STUDIES OF A MURINE STRAIN OF POLIOMYELITIS VIRUS IN COTTON RATS AND WHITE MICE*

By CLAUS W. JUNGEBLUT, M.D., and MURRAY SANDERS, M.D.

(From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York)

Plates 18 to 20

(Received for publication, July 11, 1940)

Since the production of experimental poliomyelitis in monkeys, numerous efforts have been made to find other laboratory animals susceptible to this virus. However, all attempts to transmit the disease to rabbits, guinea pigs, ferrets, rats, and mice have either failed or produced inconclusive results (1). As a matter of fact, failure to infect animals other than monkeys has come to be generally accepted as one of the criteria necessary for the identification of freshly isolated strains suspected of being poliomyelitis virus.

In 1939, Armstrong announced successful passage of a fresh human strain of poliomyelitis virus (Lansing strain) from the monkey to Eastern cotton rats (*Sigmodon hispidus hispidus*) (2). The disease thus produced was characterized by the occurrence of flaccid paralysis of the extremities and by the presence in the anterior horn of lesions similar to those usually observed in monkeys and man (3). The identity of the cotton rat virus with the monkey virus was further indicated by the fact that the rodent virus produced classical poliomyelitis upon intracerebral injection in monkeys; however, immunological tests carried out in cotton rats, while highly suggestive, lacked sufficient regularity to be conclusive. Once the virus was well established in cotton rats the disease could be further transmitted to white mice (4). The mouse virus thus obtained proved highly pathogenic for cotton rats, but reproduction of poliomyelitis in monkeys was evidently not accomplished in typical fashion. That the process of adapting a monkey pathogenic strain of poliomyelitis virus to rodents is by no means an easy one becomes apparent from the difficulties experienced by other investigators in their attempts to repeat Armstrong's work with other strains of poliomyelitis virus. Toomey (5), for instance, has reported only negative results in his efforts to adapt to cotton rats nine other strains of monkey virus. His success in producing paralysis in cotton rats with the Lansing strain appears to be limited to virus which had previously been passed through cotton rats (6).

Preliminary observations, similar to those of Armstrong, were recently reported by us in experiments in which the SK New Haven strain of poli-

* Supported by a grant from the Philip Hanson Hiss, Jr., Memorial Foundation.

omyelitis virus was employed (7). The present communication is a complete report of these earlier observations, supplemented by further supporting data.

Isolation of a Murine Neurotropic Virus by Passage of Monkey Poliomyelitis Virus to Cotton Rats and White Mice

Three successful passages of SK poliomyelitis monkey virus to cotton rats and white mice will be described in detail. *Sigmodon hispidus littoralis* was used throughout this work instead of *Sigmodon hispidus hispidus*. To our knowledge, the only difference between the two varieties of cotton rats is geographic, *Sigmodon hispidus hispidus* occurring mostly in the mid-Southern states of the United States and *Sigmodon hispidus littoralis* in the extreme South.

1. First Isolation.—On Feb. 6, 1940, 2 cotton rats (52, 53) were inoculated intracerebrally with 0.05 cc. of an emulsion of glycerinated SK cord in its 11th monkey passage.[1] One cotton rat (53) died the following day, apparently of trauma, and was discarded; the other one (52) succumbed one week later without observed symptoms. Upon autopsy, no lesions could be found except a markedly congested brain which proved sterile upon aerobic and anaerobic cultivation. Intracerebral transfer of the brain of this cotton rat to another cotton rat (58) resulted in the development of mild nervous symptoms within 2 days, and death on the 3rd day, with similar autopsy findings. Further passage of the brain of the second cotton rat to a third cotton rat (60) produced in the latter animal complete flaccid paralysis of both hind legs and one foreleg on the 6th day, followed by death 24 hours later. From the last 2 cotton rats (58, 60), intracerebral transfers of brain suspensions were made to groups of white mice. All injected mice developed complete flaccid paralysis of the hind legs within 3 or 4 days, followed by generalized paralysis and death. 3 monkeys (AD88, AD89, AD90) were injected intracerebrally with cotton rat brain of the 1st, 2nd, and 3rd passages (52, 58, 60). All 3 animals survived without developing paralysis. Similarly, intracerebral transfers of these cotton rat brains to rabbits, guinea pigs, and albino rats failed to produce any symptoms (Chart 1).

2. Second Isolation.—Because several subsequent efforts to reproduce passage from the monkey to cotton rats with the original SK material were unsuccessful, an empirical attempt was made to retrace the steps taken during the first isolation. What seemed to have been the pivotal point of success was the fact that subpassage of a symptomless injected cotton rat had eventually led to the production of paralysis in both cotton rats and mice. Consequently, on Apr. 14, 1940, 2 cotton rats (107, 108) were injected intracerebrally with SK monkey cord in its 16th passage. Despite their entirely healthy appearance, both animals were killed on the 8th day, their brains removed, and the pooled suspension was passed to 2 new cotton rats (115, 116). Unlike the first isolation, one of the 2 cotton rats (116) of the 2nd passage developed on the 3rd day a paresis of the hind legs; the other one (115) showed mild nervous symptoms. Both animals

[1] We are indebted to Dr. John R. Paul for supplying us with this material.

died on the 4th day. The brains were removed and the pooled suspension was passed into another cotton rat (122). This animal developed complete paralysis of all extremities on the 3rd day and died shortly thereafter. Groups of mice were inoculated with cotton rat brain from the 1st, 2nd, and 3rd passages. No symptoms were observed with mice injected from the 1st cotton rat passage (107, 108). Mice injected with material from the 2nd cotton rat passage (115, 116) showed mild nervous symptoms,

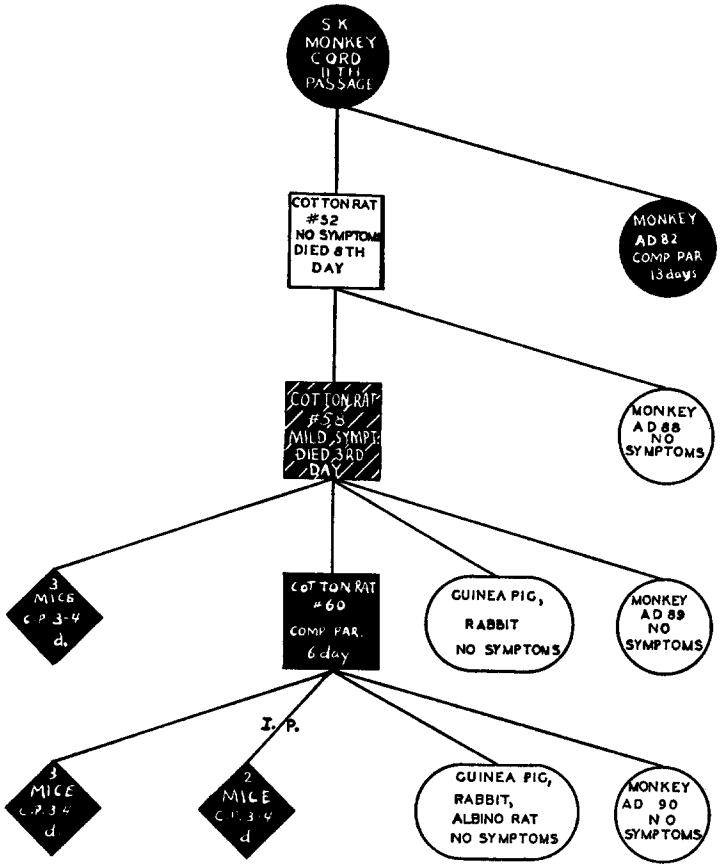


CHART 1. Genealogy of SK mouse virus (1st isolation). All injections intracerebral except where otherwise noted.

often terminated by death, but none of the animals developed manifest paralysis; by the 5th successive subpassage the virus was evidently lost. Similar symptoms were seen in the first mice inoculated with brain from the 3rd cotton rat passage (122). However, with subsequent subpassages, these symptoms became progressively more severe until frank flaccid paralysis occurred in all mice of the 5th successive subpassage.

Tests for monkey pathogenicity of the cotton rat virus were carried out at several points during the process of adaptation. Thus, 3 monkeys (AE96, AF11, AF8) were injected intracerebrally with cotton rat brain of the 2nd passage (115, 116), and another

monkey (AF5) with cotton rat brain of the 3rd passage (122). All injected monkeys showed signs of an overwhelming infection of the central nervous system. Monkeys AE96 and AF11 developed encephalitic symptoms consisting of coarse tremor and eyelid ptosis within 48 hours after injection, became prostrated and died at the end of the 2nd or 3rd day. Monkey AF8 died on the 2nd day with similar encephalitic symptoms and flaccid unilateral paralysis of the extremities (Fig. 1). Monkey AF5 succumbed with essentially similar symptoms on the 2nd day after injection. Sections from the brain and from various levels of the cord of these monkeys revealed a severe polio-encephalomyelitis, with widespread perivascular infiltration and pathognomonic destruction of ganglion cells (Fig. 2). By the time the cotton rat virus had produced typical paralysis in subpassaged mice, another monkey (AF44) was inoculated intracerebrally with brain from a paralyzed mouse. This monkey survived without showing any paralytic symptoms. Similarly, intracerebral transfers to rabbits, guinea pigs, and albino rats failed to produce symptoms of any sort (Chart 2).

As is apparent from the second genealogical chart, virulence for monkeys was present only during the early stages of the adaptive process when the infectious agent showed little or no pathogenicity for mice. After continued passage through mice had permitted the virus gradually to adapt itself more thoroughly to that host, virulence for monkeys was evidently lost. An impression had been gained during the first isolation that mouse pathogenicity and monkey pathogenicity of the SK virus might be mutually exclusive; but since transition of host pathogenicity had occurred rather suddenly, only the extreme end-points of this supposed selective action could be observed. In the second isolation, however, a definite oscillation of pathogenicity between the monkey and the mouse—mediated by passage through cotton rats—became clearly perceptible, probably because of the more gradual evolution of the adaptive process.

3. Third Isolation.—On June 7, 1940, a third attempt was made to repeat passage of SK monkey virus to cotton rats and white mice. The infectious material was a fresh cord, non-glycerinated, obtained from the 18th monkey passage of the SK strain. The freshness of the cord is emphasized since it may have had some bearing upon the unexpected variation in the outcome of this experiment. In contrast to previous isolations, which involved the principle of initial blind passage through cotton rats, the very first cotton rat (129) developed complete paralysis 2 days following intracerebral inoculation with SK monkey cord. The virus could be transmitted without difficulty by serial passage to other cotton rats (131, 133) and to white mice, producing in all injected animals severe and typical paralysis. Here again, pathogenicity for the monkey (AE99) was no longer present after a sudden explosive change in host virulence for mice had taken place. At the time of injecting the first cotton rat with virulent monkey cord, a portion of the same material had been inactivated by heating for one-half hour at 65°C. and the heated material injected into another cotton rat (130). This animal remained symptomless. Pursuing the plan of previous isolations, the brain of this cotton rat was passed serially to other cotton rats (132, 134) and to white mice. None of the injected animals developed any symptoms whatsoever (Chart 3).

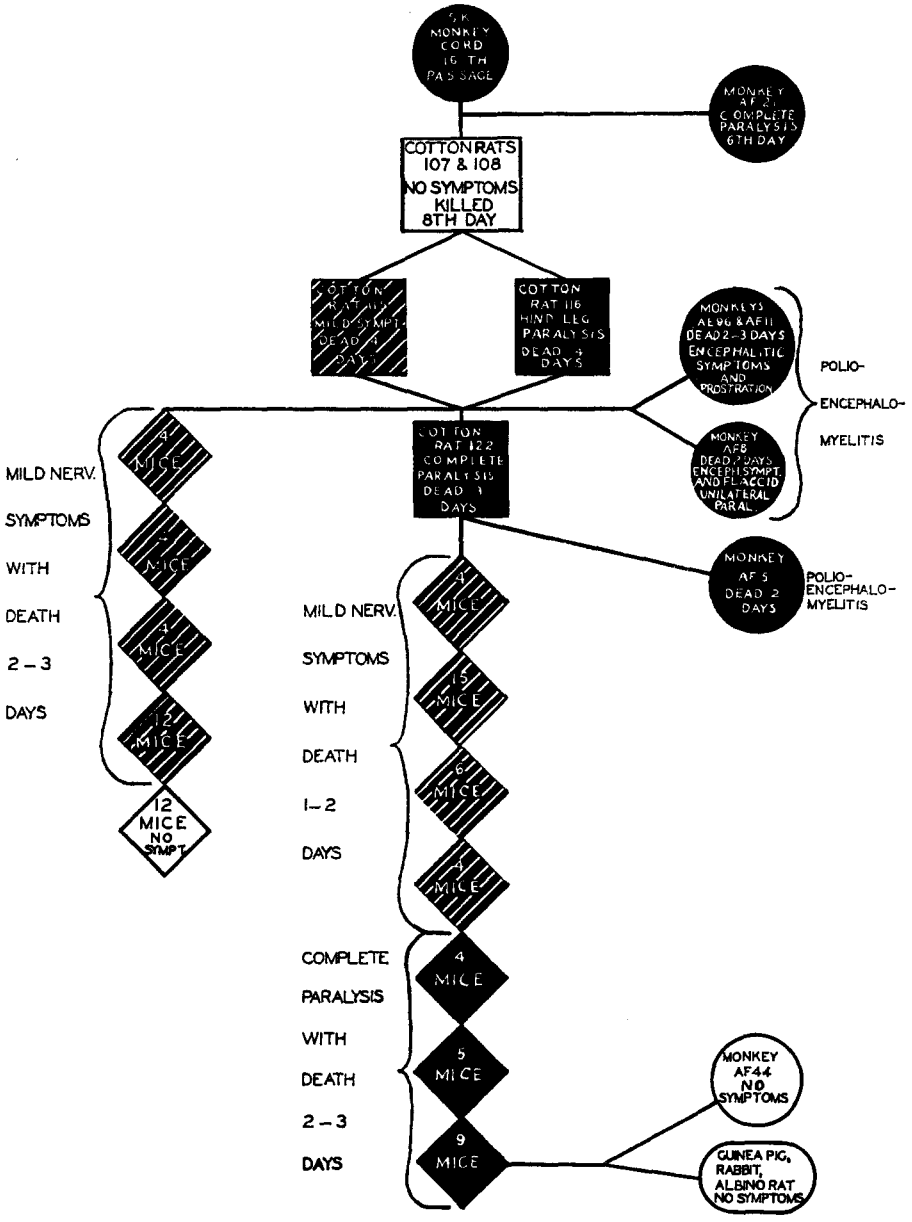


CHART 2. Genealogy of SK mouse virus (2nd isolation). All injections intracerebral.

As far as we have been able to determine, the three murine strains which were isolated on different occasions are identical in the symptomatology they produce, their host range, and their immunological characteristics.

The first and third strain appear to be slightly more virulent for mice (1:1,000,000) than the strain obtained upon second isolation (1:500,000). Judging from these facts and the complete agreement obtained in repeated experiments, there can be little doubt that the virus which produced paralysis in mice was the same agent, *i.e.*, SK virus, which produced paralysis in the monkey.

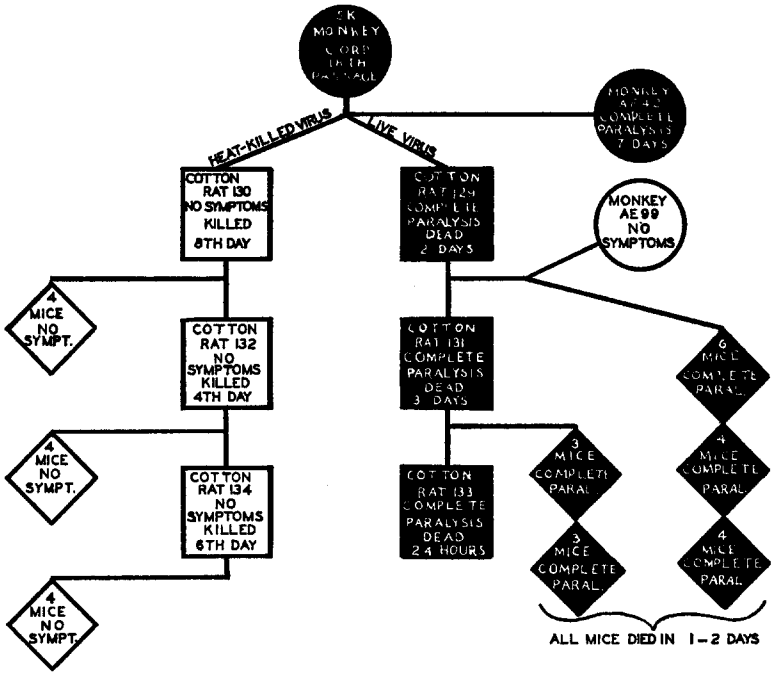


CHART 3. Genealogy of SK mouse virus (3rd isolation). All injections intracerebral.

Survival of SK Poliomyelitis Virus in the Cotton Rat

The above experiments suggested the desirability of determining more closely, if possible, the fate of SK monkey virus after its introduction into the brain of the cotton rat. The important question which posed itself was whether or not virus survived for a given length of time in the central nervous system of this rodent in a phase pathogenic for monkeys but not pathogenic for mice. Accordingly, four cotton rats were injected intracerebrally with 0.05 cc. of a 10 per cent suspension of glycerinated SK monkey cord. At various intervals following infection the symptomless cotton rats were killed, their brains removed and transferred intracerebrally to monkeys and to white mice. The results of this experiment are given in Table I.

It appears from Table I that monkey pathogenic SK virus could be recovered from a cotton rat 96 hours after infection and that this same material proved non-pathogenic for mice. Transfers made at the earlier and later intervals yielded negative results throughout. The data obtained are too few to permit of any generalizations as to the time factors which govern the carrier state in the cotton rat, or its precise chronological relation to the generation of the murine virus variant. They furnish additional evidence, however, that the SK virus, as long as it retains its power to paralyze monkeys, has not acquired pathogenicity for the mouse.

Physical Properties of the Murine Virus

Highly virulent brain or cord suspensions from paralyzed mice stained by various methods (ordinary bacterial stains and Giemsa stain), when

TABLE I

Survival of SK Poliomyelitis Virus in the Cotton Rat

Intracerebral infection of cotton rats with SK monkey virus	Interval of sacrifice	Intracerebral transfer of cotton rat brain to	
		Monkeys	Mice
Cotton rat 76	24 hrs.	AE10, no symptoms	4 mice: no symptoms
" " 77	48 "	AE11, " "	" " " "
" " 78	96 "	AE15, complete paralysis, 14 days	" " " "
" " 79	7 days	AE24, no symptoms	" " " "

examined microscopically, show no evidence of the presence of any characteristic morphological unit; the same is true for unstained material examined by dark field illumination. Seeding of common and special bacterial culture media, such as blood agar, broth, or 10 per cent serum broth, results in no growth visible in the gross or microscopically after prolonged aerobic or anaerobic incubation. On the other hand, the infectious agent passes through V, N, and W Berkefeld filters without appreciable diminution in virulence. The fact that the active principle is invisible but filters through the finer grade candles and that it refuses to grow on dead media, whereas multiplication is readily obtained *in vitro* in the presence of living cells (see later section on tissue cultures), establishes its virus nature beyond any question. The ease with which it filters through a W Berkefeld candle suggests a particle size consistent with the known dimensions of poliomyelitis virus.

Like the virus of classical poliomyelitis the mouse virus resists phenol up to 1 per cent concentration and tolerates acid to pH 4. In 50 per cent

glycerin it has remained viable, so far, up to a period of 4 months in the ice box. On the other hand, it is completely destroyed by heat for one-half hour at 60°C., and by exposure to ultraviolet light for one minute. High dilutions of virus suspensions in saline tend to deteriorate rather rapidly, particularly when kept at 37°C. Preliminary tests also indicate a very poor resistance of the virus against drying in the Flosdorf-Mudd apparatus, a fact which is of considerable interest in view of similar recent findings in the case of poliomyelitis monkey virus (8). Vitamin C inactivates the murine virus *in vitro* in a proportion of 1 mg. ascorbic acid to 5000 minimum paralytic doses of virus.

Cultivation of the Murine Virus in Tissue Culture

Since the growth requirements of this virus were utterly unknown, the three principal variables involved in its cultivation, *i.e.*, type of tissue, optimum temperature, and incubation period, were investigated systematically.

0.25 cc. of a virus brain emulsion from the 6th mouse passage was inoculated into serum ultrafiltrate tissue cultures. This technique had previously been utilized by Sanders (9) for the propagation of the lymphogranuloma venereum virus and had later been successfully adapted by Molloy (10) to the cultivation of the St. Louis encephalitis virus. The cultures were divided into three different groups: one group consisted of whole minced chick embryo (minus eyes and limbs), another of embryonic guinea pig brain, and the third of embryonic mouse brain. Parallel series of cultures were maintained in the incubator and at room temperature (23°C. ± 4°C.). Subcultures were made every 3 or 6 days by transferring either supernatant fluid or whole culture emulsion. Potency was determined at the 6th serial passage, when the original inoculum had been diluted beyond the point of possible infectivity (2×10^{-9}), by injecting mice intracerebrally with 0.03 cc. of supernatant or emulsion. Titration established the optimum conditions for cultivation of this virus as follows: (*a*) Embryonic mouse brain in serum ultrafiltrate, kept at 37°C., with 0.25 cc. of the supernatant fluid transferred every 3 days, was clearly the most effective type of culture, since an end-point of activity was not reached in spite of diluting the supernatant fluid 10,000,000 times. (*b*) Embryonic guinea pig preparations, under the same cultural conditions, were active up to a 1:10,000 dilution. (*c*) Embryonic chick cultures were the least effective, various combinations with this tissue yielding either no virus at all or merely traces of virus (Chart 4).

A control group consisted of flasks containing serum ultrafiltrate, but no tissue. 0.25 cc. of mouse virus suspension was passed in serial transmission through three such preparations. Tests for potency with the last preparation revealed no trace of virus in this medium.

Having established a basis for cultivation, a new series was started with 0.25 cc. of a virus brain emulsion from the 12th mouse passage. Now in

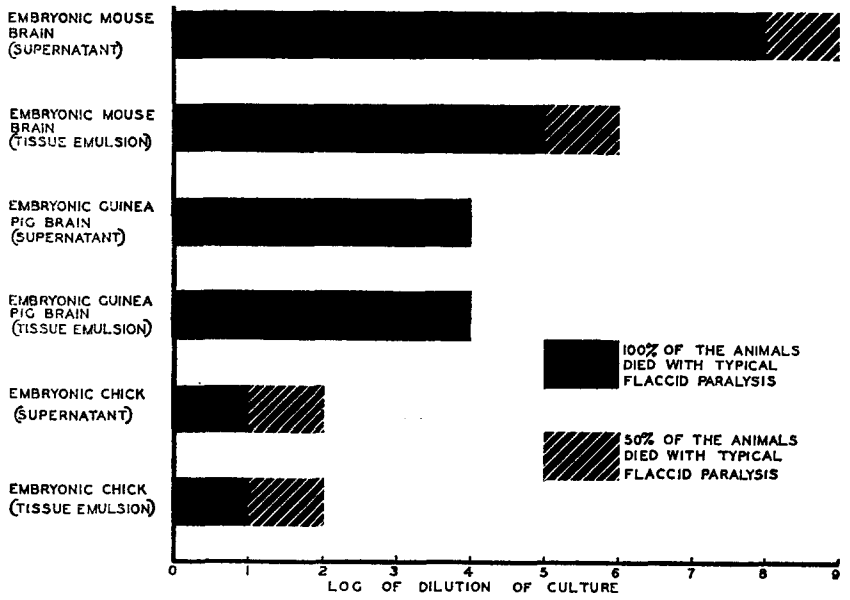


CHART 4. Cultivation of mouse virus in serum ultrafiltrate tissue cultures. Quantitative estimate of virus potency by mouse test.

TABLE II

Titration of the 14th Subculture of the Murine Virus in Serum Ultrafiltrate Tissue Culture

Dilution	Development of symptoms on successive days						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
10^{-4}	+, −, −, −	D, −, −, −	++, +, −	D, ++++, ++	D, D		
10^{-5}	+, −, −, −	+++, −, −, −	D, ++++, +, −	D, D, ++	D		
10^{-6}	−, −, −, −	−, −, −, −	++++, +, −, −	D, D, +, +	D, D		
10^{-7}	−, −, −, −	−, −, −, −	D, ++, −, −	D, ++, −	D, ++	D	
10^{-8}	−, −, −, −	−, −, −, −	−, −, −, −	+, −, −, −	D, ++, +, −	D, ++++, +	D, D
10^{-9}	−, −, −, −	−, −, −, −	−, −, −, −	++, ++, −, −	D, ++, −, −	D, −, −	−, −

Dilutions were made from supernatant fluid of tissue culture; 4 mice were injected intracerebrally with 0.03 cc. of each dilution.

− = no symptoms.
+ = a definitely sick animal showing awkwardness or paresis but no paralysis.
++ = one limb paralyzed.
+++ = two limbs paralyzed (usually hind legs).
++++ = near death. Complete paralysis.
D = died.

its 26th continuous passage, this series has consistently maintained an extraordinarily high level of potency. When tested in its 14th generation, the titer of the culture reached one to a billion (Table II).

Symptomatology of the Murine Infection and Virulence of the Virus for Mice

White mice of several breeds (Swiss, Paris, Rockland) may be successfully infected by any one of the following routes: intracerebral, intranasal, intraperitoneal, intravenous, subcutaneous, and by feeding. The virulence of the virus depends upon the infectious dose and the route of injection, but its unusual pathogenicity for mice has been an outstanding and regular characteristic throughout this work. When injected intracerebrally a constant titer of 1:1,000,000 is obtained, with an occasional end-point of 1:20,000,000. With larger doses, the incubation period may be as short as 48 hours, becoming progressively lengthened with increasing dilutions of the virus. The maximum incubation period so far has not exceeded one week. Intraperitoneal injection of 0.1 cc. of 1:10,000 dilution of a virus brain suspension has uniformly produced paralysis within from 3 to 5 days, with not a single recovery. The fact that infection may be obtained from the gastro-intestinal tract under conditions simulating natural exposure is of particular interest; first, because the original SK strain was isolated from feces of a human abortive case, and second, because unforeseen spread of the infection may occur in cages containing multiple animals due to the cannibalistic habits of mice. The average weight of the mice was 15 to 20 gm., but older (20 to 25 gm.) and younger (10 to 15 gm.) mice were also used on occasion. Within these brackets no appreciable difference could be detected in age susceptibility to intracerebral or peripheral infection. A summary of the results is given in Table III.

As previously stated, the outstanding and often single symptom of the infection in mice is a flaccid paralysis of the hind legs, usually bilateral, but occasionally affecting only one limb; the front legs are seldom involved. Most animals show a fine tremor over the ears and present a sleek fur. Once paralysis has set in, the disease rapidly progresses to a fatal end; no animal reaching the definitely paralytic stage was seen to recover. Beginning with the 26th serial mouse passage, a certain number of infected mice went through an encephalitic phase before the development of flaccid paralysis. This syndrome consisted of a coarse tremor, hunched back, rough coat, and general uncertainty of movements. It was thought that the appearance of these symptoms was caused, in part, by the rapid intracerebral passage of virus brain from mouse to mouse. However, 14 parallel series in which one group of mice was infected with spinal cord emulsions, whereas the other was continued with brain emulsions, revealed no difference in the proportion of encephalitic mice between the two groups. At the present writing, the murine virus is in its 46th serial mouse passage; all of the infected animals develop flaccid paralysis, but approximately 30 to 40 per

cent show, in addition, transitory encephalitic symptoms. It is of interest to note that virus brain from the early passages, which has been preserved in glycerin for several months, still produces the clear mono-symptomatic picture of the disease which was so highly characteristic of all three fresh isolations (Fig. 3).

In view of the fact that the described symptoms bear some resemblance to those of Theiler's spontaneous encephalomyelitis in mice, a group of 25 mice from a colony immune to Theiler's virus were infected intracerebrally

TABLE III

Virulence of the Murine Virus for Mice by Different Routes of Infection

Route of infection	No. of mice	Virus dilution	Results		
			Length of incubation period	Percentage of mice paralyzed and dead	
			days	*per cent*	*days*
Intracerebral	136	1:10	1–2	100	(1–2½)
	20	1:100	1–2	"	(1–3)
	293	1:1000	1–3	"	(2–4)
	61	1:10,000	2–3	"	(3–5)
	93	1:100,000	3–4	"	(4–7)
	38	1:1,000,000	4–6	97.4	(5–7)
	12	1:10,000,000	4–6	91.7	(5–8)
Intraperitoneal	22	1:10	2–3	100	(2–4)
	29	1:1000	3–4	"	(4–5)
	31	1:10,000	3–5	"	(4–7)
	20	1:100,000	4–6	"	(5–7)
	6	1:1,000,000	4–6	83.3	(5–7)
Intranasal	12	1:10	2–3	100	(3–4)
	15	1:1000	3–6	"	(5–7)
Intravenous	12	1:100	2	"	(3)
Feeding					
(*a*) by dropper	12	1:10	4–6	"	(5–9)
(*b*) by bread soaked in virus	6	1:10	3–5	"	(6–8)

or intraperitoneally with the murine poliomyelitis virus. These mice developed the disease in exactly the same manner as an accompanying group of 25 normal controls. The apparent lack of cross-protection in this experiment clearly indicates the separate nature of the two viruses.

Pathogenicity of the Murine Virus for Laboratory Animals Other than Mice

The mouse virus is easily transferable to cotton rats and produces in that species all the symptoms that are observed in mice (Fig. 4). In marked contrast to the white mouse and cotton rat, the murine virus induces no symptoms whatsoever after repeated injections by a variety of routes in

other rodents, such as albino rats (8 animals) and guinea pigs (9 animals). Similarly, intracerebral, intravenous, and subcutaneous injections of large amounts of virus into rabbits (9 animals) have failed to produce symptoms of any sort.

The pathogenicity of the mouse virus for *rhesus* monkeys is questionable. Let it be said at the outset that we have never observed the occurrence of frank paralysis in a single monkey within a sizable group of animals which had received large amounts of fixed mouse virus by repeated intracerebral, intravenous, or subcutaneous injection. However, while the virus has failed to paralyze, closer inspection of infected monkeys revealed the fact that many of these animals had reacted to the virus inoculations with sharp rises in temperature and the development of transitory nervous symptoms. This febrile response is usually seen within 24 hours and, after reaching a peak of 1–4°F. above normal, subsides within 2 or 3 days. The nervous symptoms consist of restlessness, faint tremor, disturbances of the eye muscles (eyelid, pupils), awkwardness or weakness of the hind limbs. Within one week the above symptoms, without exception, had again completely vanished. Of a total of 22 monkeys injected with the mouse virus on various occasions, 18 animals reacted in the above described manner. 3 monkeys injected intracerebrally with brain suspensions from cotton rats, paralyzed by mouse virus, presented essentially the same picture (Fig. 5). Intracerebral transfer of the brain and cord of these monkeys, sacrificed at the height of the syndrome, failed to produce any symptoms in new monkeys.

Pathology of the Murine Infection in Mice and in Rhesus Monkeys

Upon autopsy of paralyzed mice, a brain congested in the gross is found. In some parts of the brain diffuse proliferation of glia cells and occasional foci of perivascular infiltration may be observed. Sections of the cord reveal severe damage of the ganglion cells in both anterior horns. This damage extends all the way from loss of Nissl substance and irregular staining of the nucleus to a complete breakdown of the nerve cell with subsequent neuronophagia. Microglial proliferation is widespread at some levels as is perivascular infiltration. The intensity of the pathological process increases with the time interval that has elapsed since the onset of symptoms. In mice which have died following a period of protracted paralysis, it is often difficult to discover any intact nerve cells in the entire area of a given anterior horn. Characteristic morbid changes in organs other than the central nervous system have so far not come to our attention. Similar lesions may be observed in the brain and cord of paralyzed cotton

rats (Fig. 6). The lesions in the paralyzed rodents are therefore entirely consistent with the accepted pathology of poliomyelitis in man and monkey (Figs. 7 to 10).

It will be remembered that the fixed mouse virus, although failing to paralyze monkeys upon intracerebral inoculation, sets up a train of fairly definite clinical symptoms during the first few days following infection. It is therefore not surprising that careful examination of the spinal cord of monkeys which had been sacrificed at intervals after intracerebral injection of mouse virus should yield evidence of the presence of certain abnormal changes. These changes, in their early stages, concern essentially the structure of the ganglion cells in the anterior horn. While many nerve cells remain intact, others show various cytological alterations ranging from poor staining and dissolution of the nucleolus and nucleus to a disappearance or aggregation of Nissl substance along the cell wall. Occasionally, cells are found which present a coalescent appearance of the entire cytoplasm, but actual cellular destruction has not been observed. The more severely affected nerve cells often are surrounded by clear retraction zones; others are heavily satellized by groups of phagocytic cells. Diffuse gliosis and multiple hemorrhages are well marked in some sections, particularly those from monkeys which had been sacrificed at the later intervals (Figs. 11 to 14).

Systemic Distribution of the Murine Virus in Susceptible and Refractory Animals

The characteristic paralysis produced in susceptible animals (mice, cotton rats), taken together with the unique restriction of lesions to the central nervous system, clearly point to a marked neurotropism of the murine virus. Even in the relatively insusceptible monkey, the infectious agent is capable of inducing nervous changes below the threshold of actual paralysis. However, since the virus possesses the ability to infect mice by many peripheral routes, the question arises as to whether virus is strictly confined to the tissue in which the ultimate damage occurs, *i.e.*, the central nervous system, or whether active virus is also present in other organs and in the blood stream. Coupled with this problem is the uncertainty whether or not seemingly refractory animals can function as latent carriers of the virus without showing manifest paralysis. In an effort to answer these questions, a series of experiments were carried out in which attempts were made to recover the virus, at several intervals after infection, from various tissues of susceptible as well as refractory animals.

1. Recovery of Mouse Virus from Infected Mice.—5 mice infected intra-

cerebrally with mouse virus were killed at the height of paralysis. The brain, cord, spleen, liver, suprarenal, and heart's blood were tested for the presence of virus by intraperitoneal and intracerebral inoculation of the respective tissue emulsions into groups of mice. All mice inoculated developed typical flaccid paralysis and died, indicating a widespread distribution of the infectious agent over neural and extraneural tissues at the time the disease had been fully developed.

A second experiment was planned in order to study the dissemination of the virus at various phases of the evolution of the disease. Groups of mice were infected intravenously or intraperitoneally with a heavy dose of virus (brain emulsion 1:10) and killed at intervals of 2, 4, 7, 12, 24, and 48 hours following infection. When blood and brain were tested by intracerebral and intraperitoneal transfer to mice, virus could be recovered at all intervals from both sites. The extraordinary invasiveness of the infectious agent was indicated by the fact that as early as 2 hours after intraperitoneal injection the virus was present in the blood stream. Admittedly, the experimental set up does not permit of any clear quantitative interpretation of the results. However, since mice receiving blood from infected animals at the 24 hour interval showed a marked delay in the appearance of symptoms (72 hours), it was thought likely that the circulating virus concentration may have undergone a temporary depression at that time. By 48 hours the virus content of the blood appeared to have returned to its high level, judging from the rapidity with which these transfers produced symptoms. No such fluctuations in the length of the incubation period were observed with mice which had received brain tissue of the infected animals (Table IV).

A correct evaluation of the above results is difficult because of the enormous virulence of the infectious agent for mice. However, the data seem to suggest that the infectious process, from its very beginning, is composed of a neural, as well as an extraneural, systemic phase.

2. Recovery of Mouse Virus from Infected Guinea Pigs, Albino Rats, and Rabbits.—Pairs of guinea pigs, albino rats, and rabbits received intracerebral injections of a large dose of mouse virus (1:10 virus brain suspension). At the end of 48 hours, one animal from each group was sacrificed and the brain, cord, liver, spleen, and blood were tested for the presence of virus by intracerebral and intraperitoneal transfer of the respective tissue emulsions into mice. The same procedure was repeated at the 96 hour interval with the remaining animals of each pair. It will be seen from Table V that active virus was recovered, at the early interval, from practically every tissue of the guinea pig and the albino rat. On the other hand, all rabbit

tissues proved sterile, excepting one dubious early transfer from the site of inoculation.

TABLE IV

Recovery of Murine Virus from Infected Mice

Mode of infection	Interval of sacrifice	Virus content of brain*	Incubation period	Virus content of blood*	Incubation period
	hrs.		*days*		*days*
Intravenous	2	++	3-4	+++	2-3
	4	+++	2-3	+++	2-3
	7	++++	1-2	+++	2-3
	12	++++	1-2	+++	2-3
	24	++++	1-2	++	2-3
	48	++++	1-2	++	3-4
Intraperitoneal	2	++	3-4	++++	1-3
	4	+++	2-3	++++	1-3
	7	++++	1-2	++++	1-3
	12	++++	1-2	+++	2-4
	48	++++	1-2	++	3-4

* Amount of virus present in tissue evaluated by the length of the incubation period in injected animals.

TABLE V

Recovery of the Murine Virus from Refractory Animals after Intracerebral Injection

Animal	Interval of sacrifice	Tissues tested for virus content				
		Brain	Cord	Liver	Spleen	Blood
	hrs.					
Albino rat	48	+++	++	−	++	+++
	96	+++	++	+	−	−
Guinea pig	48	+++	−	+	+++	
	96	+++	+	−	−	−
Rabbit	48	−	−	−	−	−
	96	−	−	−	−	−

Tissues were transferred to mice intracerebrally as well as intraperitoneally.

− = no virus present.

\+ = trace of virus present as indicated by long incubation period (5 to 8 days). Only one of the two routes of inoculation produced paralysis in mice.

++ = paralysis in mice after a short incubation period (1 to 3 days). One of the two routes effective.

+++ = paralysis in mice after a short incubation period by both intracerebral and intraperitoneal routes.

The apparent selectivity in the reaction of the guinea pig and albino rat to the murine virus, as contrasted with the reaction of the rabbit, seems to be of special significance in view of the close zoological relationship which exists between the first mentioned group of rodents and the mouse

and cotton rat. The nature of the factor in the rabbit responsible for the rapid destruction of the murine virus is still obscure.

3. Recovery of Mouse Virus from Infected Rhesus Monkeys.—6 normal *rhesus* monkeys each received a dose of 1 cc. of mouse virus (brain emulsion 1:10) intracerebrally. At intervals of 24, 48, 96 hours, 1 and 2 weeks following infection, these animals were sacrificed and their organs tested for virus content in the manner previously described. Virus was present in all tissues during the early intervals. By the time one week had elapsed, however, the virus had left the blood and liver and was encountered only in the central nervous system and in the spleen. No virus could be demonstrated at the end of 2 weeks.

Since this experiment had shown the persistence of the mouse virus for at least one week in normal monkeys, it became of interest to determine the fate of the virus in monkeys which had recovered from poliomyelitis. A total of 6 convalescents (4 SK convalescents and 2 Aycock convalescents) were injected intracerebrally with the same dose of mouse virus as was used before, and were sacrificed at similar intervals for the transfer of brain and cord to mice. Virus was recovered irregularly from the SK group, but in larger amounts from the Aycock convalescents.

A third experiment was run in which 2 normal and 2 SK convalescent monkeys received a heavy dose of mouse virus (4 cc. of a 1:10 brain emulsion) by subcutaneous injection. The 4 animals were sacrificed in pairs, 48 and 96 hours after infection, and the virus content of their organs was determined by the usual method of transfer to mice. In the 48 hour test, virus was recovered in large amounts from every neural and extraneural tissue of the normal monkey, but not from the convalescent monkey. At 96 hours, virus was present only in the spleen of both animals.

The results of all three experiments are brought together in Table VI. It appears from this summary that normal *rhesus* monkeys, infected intracerebrally with the murine virus, continue to carry large amounts of the infectious agent in the brain and cord for approximately one week; also, that subcutaneous infection leads to an actual invasion of the central nervous system, with active virus being demonstrable for a period of at least 48 hours. The early presence of the virus outside of the central nervous system, particularly in the spleen, is analogous to similar observations with other species of infected animals; but the neurotropism of the virus emerges more clearly from these experiments because of the transitory nature of such extraneural carriage. The fact that the virus survives in the normal monkey for considerable time, without producing paralysis, substantiates its peculiarly limited pathogenicity for that host, which had already been suggested by the clinical and pathological findings.

These experiments show, furthermore, that the tissues of poliomyelitis convalescent monkeys, infected intracerebrally with mouse virus, are found relatively, if not absolutely, free of the infectious agent. Again, since peripheral injection of the murine virus was not followed by any invasion

TABLE VI

Recovery of the Murine Virus from Normal and Convalescent Monkeys after Intracerebral or Subcutaneous Injection

Monkey	Route of infection	Interval of sacrifice	Tissues tested for recovery of virus					
			Brain	Cervical cord	Lumbar cord	Liver	Spleen	Blood
		hrs.						
Normal	Intracerebrally	24	+++	+++	+++			?
"	"	24	+++	+++	+++	+++	++	+++
"	"	48	+++	+++	+++			
"	"	96	+++	+	+++			
		wks.						
"	"	1	+++	+++	+++	−	++	−
"	"	2	−	−	−	−	−	−
		hrs.						
SK convalescent	"	24	?	?	+			?
" "	"	48	?	+	?			
" "	"	D 72	+	?	?			
" "	"	72	?	?	?			
Aycock convalescent	"	D 24	+++	+++	?			
" "	"	48	?	?	+++			
Normal	Subcutaneously	48	+	+++	+	+++	+++	+++
"	"	96	−	−	−	−	++	−
Convalescent	"	48	−	−	−	−	−	−
"	"	96	−	−	−	−	+	−

? = atypical.
− = no virus present.
+ = trace of virus present as indicated by long incubation period (5 to 8 days). Only one of the two routes of inoculation produced paralysis in mice.
++ = paralysis in mice after a short incubation period (1 to 3 days). One of the two routes effective.
+++ = paralysis in mice after a short incubation period by both intracerebral and intraperitoneal routes.

of the central nervous system in one convalescent monkey, the operation of some cross-immunity factor may well be suspected.

Neutralization in Vitro of the Murine Virus by Poliomyelitis Antisera and of Poliomyelitis Virus by Mouse Virus Antisera

Inasmuch as neutralization by serological methods is generally considered an important aid in the identification of a given virus, the murine virus was tested for its neutralizability by various antisera.

The following sera were used: monovalent immune sera from monkeys convalescing from infection with RMV, Aycock, or SK virus; hyperimmune RMV horse serum; pooled human convalescent serum, as well as normal serum from man, monkey, and horse. The monkey convalescent sera were tested and proved virucidal for their homologous strains of virus; cross-neutralization was also obtained between SK virus and Aycock convalescent serum but not with RMV convalescent serum. However, the RMV hyperimmune horse serum also inactivated SK virus. For purposes of control, several antiviral immune sera against other neutropic viruses (Theiler mouse encephalitis, equine encephalomyelitis, rabies, St. Louis encephalitis, and herpes) were also included. The technique employed was as follows: 0.02 cc. of virus in dilutions ranging from 1:20,000 to 1:100 was mixed with 0.18 cc. of the test serum (ratio 1:10) and incubated at 37°C. for 1½ hours. After remaining in the ice box overnight, the serum-virus mixtures, in a volume of 0.03 cc., were inoculated intracerebrally into groups of 4 to 6 mice.

The results obtained in repeated tests may be summarized as follows: Whereas the normal animal and human sera, as well as the other antiviral immune sera, failed uniformly to bring about inactivation of the murine virus at its highest effective dilution, *i.e.*, 1:200,000, convalescent Aycock and SK monkey sera and convalescent human serum neutralized at the same level or slightly below, *i.e.*, 1:100,000 to 1:50,000. RMV monkey convalescent serum failed to neutralize the virus, but a high degree of neutralization, extending through a virus concentration of 1:1000, was obtained with the RMV antipoliomyelitis horse serum.[2] The seemingly contradictory action of the two RMV sera on the murine virus is paralleled by their analogous action on the SK virus in monkeys. Evidently, the enormous potency of the hyperimmune horse serum sufficed to break down immunological strain differences based on neutralization with comparatively weak convalescent sera.

The results of these experiments are shown in Chart 5. So as to illustrate better the uniformity in the inactivation obtained with the hyperimmune horse serum, a typical example of a neutralization test with this serum is given in Table VII.

In order to complete the serological analysis, neutralization tests were also carried out in which mouse virus antisera were tested for their ability to inactivate poliomyelitis virus in monkeys.

Immune sera against the murine virus were prepared by injecting one group of rabbits with a set of 12 gradually increasing doses of mouse virus brain and another group with tissue culture mouse virus. Control sera were prepared by injecting two additional groups of rabbits with either normal mouse brain or with uninoculated

[2] This serum (pseudoglobulin fraction) was obtained through the courtesy of Dr. M. Schaeffer, from the New York City Department of Health, and was stated to neutralize RMV virus in monkeys up to a dilution of 1:1500.

tissue culture medium. The emulsified mouse brains were given intraperitoneally, the tissue cultures by both the intravenous and intraperitoneal routes. All animals were bled after the 6th and 12th injection, respectively, and the sera of each group were

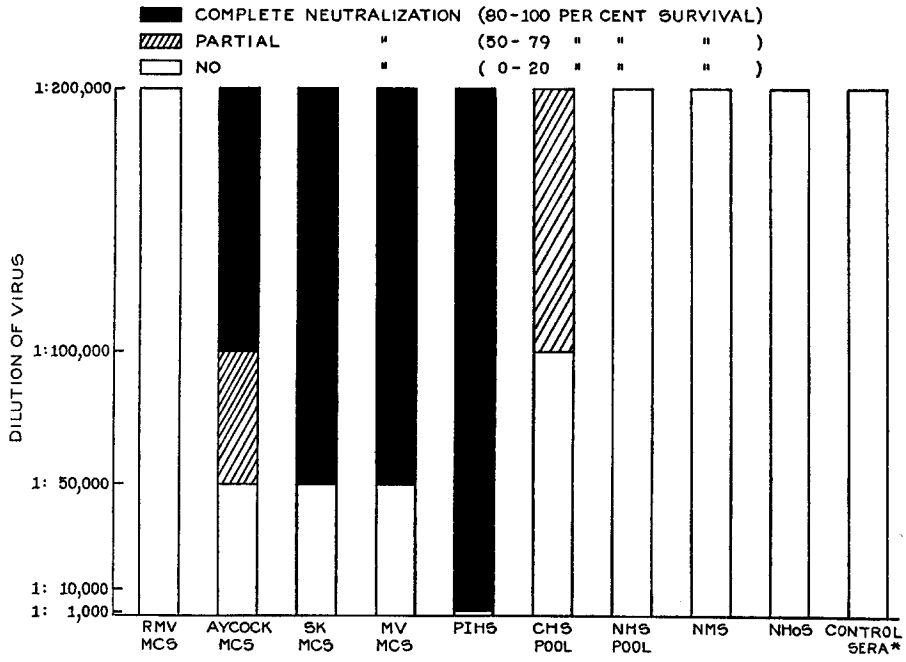


CHART 5. Comparative levels of mouse virus neutralization by various poliomyelitis convalescent, immune, normal, and other antiviral sera.

Technique.—Sera were used undiluted; ratio of virus to serum = 1:10; serum-virus mixtures were injected intracerebrally into groups of mice (4 to 6) following incubation for 1½ hours at 37°C. and overnight in the ice box.

RMV MCS = RMV monkey convalescent serum.
Aycock MCS = Aycock monkey convalescent serum.
SK MCS = SK monkey convalescent serum.
MV MCS = mouse virus monkey convalescent serum.
PIHS = poliomyelitis immune horse serum.
CHS = convalescent human serum.
NHS = normal human serum.
NMS = normal monkey serum.
NHoS = normal horse serum.

* Immune sera against the following viruses: spontaneous mouse encephalitis, rabies, Eastern equine encephalomyelitis, Western equine encephalomyelitis, St. Louis encephalitis, herpes.

pooled. Neutralization tests were carried out with the immune and control sera against the murine virus in mice and against three strains of poliomyelitis virus in monkeys.

The results are brought together in Table VIII.

It appears from this table that the two antiviral sera inactivated the

mouse virus through virus dilutions between 1:1000 and 1:10,000, whereas the two control sera had no demonstrable virucidal power. Somewhat irregular results were obtained with respect to the ability of the same immune sera to neutralize poliomyelitis virus. While the antiviral sera were capable of inactivating the Aycock virus in repeated tests, neutralization of the SK virus occurred only once. No neutralization could be obtained against the RMV virus in any of these tests.

TABLE VII

Neutralization of the Murine Virus by Antipoliomyelitis (RMV) Horse Serum (Pseudoglobulin Fraction)

Virus dilution	Daily readings								
	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
1:100	1 D post-inoc., −, −	+, ++	D, D						
1:1000	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −
1:10,000	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −
1:50,000	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −
1:100,000	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −
1:1,000,000	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −	−, −, −
Control Serum: Normal Horse Serum									
1:100	−, −, +	+, +++, D	++, D	D					
1:1000	−, −, +	++, ++, ++++	D, D, ++	D					
1:10,000	−, −, −	−, +, +	+, D, D	D					
1:50,000	−, −, −	−, +, +	++, ++, D	D, D					
1:100,000	−, −, −	−, −, +	−, +, D	+, +	+, +	+++, +	D, −	−	−
1:1,000,000	1 D post-inoc., −, −	+, +	+, +	++++, D	D				

− = no symptoms.
\+ = a definitely sick animal showing awkwardness or paresis but no paralysis.
++ = one limb paralyzed.
+++ = two limbs paralyzed (usually hind legs).
++++ = near death. Complete paralysis.
D = death.

Similar neutralization tests were carried out with sera obtained from monkeys which had been immunized by a series of subcutaneous injections of either mouse brain virus or mouse tissue culture virus (see later section on immunization). The results are shown in Table IX. Considering the data as a whole, it will be gathered that neutralization of SK virus was obtained in 5 instances and neutralization of Aycock virus in 4 instances, whereas the same immune sera, with one exception, failed to inactivate RMV virus. All sera showed fair virucidal action (1:10,000 to 1:50,000) against the murine virus in mice.

TABLE VIII

Neutralization of the Murine Virus and of Poliomyelitis Virus (SK, Aycock, RMV) by Antimurine Virus Rabbit Serum

Antiserum against mouse virus	Neutralization of the murine virus in mice Titer *	Neutralization of poliomyelitis virus in monkeys		
		SK 0.9 cc. serum + 0.1 cc. 10% virus	Aycock 0.9 cc. serum + 0.1 cc. 10% virus	RMV 0.9 cc. serum + 0.05 cc. 10% virus
Mouse brain virus serum 1st bleeding	1:1000	Paralyzed	No paralysis	Paralyzed
Control: Normal mouse brain serum 1st bleeding	No neutralization through 1:100,000	"	Paralyzed	"
Mouse brain virus serum 2nd bleeding	1:10,000	No paralysis	No paralysis	"
Control: Normal mouse brain serum 2nd bleeding	No neutralization through 1:100,000	Paralyzed	Paralyzed	"
Tissue culture virus serum 1st bleeding	1:1000	"	"	"
Control: Normal tissue culture serum 1st bleeding	No neutralization through 1:100,000	"	"	"
Tissue culture virus serum 2nd bleeding	1:10,000	Not done	Not done	Not done
Control: Normal tissue culture serum 2nd bleeding	No neutralization through 1:100,000	" "	" "	" "

* Largest amount of virus inactivated by undiluted serum.

TABLE IX

Neutralization of the Murine Virus and of Poliomyelitis Virus (SK, Aycock, RMV) by Antimurine Virus Monkey Serum

Antiserum against mouse virus	Neutralization of the murine virus in mice Titer *	Neutralization of poliomyelitis virus in monkeys		
		SK 0.9 cc. serum + 0.1 cc. 10% virus	Aycock 0.9 cc. serum + 0.1 cc. 10% virus	RMV 0.9 cc. serum + 0.05 cc. 10% virus
Mouse brain virus serum 1	1:10,000	No paralysis	No paralysis	No paralysis
" " " " 2	1:10,000	Paralyzed	" "	Paralyzed
" " " " 3	1:10,000	No paralysis	Paralyzed	"
" " " " 4	1:50,000	" "	"	"
" " " " 5	1:10,000	Paralyzed	"	"
" " " " 6	1:50,000	"	Died	"
" " " " 7	1:10,000	Died	Paralyzed	"
" " " " 8	1:10,000	Paralyzed	"	"
Control: Normal monkey serum	No neutralization through 1:100,000	"	"	"
Pooled mouse brain virus serum	1:50,000	No paralysis	No paralysis	"
Pooled tissue culture mouse virus serum	1:50,000	" "	Died 9th day no paralysis	Died 5th day no symptoms
Control: Normal monkey serum	No neutralization through 1:100,000	Paralyzed	Paralyzed	Paralyzed

* Largest amount of virus inactivated by undiluted serum.

The results of the experiments described in this section indicate that convalescent sera which are capable of inactivating SK and Aycock poliomyelitis virus in monkeys also possess neutralizing power for the murine virus in mice and, *vice versa*, that immunization with mouse virus leads to the formation of antibodies which are virucidal for the murine virus as well as for SK and Aycock monkey poliomyelitis virus. Why the antimurine virus sera prepared in monkeys should inactivate poliomyelitis virus better than those prepared in rabbits is not clear, even though the rabbit is known to function ineffectively in the production of antipoliomyelitis serum (11, 12); were it not for the fact that the rabbit immune sera possessed strong virucidal properties against the murine virus, one might feel inclined to attribute this defection to the rapid destruction of the antigen in the rabbit (see earlier section). The inability of the antimurine virus sera to inactivate RMV virus as well as the absence of neutralization between RMV convalescent serum and the murine virus are in good agreement with the serological characteristics of the original SK strain, as described by Trask, Paul, and Vignec (13) in their identification of this virus upon its first isolation from man. The close parallelism in antigenic pattern, therefore, between SK monkey virus and the murine virus is additional evidence of the essential identity of the two infectious agents.

We should briefly mention in passing that diphtheria antitoxin (pseudoglobulin), in several tests, proved capable of inactivating comparatively large doses of the murine virus (1:10,000 to 1:50,000). In further experiments it was found that mice which had previously been immunized with diphtheria toxoid showed some increased resistance against subsequent intracerebral or intraperitoneal infection with the murine virus. The significance of these non-specific phenomena, at present, remains unexplained.

Immunization of Monkeys with the Murine Virus against Infection with Poliomyelitis Virus

In order to determine whether the murine virus was capable of cross-protecting against infection with monkey poliomyelitis virus, monkeys were immunized with suspensions of live mouse virus and subsequently infected with monkey poliomyelitis virus. Two such experiments were carried out.

In the first experiment, 8 animals received subcutaneous injections, twice weekly, for 3 weeks of 1 cc. of a 30 per cent emulsion of live mouse virus brain. At the end of immunization the animals were given a rest period for 10 days and divided into three groups. The first group (3 monkeys) was infected with 1 cc. of a 1:10 dilution of a 10 per cent suspension of SK monkey virus, the second (3 monkeys) with a similar dose of

Aycock virus, and the third (2 monkeys) with 1 cc. of a 1:50 dilution of a 10 per cent suspension of RMV virus. The virus in all cases was given intracerebrally. 8 normal control monkeys were infected with identical doses of the three viruses. No paralysis of the extremities occurred in a group of 3 immunized monkeys following infection with the homologous monkey virus, *i.e.*, SK, although one animal showed a faint transitory facial paresis. On the other hand, all immunized monkeys which were infected with the two heterologous strains, *i.e.*, Aycock or RMV virus, became paralyzed. All 8 controls succumbed to the disease.

In a second experiment, a group of 7 monkeys were immunized by similar methods, except that an attempt was made to induce a greater degree of immunity by administering larger doses of virus over a longer period of time. 4 of the animals received 9 subcutaneous injections of a 30 per cent emulsion of mouse virus brain in increasing dosage

TABLE X

Immunization of Monkeys with Live Murine Virus against Infection with Various Strains of Monkey Poliomyelitis Virus

	No. of monkeys	Intracerebral infection with poliomyelitis virus	Result	
			Paralysis	No paralysis
Experiment I	3 immunized	SK	0	3*
	3 controls	"	3	0
	3 immunized	Aycock	3	0
	3 controls	"	3	0
	2 immunized	RMV	2†	0
	2 controls	"	2†	0
Experiment II	7 immunized	SK	3	4
	6 controls	"	5	1

* One monkey showed slight transitory facial paresis.

† One monkey found dead on 10th day; sections showed typical cord lesions.

(1 cc. to 5 cc.) twice a week; the 3 remaining monkeys received similar amounts of supernatant fluid of virus tissue cultures. After a rest period of 10 days, the 7 immunized monkeys, together with 6 normal control animals, were infected intracerebrally with 1 cc. of a 1:10 dilution of a 10 per cent suspension of SK monkey virus. The results of this experiment were as follows: 4 of the 7 immunized monkeys remained entirely free from any symptoms, whereas 3 animals developed paralysis. In contrast herewith, 5 of the 6 controls became paralyzed, while 1 monkey apparently escaped the disease after a marked febrile period which lasted for 7 days.

Although the result of the second experiment is, perhaps, not as good as that of the first, the fact remains that in these two experiments 7 of 10 immunized animals were protected against infection with the homologous monkey virus, *i.e.*, SK, whereas 8 of 9 similarly infected controls developed typical and severe paralysis (Table X).

Interference of the Murine Virus with Poliomyelitis Virus in Infected Monkeys

Since it had previously been observed that the murine virus, 24 or 48 hours after intracerebral or subcutaneous injection into monkeys, invades the entire central nervous system of this animal, an experiment was run to investigate whether the murine infection would modify the course of poliomyelitic infection induced at close intervals.

TABLE XI

Interference Phenomenon of the Murine Virus with Poliomyelitis Virus (SK, Aycock, RMV) in Infected Monkeys

Monkey No.	Mode of infection	Result
AF43	0.5 cc. of murine virus (10% brain emulsion) + 0.5 cc. SK monkey virus (10% cord suspension) injected intracerebrally	No paralysis
AF64		" "
AF46	0.5 cc. murine virus (10% brain emulsion) injected intracerebrally, 48 hrs. later 0.1 cc. SK monkey virus (10% cord suspension) injected intracerebrally	" "
AF41	4 cc. murine virus (10% brain emulsion) injected subcutaneously, 48 hrs. later 0.1 cc. SK monkey virus (10% cord suspension) injected intracerebrally	" "
AF49	0.1 cc. SK monkey virus (10% cord suspension) injected intracerebrally, 48 hrs. later 0.5 cc. murine virus (10% brain emulsion) injected intracerebrally	" "
Control AF65	0.5 cc. SK monkey virus (10% cord suspension) + 0.5 cc. normal mouse brain emulsion (10%) injected intracerebrally	Paralyzed
Control AF42	0.1 cc. SK monkey virus (10% cord suspension) injected intracerebrally	"
AF40	0.5 cc. murine virus (10% brain emulsion) + 0.5 cc. Aycock monkey virus (10% cord suspension) injected intracerebrally	No paralysis
Control AF59	0.1 cc. Aycock monkey virus (10% cord suspension) injected intracerebrally	Paralyzed
AF65	0.5 cc. murine virus (10% brain emulsion) + 0.5 cc. RMV monkey virus (10% cord suspension) injected intracerebrally	"
Control AF63	0.05 cc. RMV monkey virus (10% cord suspension) injected intracerebrally	"

2 monkeys received a simultaneous intracerebral injection of 0.5 cc. 10 per cent mouse virus brain emulsion and 0.5 cc. 10 per cent SK monkey virus cord emulsion, another monkey a similar mixture of mouse virus and Aycock virus, and a fourth monkey a similar mixture of mouse virus and RMV virus. 2 additional monkeys were infected intracerebrally with mouse virus 48 hours before or after intracerebral infection with SK monkey virus. A last monkey was infected intracerebrally with SK monkey virus 48 hours following the injection of a heavy dose of murine virus (4 cc. of a 1:10 brain emulsion) by the subcutaneous route. 4 normal control monkeys infected with the three strains of poliomyelitis virus alone accompanied this experiment.

The results are summarized in Table XI. It will be seen from this table that all monkeys receiving the murine virus and SK or Aycock monkey

virus, in various combinations, survived without showing any symptoms; in contrast herewith, the monkey receiving the murine virus-RMV virus mixture, as well as all control animals, succumbed to the disease in a typical manner. The results of this experiment clearly show that the presence of murine virus interferes in a critical way with the propagation in the central nervous system of the homologous and of one related heterologous strain of monkey virus, while no such interference is observable with the unrelated heterologous strain.

DISCUSSION

The data presented in this paper leave little doubt that SK monkey virus was transmitted to mice by intermediary passage through cotton rats. Justification for this belief comes from the fact that an identical virus was isolated in mice on three different occasions, that the symptomatology and pathology of the murine infection compare in all respects with those of SK infection in monkeys, and that immunological tests have established cross-neutralizability by specific antisera as well as protection against cross-infection. The circumstances under which we succeeded with the third isolation dispose of the remote possibility that the murine infection was caused by a poliomyelitis-like, latent, cotton rat virus. Prior to this, our failure to come across such a hypothetical virus in numerous passages of cotton rats injected with inert material had rendered such an assumption most unlikely.

The necessity for interposing the cotton rat between the monkey and the mouse in order to accomplish the adaptive process is obvious from the negative results previously reported by the discoverers of this strain (13), in their attempts to infect mice directly with SK monkey virus, and from our own similar observations. Whether or not strains of monkey poliomyelitis virus, other than the Lansing and the SK virus, can be adapted to rodents in the same manner is a question for which the answer is missing at this time. Such a possibility, however, cannot be denied in spite of the apparent lack of success reported by Toomey (5), as well as by ourselves (7), with several other strains, since no systematic attempt was made to apply those principles of host passage which were recognized as being of crucial importance. In future efforts to adapt new strains it would be well to keep in mind the possibility that broad peripheral carrier strains, such as the SK virus, which was recovered from the feces of an abortive case of poliomyelitis, may lend themselves better to biological grafting than highly specialized strains derived from the central nervous system.

According to the information gained from our study of the mechanism

involved in the transition of the virus from monkeys to rodents, it would appear that the process of virus adaptation may assume at least three distinct forms: First, the infectious agent may be harbored by cotton rats in a phase pathogenic for monkeys but not for mice. Under such conditions, the cotton rat seems to be able to carry the virus in a state of complete latency or subclinical infection. Second, the virus, in paralyzing cotton rats, may pass through a period of genetic instability when it preserves some measure of monkey pathogenicity and gradually assumes virulence for mice. The third type of adaptation is evidently represented by an explosive mutation characterized by a sudden and complete loss of paralyzing power for monkeys, synchronous with the appearance of maximum pathogenicity for mice. What factors determine the course in the chain of events are not as yet clear; and our varied experience illustrates that the precise outcome of a given adaptation cannot be predicted. But it is conceivable that the initial degree of fixation for the monkey, the freshness of the virus, and passage at certain time intervals may be of signal importance. Examples are not wanting of similar modifications in the pathogenicity of viruses as the result of experimentally induced passage to new hosts. To quote from Watson (14): "Viruses that change in type and reach highest virulence in the passage animals may, at the same time and in the same process, become greatly attenuated for the original host species."

Evidence of the *in vitro* cultivation of monkey poliomyelitis virus in human embryonic nerve tissue was first presented by Sabin and Olitsky (15). Demonstration of the fact that the murine virus can be grown in tissue culture is interesting from several points of view. The truly phenomenal rate of viral propagation in media containing nervous tissue from the mouse, its host *par excellence*, as contrasted with the exceedingly poor multiplication in ordinary tissue cultures consisting of chick embryo, serves to throw new light on the extreme degree of neurotropic specialization in the growth requirements of this virus. The occurrence of a fair degree of propagation in nervous tissue from the guinea pig may foreshadow a possibility of training the infectious agent to produce ultimately disease in that rodent as well. Finally, since the virus escapes in large amounts into the ambient protein-free menstruum, *i.e.*, serum ultrafiltrate, an unusual opportunity seems to offer itself for further attempts to obtain the active principle in a form approaching chemical purity.

The study of the course of infection in mice adds new information to our knowledge of the extent of systemic distribution of the infectious agent. Thus, virus was found to be present not only in the brain and cord, but also in the blood, spleen, liver, and suprarenals of infected mice until the time of their death. When injected into monkeys, the murine virus could be

recovered from both neural and extraneural sites during the early intervals after infection; it persisted, however, only in the central nervous system for longer periods of time. Since the mouse virus possesses such extraordinary virulence for mice, the murine infection might well serve as a better experimental approach to the solution of certain problems concerning the pathogenesis of poliomyelitis, which are rendered difficult or impossible in monkeys because of the much lower level of infectivity of the virus in that species. Although the ability of the virus of poliomyelitis to migrate along nervous pathways can be regarded as an established fact, there is still considerable uncertainty whether direct peripheral invasion is the only and exclusive mechanism by which infection of the central nervous system takes place. It will be recalled that, on a basis of pathological and clinical grounds, the existence of a systemic phase of poliomyelitis has long been postulated by some authors. The occasional recovery of the virus from extraneural sites, such as blood and lymphatic system, especially spleen, in monkey and man is in keeping with this hypothesis (16). Experimental evidence has also been presented for the migration of poliomyelitis virus to the nasal mucosa by way of the blood (17). As pointed out by Gordon and Lennette (18), "if the vascular system were shown to be concerned in the pathogenesis of poliomyelitis some of the problems of immunity in this disease would be less obscure." In the light of what has been said, it would seem as if the conventional definition of the virus of poliomyelitis as "strictly neurotropic" is, perhaps, now open to some revision.

Our experiments finally suggest that immunization of monkeys with the live murine virus, in the form of mouse brain emulsion or tissue culture virus, may produce a certain degree of resistance against intracerebral infection with the homologous strain of poliomyelitis monkey virus, *i.e.*, SK, but not against infection with two heterologous strains, *i.e.*, Aycock and RMV virus. This protective effect apparently is not absolute but varies considerably in intensity. Thus, of a total of 10 immunized monkeys which were subsequently infected with SK virus, 7 were obviously protected, whereas 3 animals succumbed to the disease. Judging from the limited data at hand, the interesting fact can be recorded that immunization with the murine virus variant, without producing paralysis, apparently induced a state of relative resistance towards infection with the original highly virulent monkey strain. Although the presence of neutralizing antibodies against SK and Aycock monkey virus could be demonstrated in the serum of some of the immunized monkeys, the relationship, if any, between these serological phenomena and resistance to infection cannot be said to have been a very close one.

The favorable results obtained in these immunization experiments are

at variance with previous experience, all of which indicates that protection against infection with poliomyelitis virus cannot be obtained without the production of actual paralysis. It therefore appears extremely significant that we were also able to induce solid protection in 5 monkeys which had been injected simultaneously, or at short separate intervals, with the murine virus and with the monkey virus. The experimental set up, in this case, clearly did not permit the development of any immunity principles in the orthodox sense of the word. Whatever protection followed in its wake must therefore have been due to some interference between the mode of propagation of the murine virus and the monkey virus, similar, perhaps, to the sparing effect of lymphocytic choriomeningitis virus upon subsequent poliomyelitic infection, as described by Dalldorf (19). Another analogy may be found in Findlay and MacCallum's (20) observation of an antagonistic action between pantropic Rift Valley fever virus and neurotropic yellow fever virus when the two agents are introduced simultaneously into the same animal. The mechanism of such interference is unknown and one might speculate on the exhaustion of specific growth factors in the affected ganglion cells as being responsible for the phenomenon. Whatever the explanation, the fact remains that the murine virus, because of its rapid invasion of the central nervous system, exercises some blockading effect against the slower invasion of the monkey virus. The apparent success of our immunization experiments, and, indeed, the principal mechanism of protection in poliomyelitis, may therefore rest on some such basis rather than the evolution of any classical immunity.

CONCLUSIONS

1. A neurotropic murine virus was isolated by passing poliomyelitis virus (SK strain) from the monkey to cotton rats and white mice.

2. The murine virus has been grown in tissue culture consisting of embryonic mouse brain in ox serum ultrafiltrate.

3. The symptoms and lesions produced by the murine infection compare in all respects with those of poliomyelitis in monkey and man.

4. The murine virus, while highly pathogenic for mice and cotton rats, is non-pathogenic for albino rats, guinea pigs, and rabbits. It possesses limited pathogenicity for *rhesus* monkeys.

5. Although producing no paralysis in the above mentioned refractory animals, the murine virus may be recovered in active form from neural and extraneural sites of infected albino rats, guinea pigs, and monkeys, but not from rabbits.

6. The identity of the murine and monkey virus is further suggested by cross-neutralization between the murine virus and homologous (SK) and re-

lated (Aycock) antipoliomyelitis sera, as well as between homologous and related monkey poliomyelitis virus and antimurine virus sera.

7. Immunization of monkeys with live murine virus, in the form of mouse brain or tissue culture, seems to confer some degree of resistance against subsequent infection with the homologous poliomyelitis monkey virus.

8. The presence of the murine virus in the central nervous system of infected monkeys appears to interfere with the propagation of SK and Aycock poliomyelitis monkey virus in the same animal.

The authors wish to express their gratitude to Mr. Frank Vasi and to Miss Frieda Gersh for their assistance during this work.

BIBLIOGRAPHY

1. (*a*) Harmon, P. H., Shaughnessy, J. H., and Gordon, F. B., *J. Prevent. Med.*, 1930, **4,** 59, (*b*) 89. (*c*) Brodie, M., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32,** 832. (*d*) Gavrilov, W., and Fester, A., *Arch. Virusforsch.*, 1940, **1,** 404.
2. Armstrong, C., *Pub. Health Rep., U. S. P. H. S.*, 1939, **54,** 1719.
3. Lillie, R. D., and Armstrong, C., *Pub. Health Rep., U. S. P. H. S.*, 1940, **55,** 115.
4. Armstrong, C., *Pub. Health Rep., U. S. P. H. S.*, 1939, **54,** 2302.
5. Toomey, J. A., and Takacs, W. S., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43,** 536.
6. Armstrong, C., personal communications.
7. Jungeblut, C. W., and Sanders, M., *Proc. Soc. Exp. Biol. and Med.*, 1940, **44,** 375.
8. Gavrilov, W., and Fester, A., *Arch. Virusforsch.*, 1940, **1,** 404.
9. Sanders, M., *J. Exp. Med.*, 1940, **71,** 113.
10. Molloy, E., *Proc. Soc. Exp. Biol. and Med.*, 1940, **44,** 563.
11. Thompson, R., *J. Exp. Med.*, 1930, **51,** 777.
12. Schultz, E. W., and Gebhardt, L. P., *J. Immunol.*, 1934, **26,** 93.
13. (*a*) Trask, J. D., Paul, J. R., and Vignec, A. J., *Proc. Soc. Exp. Biol. and Med.*, 1939, **41,** 241, (*b*) 246.
14. Watson, E. A., in Moulton, F. R., The genetics of pathogenic organisms, The American Association for the Advancement of Science, 1940, No. 12, 18.
15. Sabin, A. B., and Olitsky, P. K., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34,** 357.
16. (*a*) Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, **54,** 1140. (*b*) Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, **57,** 1685. (*c*) Leiner, C., and von Wiesner, R., in Poliomyelitis, International Committee for the Study of Infantile Paralysis, Baltimore, 1932. (*d*) Clark, P. F., Fraser, F. R., and Amoss, H. L., *J. Exp. Med.*, 1914, **19,** 223. (*e*) Lennette, E. H., *J. Exp. Med.*, 1937, **66,** 549. (*f*) Kempf, J. E., and Soule, M. H., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43,** 476.
17. (*a*) Lennette, E. H., and Hudson, N. P., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32,** 1444. (*b*) Armstrong, C., *Pub. Health Rep., U. S. P. H. S.*, 1936, **51,** 241. (*c*) Sabin, A. B., and Olitsky, P. K., *J. Am. Med. Assn.*, 1937, **108,** 21.
18. Gordon, F. B., and Lennette, E. H., *J. Infect. Dis.*, 1939, **64,** 97.
19. (*a*) Dalldorf, G., Douglass, M., and Robinson, H. E., *Science*, 1937, **85,** 184. (*b*) Dalldorf, G., and Douglass, M., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39,** 294. (*c*) Dalldorf, G., Douglass, M., and Robinson, H. E., *J. Exp. Med.*, 1938, **67,** 323, (*d*) 333.
20. Findlay, G. M., and MacCallum, F. O., *J. Path. and Bact.*, 1937, **44,** 405.

EXPLANATION OF PLATES

PLATE 18

FIG. 1. Bilateral ptosis; monkey AF8 injected intracerebrally 2 days previously with pooled brain emulsion from 2 cotton rats (115, 116) which had shown paresis and mild nervous symptoms. Associated with the bilateral ptosis, were coarse tremors and flaccid paralysis of one leg.

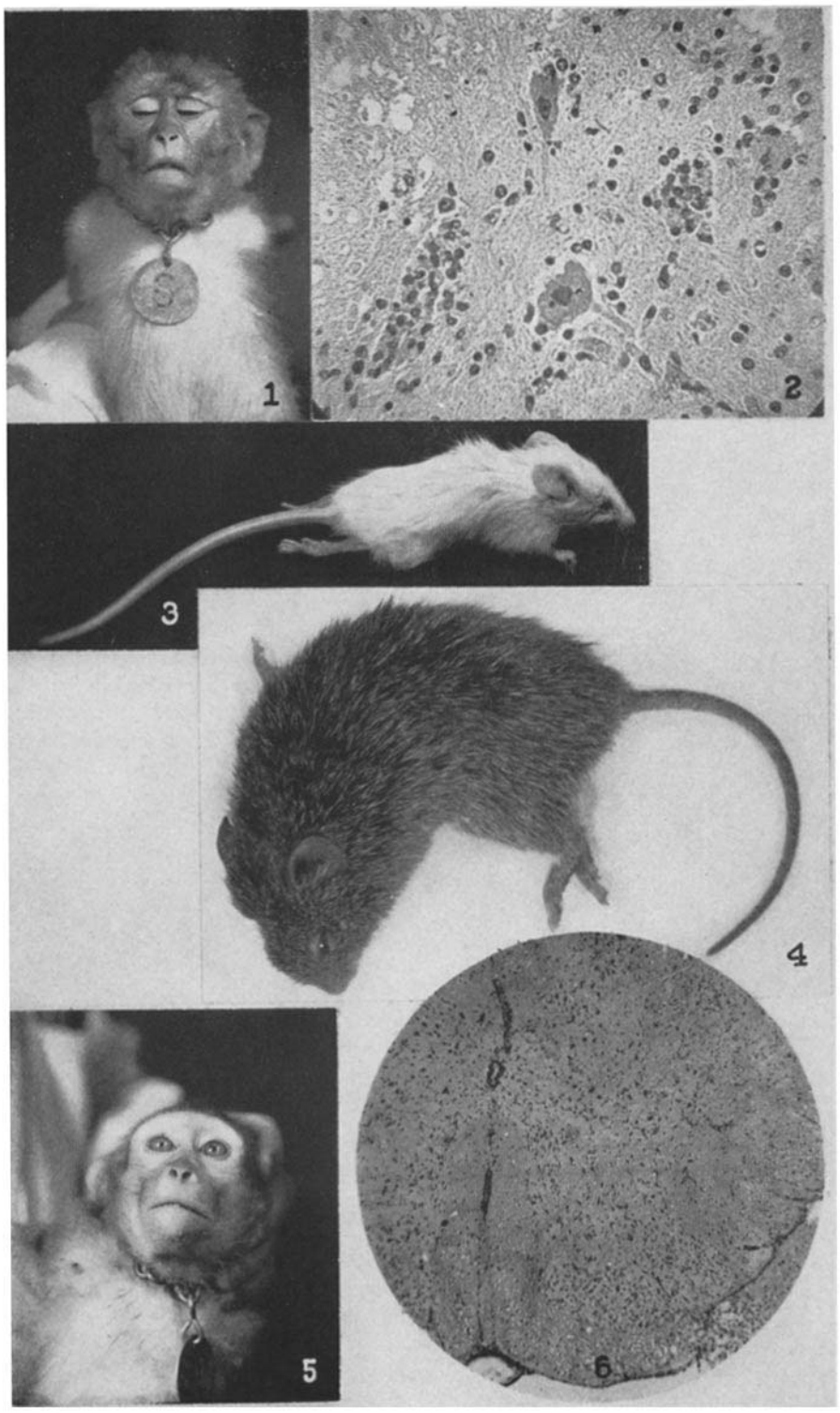
FIG. 2. Section through anterior horn at the lumbar level of spinal cord from monkey AF8. Note neuronophagocytosis and ganglion cell destruction. Perivascular infiltration was marked in adjacent areas. Hematoxylin and eosin stain.

FIG. 3. White mouse. Complete flaccid paralysis of both hind legs 48 hours post-inoculation. This mouse received 0.03 cc. intracerebrally of 1:1000 murine virus, first isolation.

FIG. 4. Cotton rat. Complete flaccid paralysis of hind legs and right foreleg 48 hours after intracerebral injection of murine virus.

FIG. 5. Monkey injected intracerebrally with brain suspension of cotton rat paralyzed by murine virus. Note uneven pupils. Nervous symptoms and rise in temperature accompanied pupillary changes.

FIG. 6. Section through lumbar level of spinal cord of paralyzed cotton rat (131) (third isolation). The animal was injected intracerebrally with brain emulsion 48 hours previously. Note perivascular infiltration, neuronophagia, and infiltration of gray matter. Hematoxylin and eosin stain.



(Jungeblut and Sanders: Murine strain of poliomyelitis virus)

PLATE 19

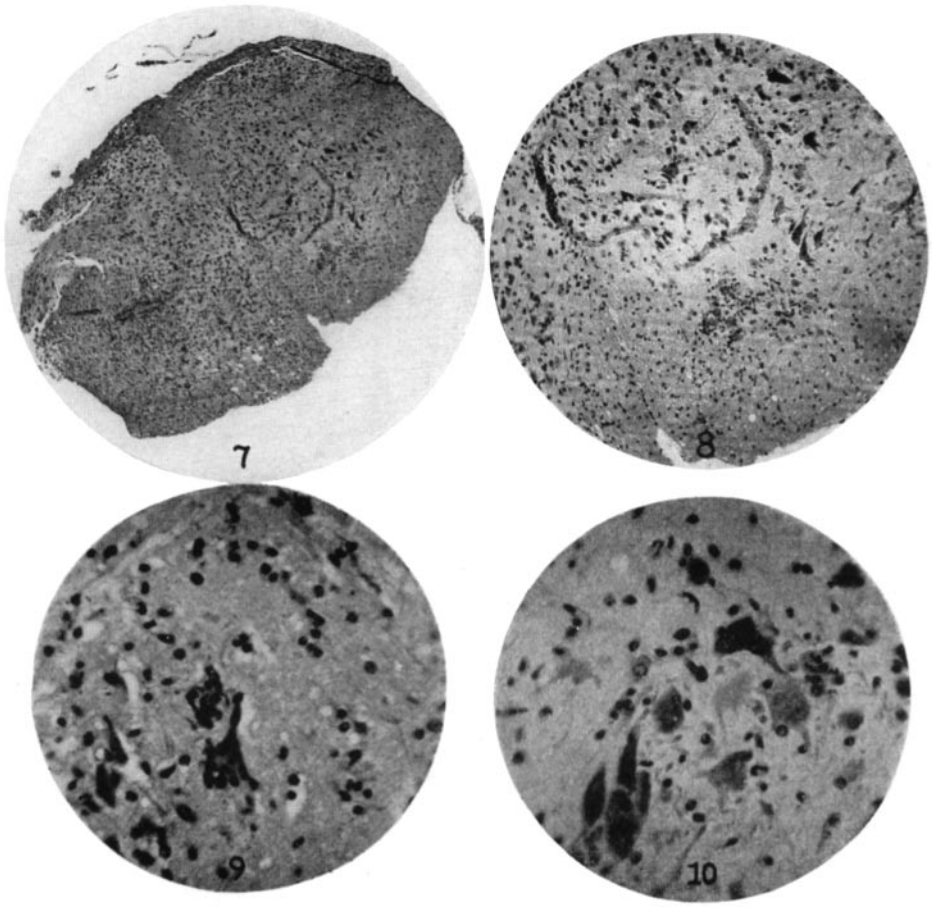
Figs. 7, 8, 9, 10. Sections through lumbar levels of spinal cords of paralyzed mice. Murine virus, first isolation, injected intracerebrally. Hematoxylin and eosin stains.

FIG. 7. Note neuronophagia, perivascular infiltration, microglial proliferation. Widespread disappearance of large ganglion cells in gray matter.

FIG. 8. Higher magnification of Fig. 7.

FIG. 9. Anterior horn. Note ring of leukocytes around ganglion cell.

FIG. 10. Varying degrees of degeneration of anterior horn nerve cells are present; also polymorphonuclear infiltration and microglial proliferation.



(Jungeblut and Sanders: Murine strain of poliomyelitis virus)

PLATE 20

Figs. 11, 12, 13, 14. Sections through anterior horns of lumbar levels of spinal cords of monkeys injected intracerebrally with murine virus, first, second, and third isolations. Hematoxylin and eosin stains.

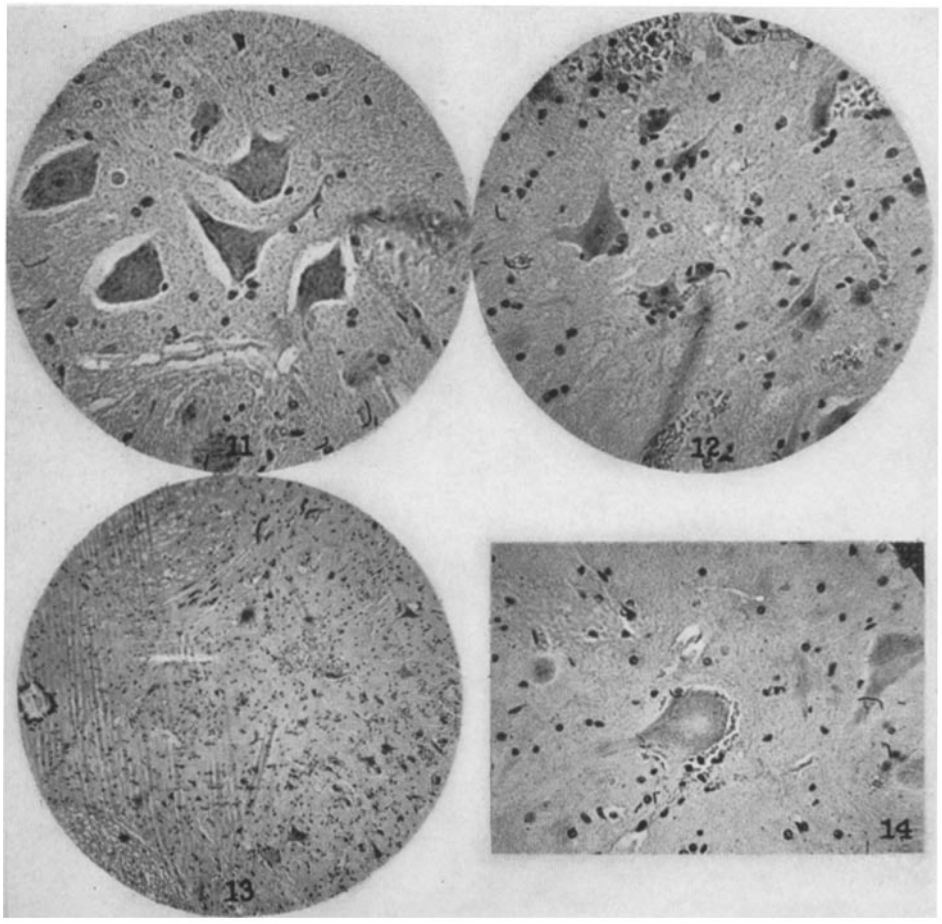
The rod-like structures in Figs. 11 to 14 are artefacts in the preparation. They should be disregarded.

FIG. 11. Poor staining but intact ganglion cells. Note retraction of cell wall and dissolution of nucleus and nucleolus.

FIG. 12. Multiple hemorrhages and moderate satellitosis of nerve cells.

FIG. 13. Pyknosis of ganglion cells. Multiple small perivascular hemorrhages. Diffuse gliosis in anterior horn.

FIG. 14. Coalescent ganglion cell, staining homogeneously without cellular detail.



(Jungeblut and Sanders: Murine strain of poliomyelitis virus)