brain or cord	Result			
			Negative*	Encephalitic syndrome	Paresis	Paralysis
1st	I	2	1		1	
1st	II	7	5	1	1	
1st	III	2	2			
1st	IV	6	3	1	1	1
1st	V	2	1	1		
2nd	I	2	2			
2nd	II	2	2			
2nd	III	2	2			
2nd	IV	8	7	1		
3rd	VIII	1	1			
3rd	XXI	1		1		
1st-3rd	I-XXI	35	26	5	3	1

* Monkeys listed as negative had either no symptoms whatsoever or showed a transient fever (103-107°F.) with occasional awkwardness in movements.

no manifest symptoms of disease, although certain animals may develop an encephalitic syndrome, with or without localized pareses. In one instance, however, classical poliomyelitis occurred in a monkey following injection with cavian virus obtained from a passage which failed to transfer further to guinea pigs, but was still paralyzing mice. The full significance of these observations for the mechanism of virus adaptation from monkey to rodents, and vice versa, is as yet not immediately apparent.

Recovery of Virus from the Tissues of Poliomyelitis-Convalescent Monkeys Following Injection with Cavian Virus

The results of previous experiments (1) will be recalled which had shown that the tissues of poliomyelitis-convalescent monkeys, when tested 24 to 96 hours

after injection with murine virus, were relatively, if not absolutely, free of the infectious agent. Since a highly effective clearing mechanism had just been demonstrated in the tissues of guinea pigs convalescing from cavian paralysis or latently immune to this virus, it became of interest to investigate the fate of cavian virus after injection into poliomyelitis-convalescent monkeys. A total of 9 monkeys which had survived a previous poliomyelitic infection with SK or Aycock virus were available for this purpose. All showed considerable residual paralysis at the time of this experiment, but the interval between their paralytic attack and the injection of cavian virus varied from 2 weeks to as

TABLE VII
*Recovery of Virus from Tissues of Poliomyelitis-Convalescent Monkeys Following Injection with Cavian Virus As Determined by Transfer to Mice**

Monkey	Interval between paralysis and injection of cavian virus	Route of injection with cavian virus	Recovery of virus from monkey tissues			
			Brain	Cord	Spleen	Blood
AG72 Normal.....	—	Intracerebral	+	+	+	+
AH18 Convalescent SK.....	3 wks.	"	—	—	—	—
AG43 " ".....	3½ mos.	"	+	+	+	+
AG19 " Aycock.....	3½ "	"	+	+	+	+
AH43 Normal.....	—	Intravenous	—	—	+	+
AH54 Convalescent SK.....	2 wks.	"	—	—	—	—
AH58 " Aycock.....	2 "	"	—	—	—	—
AG81 " SK.....	2 mos.	"	—	+	+	—
AG83 " ".....	2 "	"	+	+	+	+
AG8 " ".....	3 "	"	—	+	+	+
AG56 " ".....	3 "	"	+	+	+	+

* All monkeys were sacrificed 72 hours after the injection of cavian virus.

long as 3 months. Cavian passage virus (8 to 12th generation) was injected into these animals, either intracerebrally or intravenously, in doses of 1 cc. or 5 cc., respectively, of a 10 per cent guinea pig brain-cord suspension. Two normal monkeys received the same inoculum. After a uniform interval of 72 hours all animals were sacrificed and brain, cord, spleen, and blood were transferred to mice. The technique was the same as previously employed in similar tests with guinea pigs. The results of this experiment are given in Table VII.

The data given in Table VII show that cavian virus injected intracerebrally into a normal monkey was recovered from the central nervous system as well as from extraneural sites, while the same virus after intravenous injection was recoverable only from spleen and blood. In contrast herewith no virus, irrespective of the route of injection, could be recovered from nervous or non-nervous tissue of monkeys shortly after a preceding attack of poliomyelitis;

however, in the later stages of convalescence transfers were positive from practically all tissues tested. It would therefore seem that poliomyelitic paralysis in the monkey is associated with a well developed but transient virucidal mechanism for the elimination of cavian virus.

Immunization of Monkeys with Cavian Virus

A group of 4 monkeys were immunized with cavian passage virus (1st to 5th generation). The virus was administered by subcutaneous injection, in ten equal doses of 7 cc. of 10 per cent brain-cord suspensions, over a period of 1 month. At the end of the immunization period these monkeys were tested for immunity by intracerebral injection with 1 cc. of a 1:10 dilution of SK monkey virus. Two normal control monkeys received a similar dose of SK virus by the intracerebral route. Both control animals developed partial paralysis. Of the 4 immunized monkeys, 2 developed partial paralysis, 1 showed a transient weakness of the arms and legs, and 1 remained free from any symptoms. The degree of protection in this experiment was certainly not striking.

Interference between Cavian and Monkey Virus in Rhesus Monkeys

One attempt was made to determine whether cavian virus could be used effectively as an interfering agent in blocking poliomyelitic infection in monkeys. Six monkeys were injected intracerebrally with 0.5 cc. of RMV virus in a 1:10 dilution. The animals were then divided into two groups of 3 monkeys each, one group receiving intravenously repeated doses of 6 cc. of 10 per cent cavian virus (brain-cord suspension of 12th to 19th generation), beginning with the day of infection, the other group receiving the same dose of cavian virus 48 hours after infection. Three control monkeys were injected intracerebrally with 0.5 cc. of a 1:10 dilution of RMV virus alone. All animals in this experiment developed prostrating paralysis after an incubation period of 6 to 8 days, except one which had received cavian virus at the 48 hour interval. This monkey survived with slight paralytic involvement of both arms and legs.

The above results furnish no evidence of effective interference between cavian virus and RMV virus in monkeys under the conditions of the test. Possibly the failure was due to the low virulence (1:20) of the cavian virus in its early passages, or to the large dose of RMV virus (1:10) used in this experiment. A final conclusion can only be reached after further tests have been done under more favorable quantitative conditions.

DISCUSSION

In 1910, while studying experimental poliomyelitis in monkeys, Roemer and Joseph (6) called attention to a presumably spontaneous flaccid paralysis which

occurred in approximately 5 per cent of their normal guinea pig stock. The causative agent was proved to be a filterable virus but further identification was not carried out, probably because the virus was lost in its 5th passage through guinea pigs (7). Similar isolated observations, although less documented, were made by Neustaedter (8) in 1913 and by Picard (9) in 1925, both authors interpreting their findings as spontaneous induction of poliomyelitis in guinea pigs following close contact with poliomyelitis-infected monkeys. In the absence of further published records, it must remain undecided, first, whether the described condition actually represented authentic poliomyelitis acquired by chance contact at the implied source of infection; secondly, whether the disease was caused by a known virus, such as lymphocytic choriomeningitis or equine encephalomyelitis, which happened to occur in these guinea pigs with atypical manifestations alone or together with poliomyelitis virus; or, thirdly, whether the authors were dealing with a truly spontaneous infection in guinea pigs that imitates poliomyelitis to the same extent as does Theiler's spontaneous encephalomyelitis in mice.

Notwithstanding the above mentioned observations, it has generally been held that the guinea pig is insusceptible to experimental inoculation with human or simian poliomyelitis virus. There seems no reason now to depart from this traditional belief—as far as the power of the virus to induce paralysis is concerned—because attempts to transfer SK poliomyelitis virus directly from monkeys to guinea pigs have uniformly failed to elicit any paralytic symptoms in the hands of Trask and his coworkers (10), as well as in our own experience. However, a new impetus is given to this problem by the data presented in this paper, since adaptation of poliomyelitis virus from monkey to guinea pig apparently succeeded by subjecting the virus to intermediary passage through certain rodents, namely the cotton rat and the white mouse. Confirmatory evidence that the SK strain can be transferred from the cotton rat to the guinea pig has since been presented by Toomey and Takacs (11).

The possibility that the cavian virus described in this paper is a contaminating virus, naturally occurring in guinea pigs, can be safely eliminated because cavian virus is serologically indistinguishable from and in all probability identical with murine virus. It may be assumed, then, that the disease in guinea pigs is the result of a direct transfer of the infectious agent from mouse to guinea pig. Therefore, all other evidence suggesting the poliomyelitic nature of the SK virus in mice (1, 5) is equally applicable to identification of the virus in guinea pigs. Further support comes from the symptomatology and pathology of the guinea pig paralysis, which approaches the picture of poliomyelitis in man and monkey more closely than does the mouse paralysis. To these observations may be added the fact that attempts to transfer cavian virus to *rhesus* monkeys have occasionally resulted in localized pareses and, in one instance, in flaccid paralysis. The lack of protection in monkeys follow-

ing immunization withavian virus against intracerebral reinfection with SK monkey poliomyelitis virus cannot be weighed too much, since the same procedure would also prove unsatisfactory with authentic strains of monkey poliomyelitis virus. As far as identification by serological methods is concerned, inactivation ofavian virus in guinea pigs by poliomyelitis-convalescent monkey sera is definite enough when it occurs, although the data can hardly be said to be sufficiently convincing to bear much weight. More suggestive evidence comes from neutralization tests with potent antipoliomyelitis horse sera, one of which proved capable of completely inactivating theavian virus in repeated tests. The trend of these experiments, therefore, is not inconsistent with an assumption of some antigenic relationship between poliomyelitis virus and theavian strain of virus, although certain differences do undoubtedly exist. It is well to remember in this connection that similar difficulties limit the usefulness of the neutralization test, even when applied to the problem of identifying certain strains of poliomyelitis virus upon fresh isolation from man.

Of collateral interest is the information obtained from attempts to trace the fate ofavian virus in the tissues of normal and poliomyelitis-convalescent monkeys. As far as the available data go they show that paralyzed monkeys, shortly after an attack of poliomyelitis, possess a well marked clearing mechanism foravian virus which is not present in normal monkeys nor in recovered monkeys during the late stages of convalescence. This ability of poliomyelitis-immune tissues to dispose ofavian virus *in vivo* is analogous not only to the elimination ofavian virus from convalescent guinea pigs, but also to the fate of poliomyelitis virus in monkeys after a paralytic attack of poliomyelitis (12).

Because the study of experimental poliomyelitis for many years has been confined to observations in only one host, *i.e.* the *rhesus* monkey, accumulated knowledge has been necessarily incomplete and, to some extent, misleading. The trend of recent investigations suggests that important differences may exist in the response of other hosts to the same virus, since *cynomolgus* monkeys and chimpanzees were found to be fully susceptible to infection by the gastrointestinal route which is normally ineffective in *rhesus* monkeys. As the opportunities expand for further study of the experimental infection in new hosts, additional channels are opened up that may be expected to afford a clearer insight into the complexity of the human disease. Inasmuch as there is now presumptive evidence that SK poliomyelitis virus can be successfully propagated, as a fixed strain of virus, not only in monkeys but also in white mice and guinea pigs, it becomes of interest to compare the similarities and dissimilarities in the response of the three hosts to the same infectious agent. In the first place, one is impressed by the decided neurotropism of the virus in *rhesus* monkeys and in guinea pigs which stands in marked contrast to its wide distribution in mice. Further differences exist in the effectiveness of various avenues of infection. Thus, *rhesus* monkeys are constantly susceptible only

to intracerebral injections of the virus, guinea pigs may acquire the disease by intracerebral and intravenous injection, while mice succumb to intracerebral infection as well as to introduction of the virus by all peripheral routes, including feeding and nasal instillation. In keeping with these observations, the virulence of the virus varies considerably among the three hosts; whereas titers between 1:100 and 1:500 represent the approximate end points of activity in *rhesus* monkeys and in guinea pigs, respectively, the virus reaches levels of potency in mice that lie in the neighborhood of 1:1 billion. The higher potency in mice, together with an apparently broader basis of cellular attack, may be the reason why *in vitro* cultivation has so far clearly succeeded only with murine virus.

One phenomenon, outstanding in the course of this investigation, which deserves special consideration is the apparent cyclic variation in the transmissibility of the infectious agent from mice to guinea pigs. It will be recalled that while murine virus was being serially propagated through unbroken mouse passages and cavian virus through similar unbroken guinea pig passages, phases of positive transfer from mouse to guinea pig alternated with phases of negative transfer. It is impossible to say at present whether these fluctuations in pathogenicity were caused by periodic changes inherent in the biological nature of the virus, or whether they reflect alterations of a possibly seasonal character in susceptibility of the guinea pig which are brought out only when the virus is forced to adapt itself from one host to another. That the non-paralytic transfers cannot be regarded as "missed infections" is amply shown by the protection against reinfection which results from such symptomless attacks. A similar protection is also demonstrable in guinea pigs which have remained symptomless following injection of murine or cavian passage virus by ineffective routes. It would therefore seem as if the virus in guinea pigs may either assume the form of a manifestly paralyzing agent or of an agent which causes latent infection with subsequent development of a strong cellular and humoral immunity. The analogy between such experimentally controlled observations and the epidemiology of poliomyelitis in man is striking and one will look to further elucidation of this problem as a promising lead for a better understanding of the pathogenesis of the human disease.

CONCLUSIONS

1. Murine SK poliomyelitis virus has been transferred from mouse to guinea pig with the establishment of a fixed strain of cavian passage virus.
2. The disease thus produced in guinea pigs is characterized by the occurrence of flaccid paralysis. Typical poliomyelitic lesions are found in the anterior horn of the spinal cord.
3. Guinea pigs are susceptible to infection with murine virus by the intracerebral, intravenous, intraperitoneal, and subcutaneous route; cavian passage

virus produces paralysis only upon intracerebral or intravenous injection. Neither virus paralyzes guinea pigs by feeding or nasal instillation.

4. The potency of the virus (murine or cavian) in guinea pigs is considerably lower than in mice and compares with the titer of the original SK strain in monkeys. In paralyzed guinea pigs the virus is found only in the central nervous system and not in extraneural sites, such as blood or abdominal viscera.

5. Attempts to cultivate cavian passage virus in tissue culture have yielded evidence of some *in vitro* propagation but no passage virus has as yet been obtained by this method.

6. Cross neutralization tests with cavian passage virus in guinea pigs and with murine virus in mice have established the serological identity of the two viruses. Inactivation of cavian passage virus in guinea pigs by poliomyelitis-convalescent monkey sera is irregular. Complete neutralization has been obtained with a concentrated poliomyelitis horse serum.

7. Resistance to reinfection with potent virus can be demonstrated in convalescent guinea pigs as well as in guinea pigs which have survived a symptomless infection with either murine or cavian virus. This immunity is demonstrable by the power of the serum of such animals to neutralize the virus *in vitro* and by the ability of nerve tissue to dispose *in vivo* of the infectious agent.

8. Cavian passage virus has a limited pathogenicity for *rhesus* monkeys. Of a total of 35 monkeys injected intracerebrally with guinea pig passage virus 26 failed to respond with any manifest symptoms of disease; 8 monkeys showed various signs of definite involvement of the central nervous system consisting of tremor, convulsions, facial palsy, and localized pareses; 1 monkey developed typical flaccid paralysis.

9. Following injection with cavian virus the virus may be recovered from the tissues of normal monkeys but not from the tissues of convalescent monkeys shortly after a paralyzing attack of poliomyelitis due to SK or Aycocock virus.

10. Immunization of monkeys with early cavian passage virus by the subcutaneous route has given no clear-cut evidence of protection against intracerebral reinfection with SK poliomyelitis virus. Neither has there been any evidence of effective interference in monkeys injected intravenously with early cavian passage virus and intracerebrally with RMV poliomyelitis virus.

11. The bearing of the experimental data upon the epidemiology of the human disease is discussed.

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