

ISOLATION OF B VIRUS (HERPES GROUP) FROM THE CENTRAL NERVOUS SYSTEM OF A RHESUS MONKEY*

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(Received for publication, May 3, 1954)

The first isolations of B virus were made by Sabin and Wright (1), and independently by Gay and Holden (2), from the central nervous system of an investigator who developed a spreading, fatal infection with ascending myelitis after a bite on the hand from an apparently normal *rhesus* monkey. Gay and Holden called the agent W virus and thought it to be a highly neurotropic strain of herpes simplex virus. Sabin and Wright named it the B virus (from the initial of the laboratory worker from whom it was isolated), and subsequently Sabin showed that although related to the virus of herpes simplex (and to the virus of pseudorabies), B virus had several distinctive properties (3-6). Burnet, Lush, and Jackson (7) also reported that B virus and herpes simplex virus are closely related antigenically, but that B virus has broader antigenicity than herpes virus.

The second isolation of B virus was also performed by Sabin (8), under circumstances similar to the first. Another investigator engaged in experimental work with *rhesus* monkeys contracted a fatal infection of the central nervous system, apparently from contamination by monkey saliva of a minor cut on the finger.

To our knowledge these have been the only two recorded isolations of B virus. In both the instances the virus was isolated from persons who developed infection after close contact with the saliva of monkeys, a fact which led Sabin to presume that the virus was derived from the animals. This view was supported by the finding that some monkeys possess neutralizing antibodies against the agent (5, 7, 9), leading to the hypothesis that the monkey is the natural host of B virus. However, there has not been any report of direct isolation of this agent from the monkey. It is for this reason that we are reporting here an instance of the isolation of B virus from a *rhesus* monkey.

Materials and Methods

Virus.—For passage of the newly isolated virus, 20 per cent suspension of the central nervous system of infected animals was usually employed. The quantity of the material inocu-

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

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lated in order to produce infection varied with different animals and the route of inoculation. The kinds of animals used and the dosage indicated in Table I remained the same in all experiments, unless stated otherwise.

Neutralization Tests.—In cotton rats the tests were carried out as follows: 20 per cent brain suspension from the fourth viral passage in cotton rats was diluted in 10 per cent heat-inactivated normal horse serum to yield a suspension containing 200 LD₅₀ per 0.03 ml. of the virus. This was mixed with equal quantities of undiluted serum (0.2 ml. of virus suspension added to 0.2 ml. of serum) and incubated at room temperature for 90 minutes. The mixture was then inoculated intracerebrally into young adult cotton rats. In one experiment by the intraperitoneal route, a 20 per cent brain suspension was mixed with the undiluted serum.

TABLE I
Production of the Experimental Disease

Experimental animals	Route of inoculation	Dose per animal	Histopathologic lesion
Monkeys	Intracerebral	1.0	Meningoencephalitis
Cotton rats	"	0.03	"
" "	Intraperitoneal	0.2	Encephalomyelitis
Adult mice	Intracerebral	0.03	Meningoencephalitis
Newborn mice	"	0.01	
" "	Subcutaneous	0.02	
Hamsters	Intracerebral	0.03	Meningoencephalitis
Guinea pigs	"	0.04	"
" "	Subcutaneous	0.1	
" "	Intraperitoneal	0.1	
Rabbits	Intracutaneous	0.2	Necrosis of skin followed by myelitis
Chick embryo	Chorioallantoic	0.1	Pocks on the membrane with focal necrosis
Tissue culture:			
Monkey kidney epithelial culture		0.1	Plaque formation; giant multinucleate cells
Human epithelial culture, strain HeLa		0.1	" "

The inoculated animals were observed daily for 14 days for signs of infection which was demonstrated by ataxia, paralysis, prostration, or death.

The neutralization tests in newborn mice were carried out as follows: 20 per cent brain suspension from the fifth viral passage in newborn mice was diluted as above. The suspension containing 200 LD₅₀ of the virus was mixed with an equal quantity of serum and incubated as above. It was then inoculated intracerebrally into newborn mice, not more than 2 days old. The inoculated animals were observed daily for 14 days for signs of infection, ranging from failure to grow at the normal rate to prostration and death. The disease almost always ended in death. Some neutralization tests using various dilutions of virus with a constant amount of serum were also carried out in cotton rats and newborn mice.

The neutralization tests in rabbits were carried out by the method of Sabin (3), the source

of virus being 20 per cent suspension of the brain and spinal cord from the first passage in a rabbit. Various concentrations of CNS (10^{-1} to 10^{-5}) suspended in 10 per cent heat-inactivated normal horse serum were mixed with equal quantities of undiluted immune sera (0.2 ml. of virus plus 0.2 ml. of serum). The mixture was incubated for 2 hours at room temperature, and then inoculated intracutaneously into rabbits, each weighing about 1800 gm.

Herpes Simplex Virus.—A standard strain, HF, obtained from the Viral and Rickettsial Registry, Washington, D. C. It was used as a 20 per cent suspension of baby mouse brains in the first passage in this laboratory. Neutralization tests employing various sera were carried out in the same manner as described for the new virus. All the tests were carried out by the intracerebral route in newborn mice less than 48 hours old.

Circumstances under Which B Virus Was Isolated

The tests leading to the isolation of the strain are listed in Fig. 1. On November 11, 1950, *rhesus* 52-28 was inoculated intracerebrally with a fecal sample obtained from a patient suffering from an acute attack of poliomyelitis. After an incubation period of 7 days the monkey came down with signs suggestive of early poliomyelitis infection: it had tremors and was excited and clumsy in its movements. As the disease did not progress in the next 24 hours, the monkey was sacrificed. Six levels of the medulla and spinal cord were taken for histological examination and the remainder was stored on dry ice. Histological study showed evidence suggesting poliomyelitis infection in the medulla (perivascular cuffing, foci of inflammation, and neuronophagia; normal meninges). The spinal cord sections were negative in the five levels examined.

An attempt was made to pass the strain to monkeys and to cotton rats and mice. On December 2, 1950, a 20 per cent suspension of spinal cord and medulla of *Rh.* 52-28 was inoculated into two *rhesus* monkeys (*Rh.* 53-22 and *Rh.* 53-23), 11 cotton rats, and 12 adult albino mice. The cotton rats and the mice did not show any signs of disease. Both the monkeys, however, showed tremors, weakness of the extremities, and ataxia on the 12th day after inoculation. *Rh.* 53-23 was sacrificed the same day, while *Rh.* 53-22 went on to recovery. Later it received intraperitoneally suspensions of *Rh.* 52-28 spinal cord, and was finally bled and sacrificed on February 9, 1951. The histological findings on the medulla and cord of *Rh.* 53-23 were negative, there being neither any evidence of poliomyelitis nor of meningitis in the five levels examined. In contrast, the medulla and cord of *Rh.* 53-22, which was sacrificed about 2 months later, showed on histological examination old lesions of poliomyelitis.

On January 8, 1951, a 20 per cent suspension of the spinal cord of *Rh.* 53-23 was inoculated intracerebrally into 12 cotton rats and 12 adult albino mice in a fresh attempt to adapt to these rodents what we then thought was a new strain of poliomyelitis virus. 11 of the cotton rats came down after an incubation period of 5 to 6 days with signs of ataxia, excitement, and a little wasting, followed by paralysis, often flaccid and sometimes more marked on one side, prostration, and death. 7 of the mice died at irregular intervals over the 4 week observation period without manifesting overt illness before death.

The cotton rat brain material was successfully passed in series through fresh batches of cotton rats, and after three passages as a 10 per cent suspension the incubation period was found to be more or less constant at 4 days. The infective titer of the agent in cotton rats was found to be $10^{-3.5}$ by intracerebral inoculation, and $10^{-3.0}$ by intraperitoneal inoculation. However, the cotton rat material could be passed into adult mice only with great difficulty and irregularity. Moreover, the disease produced in the adult albino mice was markedly different from poliomyelitis, which gave rise to the suspicion that perhaps we were no longer dealing with a strain of poliomyelitis virus. The affected mice showed wasting, ruffled coats, humped positions, and a tendency to sit isolated in one corner of the cage; there were hardly any noticeable tremors. Some of them died rather suddenly the day after the signs were first

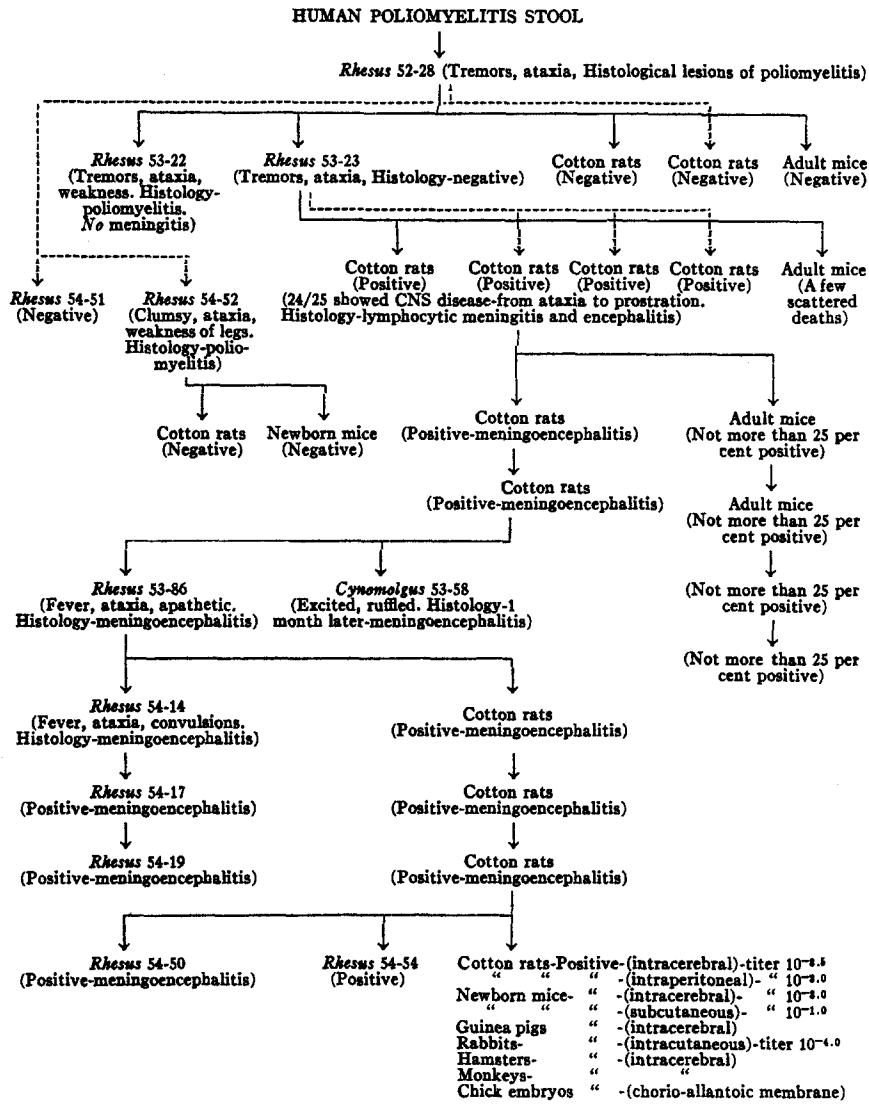


FIG. 1. "Origin" of the strain of B virus from the central nervous system of *rhesus 53-23*. Intracerebral passage of CNS tissue is indicated by arrows. Dotted lines indicate passages which were repeated at later dates.

noted, a few apparently in convulsion because the legs were found stretched out. Even though repeated serial passages were carried out in adult mice using 20 per cent brain suspensions, not more than about 25 per cent manifested signs of infection at each passage.

The suspicion was confirmed when histological sections of the brain and spinal cord of the affected cotton rats and mice showed no evidence of poliomyelitis, but rather a meningo-

encephalitis. There was extensive involvement of the meninges with lymphocytes predominating, the inflammation often extending into the superficial brain cortex giving rise to areas of necrosis. There were also found areas of rarefaction, perivascular cuffing, foci of hemorrhage, and thrombosis of capillaries with the last being especially marked in the cerebellum.

Suspensions of brains from infected animals were on numerous occasions tested in nutrient broth and on blood agar plates for the presence of bacteria and were uniformly found sterile. On several occasions direct smears of freshly removed brains from infected cotton rats and mice were made on blood agar plates and these were also found sterile. The material after passage through a bacterial filter was still found infective for cotton rats.

Having satisfied ourselves that we were dealing with a virus with properties different from those of poliomyelitis, we set out to determine the source of the virus as well as its identity.

Determination of the Source of the Virus (See Fig. 1).—The pathogenicity of the virus for monkeys was investigated. Infected cotton rat brain material was inoculated into two monkeys, one *cynomolgus* and one *rhesus* (*Cyno.* 53-58 and *Rh.* 53-86). The passage was successful. The *rhesus* was normal until the 6th day when its coat became ruffled and it showed clumsiness in walking and climbing. When undisturbed, it sat in an apathetic manner in one corner of the cage with little desire to take food. It was sacrificed the next day, and the histology of the cerebrum, cerebellum, medulla, and spinal cord revealed extensive lymphocytic meningitis with thrombosis of blood vessels similar to that found in the cotton rats, but no evidence of poliomyelitis. The *cynomolgus* on the 5th day after inoculation was found to be excited and ruffled, but it was not seriously ill, and recovered in a few days. It was sacrificed 1 month later. The histology of its brain and cord was similar to that of the *rhesus*, except that the pathological lesions were less extensive. Three serial passages in *rhesus* monkeys (*Rh.* 54-14, 54-17, 54-19) were then carried out starting with 20 per cent brain and cord suspension from *Rh.* 53-86. All these monkeys showed illness similar to that described for *Rh.* 53-86, usually with fever as shown in Fig. 2. Attempts to demonstrate the presence of the virus in the acute phase serum of these monkeys by subinoculation into cotton rats and newborn mice gave negative results.

As the virus was found pathogenic for monkeys, the following possible sources were considered as its possible origin in the laboratory: (a) the original human feces; (b) the first monkey *Rh.* 52-28 into which the feces was inoculated; (c) monkey *Rh.* 53-23, one of the pair which was inoculated with the CNS suspension of *Rh.* 52-28; and (d) the stock cotton rats. The last could be easily dismissed from further consideration because of the fact that several batches of cotton rats inoculated with spinal cord material from *Rh.* 53-23 on different occasions consistently manifested the same type of disease, while cotton rats inoculated with other materials during this period failed to do so. The virus, therefore, had to be present in one of the three earlier specimens.

The original *Rh.* 52-28 cord suspension was next reinoculated into a fresh batch of cotton rats which again failed to show any signs of disease. The new virus, therefore, was probably not present in this material. To establish this, the *Rh.* 52-28 cord suspension was inoculated into two new *rhesus* monkeys (*Rh.* 54-51 and *Rh.* 54-52) just as was done in the first instance, to see if the virus could be brought out by monkey passage. *Rh.* 54-52 after an incubation period of 14 days came down with poliomyelitis, confirmed histologically, while the other monkey did not show any signs of disease. Inoculation of a suspension of the spinal cord and medulla of *Rh.* 54-52 failed to produce disease in cotton rats or mice. This eliminated the possibility of the new virus being present in *Rh.* 52-28, (and presumably in the original fecal material that was inoculated into this monkey).

The new virus was therefore believed to have been derived from the tissues of *Rh.* 53-23, because it could be shown that it was not present in the material which had been inoculated into this monkey, while the spinal cord material from *Rh.* 53-23 when inoculated into batches of cotton rats on different occasions, always produced the characteristic infection.

Host Range of the New Virus

To aid in the identification of the new virus, the host range was studied further. The chief histological findings are summarized in Table I.

Mice.—Infection was produced in newborn animals (less than 48 hours old) on intracerebral inoculation more readily and constantly than in adult mice, but less so than in cotton rats. The baby mice showed signs of ataxia, failure to grow at the normal rate, bizarre movements, contortions, prostration, and death. After serial passage in baby mice, the intracerebral titer

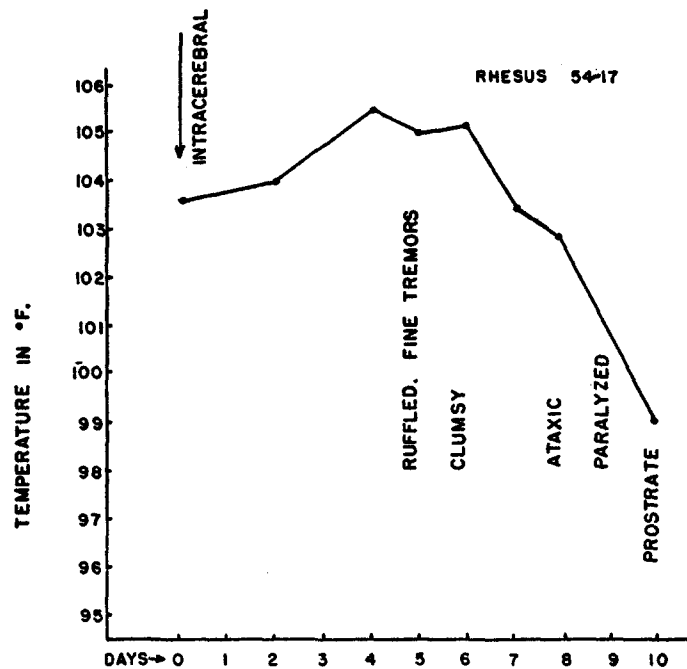


FIG. 2. Course of infection in a *rhesus* monkey inoculated by the intracerebral route.

was found to be $10^{3.0}$, and the subcutaneous titer about 10^{-1} . Satisfactory sections of the CNS were not available for study. However, numerous histological sections of the muscles failed to reveal any evidence of myositis.

Four week old mice were poorly susceptible to the virus even after serial passage. In each passage in which 12 to 20 mice were used, only 1 to 5 mice succumbed. However, the sick animals showed a meningoencephalitis. There was extensive involvement of the meninges, lymphocytes predominating, with the inflammation at times extending into the superficial layers of the brain.

Cotton Rats.—In 4 week old animals, infection was constantly produced by the intracerebral or intraperitoneal route of inoculation; the incubation period

being 4 days after intracerebral inoculation and longer by 3 to 5 days after intraperitoneal inoculation. After intraperitoneal inoculation, the spinal cord sections showed myelitis, there being foci of hemorrhage and perivascular infiltrations with neuronal degeneration. There was also an encephalitis, but the meninges showed little reaction. Histological sections of the other organs studied, namely liver, lung, spleen, kidney, and adrenals, appeared normal.

Guinea Pigs.—In young guinea pigs (about 400 gm. in weight) fatal or paralytic infection was produced irregularly after intraperitoneal and sub-

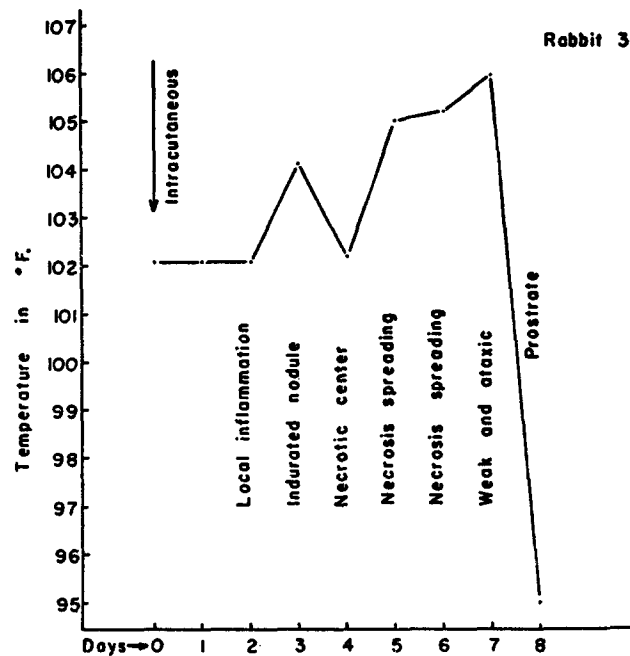


FIG. 3. Course of infection in a rabbit inoculated in the skin. The signs noted on days 2 to 6 pertain to the cutaneous area about the site of injection.

cutaneous inoculations of a 20 per cent suspension of infected cotton rat brain, but more readily by intracerebral inoculation although even here all the inoculated animals did not succumb. Histologically, after intracerebral inoculation of the guinea pig, there was extensive meningitis with infiltration of the inflammatory exudate into the brain substance. The lungs showed a secondary, patchy pneumonia, while the liver and the spleen appeared normal. Peritonitis was not observed in the few animals that were inoculated by the intraperitoneal route. Subcutaneous inoculations seemed to produce a spreading necrosis around the site of inoculation in some of the animals.

Rabbits.—Intracutaneous injection (see Fig. 3) produced a hemorrhagic, necrotic, indurated nodule at the site of inoculation about 24 to 48 hours

after inoculation as Sabin has described for B virus. This was followed by fever, ruffled coat, and finally prostration often with flaccid paralysis, sometimes of all limbs and sometimes more marked on the side of inoculation. Paralysis was usually accompanied by a sharp drop in temperature to subnormal, and then death about 8 days after inoculation. When infected rabbit brain was titered in rabbits by the intracutaneous route, the titer was found to be $10^{-4.0}$. Histologically, the skin of the rabbit showed necrosis of the epidermis infiltrated with neutrophils and mononuclear cells. In sections stained with hematoxylin and eosin, or methylene blue and eosin, intranuclear inclusion bodies as described by Sabin were identified in the nucleus of surviving epithelial cells. Spinal cord sections showed myelitis, with neuronophagia, and the same process was found to extend into the lower part of the medulla. The meninges, pons, cerebellum, cerebrum, and other internal organs studied, namely lung, liver, spleen, kidney, and pancreas appeared normal.

Hamsters.—Intracerebral inoculation of 4 week old hamsters produced signs similar to those in other susceptible rodents, namely excitement, ataxia, and prostration before death, which occurred within 7 days after inoculation. Histology of the brain showed characteristic meningoencephalitis.

Chick Embryos.—Inoculation of 10 day old embryos on the chorioallantoic membrane gave rise to pocks on the membrane similar to those described for B virus (10). The incubation period for the development of the pocks was 5 days when the virus suspension was used in a $10^{-2.0}$ concentration in a quantity of 0.1 ml. During these early passages in eggs, the eggs were harvested 7 days after inoculation, and the embryos were still alive at that time, even though pocks covered the membrane. Three passages were carried out successfully in the developing chick embryos.

Tissue Culture.—In epithelial cell cultures of monkey kidney (and human epithelioma, strain HeLa), the virus grew, readily producing a distinctive degeneration with the formation of giant multinucleate cells (11). Foci of infection appeared 1 day after infection and remained localized in plaques for about 2 to 3 days.

Immunological Studies

Lack of Antigenic Relationship to Other Viruses.—Because of the fact that the new virus produced an aseptic meningitis and gave rise to apparent disease in adult albino mice in some respects similar to that caused by the virus of lymphocytic choriomeningitis (LCM), our first thought was that we were perhaps dealing with an atypical strain of LCM virus. It was considered different from typical LCM virus in the following respects: (a) it produced disease in cotton rats much more readily than in adult mice; (b) the characteristic tremors produced by LCM virus were not seen in adult mice; (c) histologically, in most cases, the choroid plexus was normal; the lesion was a lymphocytic meningitis with encephalitis rather than a lymphocytic choriomeningitis.

Neutralization tests in cotton rats and newborn mice using the new virus against hyperimmune LCM sera¹ were negative, showing that this virus was not related to LCM virus (see Table II). This was confirmed by the results of the complement fixation tests in which immune monkey sera active in the neutralization test against the new virus failed to react with LCM antigen (manufactured by the Lederle Laboratories), a control LCM serum giving a positive reaction in this test. Complement fixation tests using the LCM im-

TABLE II
Neutralization Tests with the New Strain of B Virus

Immune serum	Source	Neutralization index		
		Cotton rats	Newborn mice	Rabbits
1. Lymphocytic choriomeningitis	Rabbit K	0	0	
2. " "	Rabbit W	0	0	
3. Egyptian strain of West Nile	Rabbit	0	0	
4. Fort Bragg fever	Hamster	0	0	
5. Poliomyelitis Type 1	Monkey	0	0	
6. " " 2	"	0	0	0
7. " " 3	"	>100	>10	
8. <i>Cyno.</i> 53-58 (normal)	"	>100	>100	
9. <i>Rh.</i> 54-53 (preinoculation)	"	0	0	
10. <i>Rh.</i> 54-53 (postinoculation of new virus)	"	>320	>100	>320
11. <i>Rh.</i> 54-54 (preinoculation)	"	0	0	10
12. <i>Rh.</i> 54-54 (postinoculation of new virus)	"	>320	>100	>1000
13. B virus (Sabin)	"		320	
14. " "	Rabbit	>320	>320	>1000
15. Herpes simplex	"	0	0	100
16. Human gamma globulin	United States,			
1:10	1945	>100	>100	
1:100		>100	>100	
17. Human gamma globulin	United States,			
1:10	1951	>100	>100	
1:100		>100	>100	

mune sera against antigens (prepared from a variety of sources such as infected cotton rat spleen and brain, infected guinea pig spleen and brain, and infected rabbit spleen and brain) containing the new virus were either negative or not satisfactory because of anticomplementary activity of the antigens.

Neutralization tests were then carried out (see Table II) in cotton rats and newborn mice by the intracerebral route against specific immune sera active

¹ The hyperimmune LCM sera were kindly supplied by Dr. Joseph E. Smadel, Army Medical Center, Washington, D. C., and Dr. Herbert A. Wenner, University of Kansas, Kansas City.

against other viruses, namely the Egyptian strain of West Nile virus, Lansing, Leon, and Brunhilde types of poliomyelitis virus, herpes simplex virus² and B virus.³ These sera failed to neutralize the new virus, except for the Leon type serum and two B virus sera. We do not consider the Leon reaction as specific, because the Leon immune serum was prepared in monkeys and it is possible these monkeys possessed antibodies to the new virus prior to their inoculation (see below). The complete neutralization of the new virus by the two B virus immune sera indicated its probable identity.

Additional neutralization tests were carried out in rabbits inoculated intracutaneously. The new virus was tested against herpes simplex and B virus immune sera (see Table II). Both kinds of sera neutralized the new virus but the B virus antiserum was more effective. These and other immunological tests described, together with the skin lesion produced in the rabbit and the other properties already mentioned, are in our opinion sufficient to characterize this agent as B virus.

TABLE III
Neutralizing Antibodies to B Virus in "Normal" Monkeys

	1951	1949	1947	1946	1945	1944	1943	Total
<i>Rhesus</i> positive.....	0/6*	5/9	1/6	0/1	1/2	0/1	0/2	7/27
<i>Cynomolgus</i> positive.....	2/10	0/6		0/1				2/17
Total positive.....	2/16	5/15	1/6	0/2	1/2	0/1	0/2	9/44

* 0/6 indicates that the 6 sera tested were negative.

The convalescent sera of *Rh.* 54-53 and *Rh.* 54-54 were also tested for their ability to neutralize herpes virus, and both sera were found positive. The capacity of these sera to neutralize the local strain of B virus is also shown in Table II. The sera of these monkeys prior to inoculation failed to neutralize the virus.

Antibodies in Normal Monkey Sera. Tests in newborn mice for the presence of neutralizing antibodies to the local strain of B virus showed that of 44 sera of "normal" monkeys collected in this laboratory during a period of 8 years from 1943 to 1951 (and kept in a frozen state), 9 were positive, being distributed in groups of monkeys during different years (see Table III). Of the *rhesus* sera collected in the first part of 1951 none showed any neutralizing activity while 2 *cynomolgus* sera did. It may be recalled in this connection that the 1 *cynomolgus* (*Cyno.* 53-58) which had been used experimentally in inoculation with the

² The immune serum to herpes simplex virus was kindly supplied by Dr. Edward C. Curnen.

³ The two immune sera against B virus were kindly supplied by Dr. Albert B. Sabin, University of Cincinnati, Cincinnati.

new virus developed a mild disease and recovered, and its preinoculation serum tested later showed presence of antibodies against the virus. In 1949, however, 5 of the 9 *rhesus* sera tested had antibodies, but none of 6 *cynomolgus* were positive. In 1947, 1 out of 6 *rhesus* sera tested contained antibodies, and in 1945, 1 out of 2. In the years 1943, 1944, and 1946 none of the 5 sera tested was found to contain antibodies. The positive monkey sera were also tested against herpes simplex virus, and it was found that 8 out of the 9 neutralized herpes virus in newborn mice. Of 15 sera negative against B virus, 13 also failed to neutralize herpes virus, and 2 gave equivocal results.

Antibodies to the Local Strain of B Virus and to Herpes Virus in Human Populations.—Neutralization tests in cotton rats and newborn mice showed that two batches of human gamma globulin manufactured in the United States, one in 1945 and one in 1951 neutralized the B virus strain in dilutions of 1:100 or greater of the gamma globulin. The above two samples of gamma globulin

TABLE IV
Neutralization of the Local Strain of B Virus and of Herpes Simplex Virus by Normal Sera from Bombay, India, 1951

Age group	B virus	Herpes virus
Newborns	2/6	5/6
½-6 years	0/5	1/5
7-12 "	2/13	5/13
20-30 "	6/10	8/10
31-60 "	1/6	1/6
Total	11/40	20/40

were tested for antibodies against herpes virus, and they were found also to neutralize herpes virus in dilutions of over 1:100, in conformity with the finding of Heyl, Allen, and Cheever (12).

Following these observations, neutralization tests were carried out in newborn mice with 40 human sera (undiluted) of various age groups collected from the "normal" population from Bombay, India; the results are listed summarized in Table IV. All 11 sera which neutralized B virus also neutralized herpes virus, but the reverse did not hold. Thus another 9 sera neutralized herpes virus alone.

DISCUSSION

This paper records the first instance known to us of direct isolation of B virus from the tissue of a monkey. Although B virus has been isolated twice before, on both these occasions it was isolated from a fatal human case who developed infection after close contact with saliva of monkeys. In the present

instance, the virus was obtained from the tissue of a monkey which had been inoculated with poliomyelitis virus. These experiences emphasize one of the difficulties inherent in passing viruses through animals; namely, the possibility that a virus present in the experimental animals may be picked up in the process. When a virus appears to have changed in passage, the host spectrum, the pathology of the disease produced by the new virus, as well as neutralization or other immunological tests with known specific sera, have to be resorted to in order to determine the nature of the new agent. Similar situations are known to exist in which investigators have used stock mice harboring latent and spontaneous infection, and have exchanged their virus for one indigenous to the mouse.

Concerning the manner in which the B virus existed in the monkey in the present case three possible explanations occur to us: (1) the monkey was incubating a disease due to B virus at the time it was inoculated with poliomyelitis virus, (2) the virus was present in the tissues of the monkey in a latent state and the signs observed in the monkey were due to *infection* of the nervous system with the inoculated poliomyelitis virus, and (3) the latent virus became activated on inoculation of poliomyelitis virus either because of the trauma of the inoculation or because of other unknown host factors. The virus could not have been picked up as a contaminant in the laboratory during the preparation of material for inoculation, for we did not have this or a related virus in the laboratory at the time. It will be recalled that the histological examination of the medulla and spinal cord of *Rh. 53-23* (the monkey from which the virus was obtained) showed no lesions, either of poliomyelitis or meningoencephalitis in the five levels examined. Thus, although this animal developed mild clinical signs compatible with either disease, the pathological process may not have developed sufficiently at the early stage at which it was sacrificed. Its companion, *Rh. 53-22*, however, which simultaneously developed similar clinical signs, showed lesions of poliomyelitis histologically, when it was sacrificed at a later date. It is probable that if we had per chance elected to sacrifice *Rh. 53-22* instead of *Rh. 53-23* at the time, we would never have suspected the presence of another virus coexisting in *Rh. 53-23*.

Since the first reports in 1934 of the isolation of B virus from a patient bitten by a monkey, it has been known that antibodies to this virus may at times be present in monkeys. This species has been presumed to be one of the natural hosts, if not the only one, of B virus. The assumption that the virus is carried in the monkey has now been proved to be true from our experiences.⁴

⁴ The Virus Subcommittee of the International Nomenclature Committee, at the 6th International Congress of Microbiology held in Rome in September, 1953, recommended the use of non-Linnean binomials for certain viruses (14). *Herpesvirus simiae* was selected as the binomial for B virus. The present results lend support to this designation.

Study of monkey sera available in this laboratory revealed that a number of them had antibodies to this virus among both *rhesus* as well as *cynomolgus* monkeys used in the past or at present. It is difficult to ascertain in what part of the world the monkeys became infected. Although antibodies to this virus have been detected in the sera of normal human population in India and in gamma globulin prepared from normal adults in the United States this may be the result of the partial crossing which exists between B virus and herpes simplex virus.

In view of the fact that human gamma globulin preparations are able to neutralize B virus, its administration may be suggested as a passive prophylactic measure against possible B virus infection after a monkey bite. However, using the original strain of B virus, Sabin found that 26 sera taken from adults residing in New York City were negative with the exception of 2 which neutralized 10 to 20 intradermal doses for the rabbit (13). It may be that strains of B virus differ in the extent of their antigenic crossing with herpes simplex virus. The finding that certain human sera and pooled gamma globulin neutralize our strain of B virus has been confirmed using tissue cultures of monkey kidney (11).

SUMMARY

While passaging a recently isolated strain of poliomyelitis virus through a *rhesus* monkey, another virus was procured from its central nervous system. After intracerebral inoculation, the virus produced meningoencephalitis in monkeys, cotton rats, hamsters, guinea pigs, and rabbits; after intracutaneous inoculation a necrotic skin lesion was produced in the monkey and rabbit and this was often followed by myelitis. The virus could also be passed in newborn mice less than 48 hours old and in chick embryos by inoculation of the chorio-allantoic membrane.

Immunological and host range studies revealed this virus to be related to the B virus originally described by Sabin and Wright in 1934 (1).

To our knowledge this is the first record of B virus having been isolated from a monkey, and lends support to the inclusion of this agent as the simian member of the herpes group.

The infection is not uncommon in monkey stocks, as revealed by the finding of antibodies to the virus in their sera. In the present series 9 of 44 monkeys gave positive antibody tests.

Gamma globulin prepared in the United States in 1945, 1951, and 1953, as well as a certain proportion of sera from normal individuals in Bombay, India, and elsewhere, showed neutralizing activity against the new strain of B virus, and also to herpes simplex virus. This may be the result of the partial crossing which exists between the two viruses.

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