POLIOMYELITIS BY ACCIDENTAL CONTAGION IN THE CHIMPANZEE*

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For a number of years chimpanzees have been rather extensively used for experiments on the portals of entry of poliomyelitis virus (1). The animal was selected for this purpose originally because of a report by Müller (2) which suggested that two chimpanzees in a children's zoo had accidentally contracted poliomyelitis during an epidemic of this disease. Müller's description of this event, however, was far from complete and did little more than indicate the possibility that the chimpanzee might share with man a greater susceptibility to poliomyelitis than has been demonstrated in laboratory monkeys. While the many morphological and functional similarities between man and the chimpanzee have made this seem a reasonable working hypothesis, more adequate proof has only recently been encountered. This paper will describe definite infections with poliomyelitis virus in two uninoculated chimpanzees which were kept in the laboratory for some months under conditions which rendered impossible rigid isolation from accidental sources of contamination (3).

The animals were received in October, 1941, and kept in adjoining cages (4 feet × 6 feet) which were separated by a single large mesh grill permitting the exchange of bits of food and limited grooming activities. During the next 6 months the animal house also accommodated numerous *rhesus* monkeys which were receiving intranasal inoculations of nearly a dozen different human stools containing active poliomyelitis virus. They were confined in similar cages separated from the chimpanzees by a 4 foot aisle. Water and food pans were used interchangeably, often without adequate sterilization. The same attendant went from one cage to another without change of boots or clothing. These were frequently smeared with monkey feces.

Stools from these two chimpanzees were found to contain poliomyelitis virus after the animals had been resident under the above conditions for 6 months. They had at no time received any direct inoculations of virus. The steps in the identification of the virus as poliomyelitis will be described subsequently in the paper. At this point it seems pertinent to include some excerpts from the histories of the animals in question.

Chimpanzee "Mimi" A-2-65—Received in October, 1941. Weight 25 lbs. Age approximately 3.5 years. Blood removed from arm vein for serum.

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Jan. 26, 1942. In excellent physical condition. Bled for serum.

Feb. 21. For the past week there has been a slight narrowing of the right lid slit which appears to be due to an almost imperceptible swelling of the lids. Lid movement, extraocular, and facial movements are normal. For a few days the animal seemed a bit listless, but is normally active now. (Temperatures not taken during this period.)

Apr. 27. Bled for serum. Stool collected, after which inoculated with 4 cc. of Sudeck stool[1] by mouth (this stool had been shown to contain many infective *rhesus* doses). Two control *rhesus* monkeys inoculated intranasally.

Apr. 28. Inoculation repeated.

May 16. Stool collected. No signs of poliomyelitis though the two control monkeys have been paralyzed.

June 19. Has been perfectly well. No significant temperature elevation. Bled for serum.

Sept. 2, 3, 4. Received a total of 60 cc. of Sudeck stool by mouth. *Rhesus* control inoculated intranasally.

Sept. 13. *Rhesus* control paralyzed.

Oct. 3. Chimpanzee has shown no fever or other sign of illness.

Mar. 2, 1943. Has remained perfectly well. Bled for serum. Traded for another chimpanzee.

Chimpanzee "Benito" A-2-72.—Received October, 1941. Weight 28 lbs. Age approximately 4.5 years.

Jan. 22, 1943. Bled for serum (chloroform anesthesia).

Jan. 23. Animal has conjunctivitis of left eye which may have been due to a spilling of chloroform. Is listless. Nothing made out in chest. (No temperature taken at this time.)

Jan. 24. Still inactive and eating poorly.

Apr. 27. Has dermatitis and has lost weight. There are many intestinal parasites. Bled for serum. Stool collected, after which inoculated with 4 cc. of Sudeck stool by mouth. Two rhesus controls inoculated intranasally.

Apr. 28. Inoculation repeated.

May 15. No symptoms of poliomyelitis and no temperature elevation. Two *rhesus* controls are paralyzed. Stool collected.

June 18. Being treated for worms. Dermatitis under control. Bled for serum.

Sept. 2, 3, 4. Animal in excellent condition. Received a total of 60 cc. of Sudeck stool by mouth. One *rhesus* control inoculated intranasally.

Sept. 13. *Rhesus* control paralyzed.

Oct. 3. Chimpanzee has shown no fever and no signs of poliomyelitis.

Mar. 2, 1944. Has remained in excellent condition. Bled for serum. Traded for another chimpanzee.

Several points in these histories deserve consideration. Both animals suffered temporary indispositions during the early period of observation. These were sufficiently marked to excite comment at a time when no one was seriously considering the possibility of accidental infection. It is true that these minor illnesses occurred 2 and 3 months respectively before the demonstration of virus in the stools. Since virus has been found in the stools of one chimpanzee as long as 54 days after inoculation (4) it does not seem fantastic to suspect that these slight clinical manifestations *may* have marked the onset of active infection.

[1] The Sudeck stool was collected from a patient with paralytic poliomyelitis in Baltimore in 1941.

The failure of the animals to develop signs of poliomyelitis following subsequent inoculation with many times the lethal dose for *rhesus* monkeys is possibly of some significance although certain qualifications must be made. The Sudeck strain of poliomyelitis virus has been found to be capable of producing non-paralytic infections upon oral inoculation in two other chimpanzees, although it readily paralyzes *rhesus* monkeys following intranasal inoculation. It therefore cannot be stated with certainty that "Mimi" and "Benito" were completely refractory to their actual inoculations of known virus, since they may have had a second non-paralytic infection with this new strain of virus.

The Recovery of Virus

Poliomyelitis virus was first isolated from a stool specimen of the chimpanzee "Mimi", taken on April 27, 1942, nearly 7 months after her advent into the laboratory. The monkey which received this material (A-4-32, Chart 1) was inoculated intranasally with 1 cc. of untreated stool on 6 successive days. On the 13th day it developed typical paralytic poliomyelitis. The brain was subsequently examined in serial sections and revealed a distribution of lesions in various nerve centers which is characteristic of poliomyelitis (1). Cord material from this animal subsequently produced paralytic poliomyelitis on intracerebral inoculation into six *rhesus* monkeys. One of these animals was examined microscopically and was found to have typical lesions in the brain and spinal cord. This material produced no signs of disease of the central nervous system in ten mice, two rabbits, and three guinea pigs. (An equal number of controls were inoculated with sterile broth and were also asymptomatic.) Cultures in thioglycollate medium remained sterile. These findings left little doubt that the virus isolated from the chimpanzee stool was in reality that of poliomyelitis. The occurrence, however, was so unexpected that it seemed necessary to rule out the possibility that there might have been some mixup in the original material. Accordingly the stool of April 27, 1942, was again inoculated intranasally into *rhesus* A-4-39 and A-4-71. The former showed no signs of the disease either clinically or microscopically, but A-4-71 developed typical paralytic poliomyelitis. Although intracerebral passage to *rhesus* A-5-15 failed, this in no way detracts from the validity of the result in A-4-71 which clearly indicated that there was active virus in the stool of the uninoculated chimpanzee "Mimi."

Since virus had been demonstrated in the stools of "Mimi" it seemed worth while to repeat a test, previously negative, with the stools of Benito obtained under similar circumstances (*rhesus* A-4-28, Chart 1). Accordingly the stool specimen of April 27, 1942, which had failed to produce disease on intranasal inoculation into *rhesus* A-4-28 was inoculated into *rhesus* A-6-14. As in previous inoculations the technique consisted of the instillation of 1 cc. of raw stool suspension into each nostril on 6 successive days. On the 14th day following the first inoculation the animal became quadriplegic and was sacrificed. The brain was examined in serial sections and was found to show a distribution of lesions typical of poliomyelitis. Cord emulsion was given intracerebrally to seven *rhesus* monkeys, six of which developed typical paralysis. Microscopic examination of the cord of one of these (A-7-16) showed typical lesions in the spinal cord. The same material also failed to provoke any signs of illness in ten mice and produced no growth in thioglycollate medium. It therefore seemed clear that poliomyelitis virus and no other had been present in "Benito's" stools on April 27, 1942.

Tests for Immunity

As indicated in the histories, blood serum was obtained from the chimpanzees at various times during their stay in the laboratory. This was tested for ability

Virus in Chimpanzee Stools

A-2-65 "Mimi"		
Stool of Apr. 27, 1942 (pre-inoculation)		
Rhesus 4-39 (N) CNS (N)	*Rhesus* 4-71 (P) CNS (+)	*Rhesus* 4-32 (P) CNS (+)
	Rhesus 5-15 (N) CNS (N)	*Rhesus* 5-26 (P) CNS (+)
		Rhesus 7-09 (P)
		Rhesus 7-10 (P)
		Rhesus 7-13 (P)
		Rhesus 7-14 (P)
		Rhesus 8-24 (P)
		10 mice (N)
		2 rabbits (N)
		3 guinea pigs (N)
		Cultures:
		Aerobic (N)
		Anaerobic (N)

A-2-72 "Benito"	
Stool of Apr. 27, 1942 (pre-inoculation)	
Rhesus 4-28 (N) CNS (N)	*Rhesus* 6-14 (P) CNS (+)
	Rhesus 6-33 (N) CNS (N)
	Rhesus 7-16 (P) CNS (+)
	Rhesus 7-61 (P)
	Rhesus 7-62 (P)
	Rhesus 7-65 (P)
	Rhesus 7-66 (P)
	Rhesus 8-25 (P)
	10 mice (N)
	Cultures
	Aerobic (N)
	Anaerobic (N)

(P) = Paralysis.
(N) = Negative, normal, or no paralysis.
(+) = Typical poliomyelitic lesions.

CHART 1

to neutralize Sudeck virus, a recently isolated strain then current in the laboratory, as well as the viruses actually isolated from the animals themselves (Table I).

Virus was mixed with whole serum to a final concentration of 10 per cent, 5 per cent, or 1 per cent. (In a series of 73 intracerebral inoculations no significant difference has been ob-

served between 10 per cent, 5 per cent, and 1 per cent virus inoculations. These concentrations are therefore lumped together in the table.) The mixtures were allowed to stand 2 hours at room temperature which was followed by 2 hours in the ice box. The tests were staggered as they were set up, so that approximately the same interval elapsed before inoculation of each sample. *Rhesus* monkeys of uniform size were used for each test. The animals were etherized and a trephine hole was made straddling the midline just over the motor cortex. The inoculum was then introduced in equal amounts into the lateral thalamus of each side, a total of 0.8 cc. being used for each animal. The lateral thalamus was chosen because it is a region of high susceptibility and is located sufficiently deep within the brain so that regurgitation of the inoculum is largely avoided. With this technique it was felt that some of the va-

TABLE I

Neutralization Tests—Chimpanzee Sera against Sudeck Virus

Sera A-2-65 "Mimi"	Paralysis in test animals	
	Sudeck, virus 1–10 per cent	Animal's virus 1–10 per cent
Oct. 17, 1941 (admission)		PP
Jan. 26, 1942 (4 mos. after reception)	PP	PP
Apr. 27, 1942 (date of virus isolation)	NN	NN
June 19, 1942	NN	NN
Mar. 2, 1943	NN	NN
Sera A-2-72 "Benito"		
Jan. 22, 1942 (4 mos. after reception)	NN	PPP
Apr. 27, 1942 (date of virus isolation)	NN	NNN
June 18, 1942	NN	NNN
Mar. 2, 1943	NN	
Control inoculations	77 P, 10 N	

P = Paralysis (each letter represents one test animal).
N = Normal or neutralization (each letter represents one test animal).

garies in the distribution of the inoculum (5) might be minimized. (This technique of inoculation was not employed for Sudeck tests. These were done by intracortical injection.)

It can be seen in Table I that on admission, and for 4 months thereafter, neither animal had antibody against the virus eventually isolated from it. However, both chimpanzees had developed antibody against their own virus strains by the time these were isolated from the feces. The various sera from "Mimi" behaved in identical fashion toward the Sudeck strain, suggesting that it may have been the one involved in her infection. On the other hand, "Benito's" serum neutralized the Sudeck virus from the time of the first test and continued to neutralize it throughout his entire stay in the laboratory. Titration was impossible because of the limited quantity of serum available so that his status with relation to this strain could not be determined. However, it is quite clear that "Mimi" and probably "Benito" did not develop their Sudeck

antibodies as a result of known overt inoculation with the Sudeck virus, since they both had antibody against this strain at the time the inoculations were done.

Significance of the Neutralization Tests

In evaluating these neutralization tests there are certain statistical considerations which should be mentioned. It is often a matter of conjecture whether any point is really proven with only two test animals. On the basis of our past experience with 87 tests with various strains from which paralysis should have been the outcome, but in which ten animals failed to sicken, the chance of failure of the virus in any single test is 11 per cent. Therefore, the chance of encountering unexplicable inactivity of the virus in two tests is 1.2 per cent (0.11^2)—a margin which virtually rules out sampling effects. Thus if both animals used in the test remain well, it is reasonably certain that the virus used in the test was actually neutralized and did not fail to infect for some other reason. It has been remarkable in our experience that inoculation of several strains of virus in concentrations varying from 1 per cent to 10 per cent produces an almost uniform attack rate of about 90 per cent. This suggests that the material under consideration is quite uniform and that the 87 animals represent an adequate sample of it. However, since this number of animals is relatively small, one can conceive that there might be some variability which was not detected. Assuming, therefore, that the failure rate of 11 per cent might vary by two standard deviations, it could be as low as 6 per cent, or as high as 20 per cent. The latter would be an extremely conservative estimate, but assuming for the moment that this were true, it is apparent that two tests which did not result in paralysis of the inoculated animals would be encountered in 4 per cent of them by chance. This value lies on the borderline of significance. However, if one considers sequences of tests done in pairs, it is still possible to draw valid conclusions. Thus, while there is a 4 per cent chance that "Benito's" serum of January 22, 1942, did not neutralize but that the virus failed for some other reason, there is only a 0.16 per cent chance that the sera of *both* January 22 and April 22 did not have real neutralizing power (four animals—0.04^2). This type of reasoning may be applied to runs of several pairs of tests in which event the chances of encountering false neutralization decrease quite sharply. Thus the chance that three pairs of sera would be incorrectly shown as positive is 0.006 per cent (0.04^3) while in this particular universe four pairs of falsely negative sera would be encountered in only 0.0003 per cent (0.04^4) of trials.[2]

It is of great interest that the animals had antibody against virus at the time of its isolation from the stools. Taken in conjuction with some unpublished studies on passive immunization (4) these findings raise the question whether

[2] The writers wish to thank Dr. Margaret Merrell of the Department of Biostatistics, Johns Hopkins University, for her suggestions and criticism in relation to these statistical procedures.

antibody as such has any effect upon the elimination of virus from the intestinal tract.

DISCUSSION

It is not altogether surprising that chance infections of other primates may occur with certain human strains of poliomyelitis. One should emphasize that the two chimpanzees were in more or less direct contact with human stools containing virus unchanged by animal passage. The recent demonstrations of several strains of poliomyelitis virus which seem to be similar to the Lansing strain in their ability to infect rodents on intracerebral inoculation (6, 7) indicate that the population of human poliomyelitis viruses is more heterogeneous than hitherto supposed. Last year Craigie called our attention to what appeared to be accidental infection of *rhesus* monkeys in his laboratory (8) and this phenomenon has been under investigation during the past months. The circumstances surrounding these infections are not as simple as those concerned in the cases of the chimpanzees and hence must be considered in a separate publication. The possibility that certain strains of virus may form a spectrum broad enough to include infectivity by peripheral portals in lower as well as higher primates must be kept in mind. However, the general organization of the chimpanzee is so much closer to that of man than is that of the monkey that it would be not unexpected to find it receptive to more strains of human poliomyelitis by peripheral portals than are the lower mammals.

We cannot at the moment completely rule out the possibility that in the cases just described we may be dealing with a true poliomyelitis of chimpanzees in the sense that Theiler's spontaneous encephalomyelitis is a true disease of mice. Even if this were the case there is every reason to believe that the poliomyelitis of chimpanzees would be found quite closely related to that of man, since according to our observations it would have the same host range in laboratory mammals and an identical pathogenesis. Whatever the interpretation of the presence of the virus in the stools of the uninoculated chimpanzees, this species emerges as a superior subject for poliomyelitis experiments dealing with portals of entry, resistance, and immunity.

SUMMARY

Poliomyelitis virus was isolated from the stools of two uninoculated chimpanzees which had been quartered for 6 months in cages adjoining those of *rhesus* monkeys receiving intranasal inoculations of potent human stools. Upon arrival, and for 4 months thereafter, neither chimpanzee had antibody against the virus eventually isolated from it. However, antibody had developed against the animals' own virus strains at the time these were isolated from the feces.

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