

## PASSIVE IMMUNITY TO POLIOMYELITIS IN THE CHIMPANZEE\*

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The rôle of antibody in immunity to poliomyelitis has long been in dispute. While it is generally conceded that actual infection protects to a considerable degree against second attacks experimentally induced with homologous virus, it is nevertheless apparent that even this type of immunity is relative and may be overwhelmed if sufficient virus is given or a new portal utilized (1-4, 5, chapter X). It is therefore not surprising that effective immunization with inactive virus has not been convincingly demonstrated (6-8) since experiments of this type have not in the past been specifically planned to evaluate the relativity of immunity. Passive immunization of laboratory animals has likewise been almost uniformly unsuccessful (9-12) except in a few instances (13).

Recently Kramer (14) has advanced evidence that resistance to intracerebral inoculation of Lansing poliomyelitis virus can be induced in mice by the intraperitoneal injection of relatively large amounts of immune serum. While these effects are definite, the quantities of virus and serum used are so incommensurate with what one expects to find involved in human poliomyelitis, that attention naturally turns to more physiological trials of passive immunization in man himself. Here results have been inconclusive. The study of Stokes, Wohlman, Carpenter, and Margolis (15) was inadequately controlled; Waltner (16) reported an attack rate of 0.38 per cent in a passively immunized group of children under 12 years as opposed to a rate of 0.62 per cent in a control group, but the number of cases was too small to make these differences statistically significant. Favorable results of passive immunization were also reported by Davide (17), who observed the spread of a poliomyelitis epidemic among 174 persons under 25 years of age in rural Sweden. Of this group 73 had been inoculated with 5 cc. of convalescent serum. Fourteen cases were reported in the non-immunized group and one case in the immunized. While at first sight this seems to be a convincing result, there is considerable doubt as to the diagnosis of poliomyelitis in most of the cases. The author states that some cases were paralyzed (number not stated) but that the majority of those which occurred in the control group were mild, consisting of fever, diarrhea, neck rigidity, and "delirium." No lumbar puncture or virus studies were done. There was also no attempt to demonstrate whether the serum used possessed neutralizing power. Since it was taken from convalescents as early as the 10th day after the subsidence of fever (no further details as to sources)

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Protocols of all animals are included in the paper following this one in the *Journal*.

there is reason to believe that the titer may have been low. The author himself makes no great claims for the validity of the experiments and states that further proof is required.

Because of the many difficulties attending field trials of immunization procedures it was felt that laboratory experiments had much to offer. At the same time some of the pitfalls of previous experimental work might be in some measure avoided by special attention to the choice of animal, the control of dosage, the following of antibody levels, and the determination of the effectiveness of the measures employed by means of virus studies and histopathological examinations. Accordingly, experiments have been undertaken in 6 chimpanzees. This animal has the advantage of being quite susceptible to poliomyelitis, since accidental infections have been demonstrated (18). It has also been shown to be capable of acquiring infection by the alimentary tract which presumably furnishes a closer parallel with man than is to be found in the *rhesus* monkey or the mouse (19). While the number of chimpanzees used is admittedly limited by their rarity and cost, the results which have been accumulated by the intensive study of each case are sufficiently uniform to merit consideration.

#### *Material and Methods*

Six immature chimpanzees weighing 20 to 35 pounds each were procured in lots of 3, the first group being used as unprotected controls and the second as passively immunized test animals. With one exception the animals were all obtained from a single dealer but there was evidence that each animal had already had several owners, although 2 chimpanzees in the immunized group were only recently imported from Africa. The two groups of animals were brought into the laboratory separately, in April and July respectively, to preclude the possibility of the passively immunized group having contact with active virus prior to inoculation. The control animals were all sacrificed by the middle of May so that 6 weeks elapsed before the reception of the second group. This latter group was immunized and inoculated within 5 days of reception. At all times the chimpanzee quarters, which were located in a separate wing of the animal house, were rigidly isolated. Each animal was bled for serum on arrival (2 were bled while still at the dealer's) and a stool specimen was collected on the day of inoculation which, in the case of the controls was the 19th day after reception, and for the immunized test animals the 3rd to 5th day. Stool samples and sera were collected at appropriate intervals thereafter.

Inoculation was carried out by the alimentary route with unpassaged human stool from a paralytic patient hospitalized in Memphis, Tennessee, in August, 1942. The strain is known as the Kotter virus and sufficient stool was collected to carry out the entire series of inoculations with the same lot. The original material was frozen on CO<sub>2</sub> ice after collection and transported to the laboratory where it was thawed, and prepared by the method already described (20). The stool was suspended in enough sterile distilled water to make it freely flowing, strained through gauze, and centrifuged at 3000 R.P.M. for 30 minutes. The supernatant was then refrozen in 20 cc. ampules and used as needed. The stool was selected in the beginning because of its ability to paralyze *rhesus* monkeys on intranasal inoculation and in the course of the subsequent experiments 5 monkeys were so inoculated. All of these developed paralysis.

The inoculation of the chimpanzees was carried out as follows: The animals were anesthetized lightly with chloroform and a stomach tube was passed. Through this 15 cc. of stool suspension was given and the tube was washed out with a small quantity of water and withdrawn. As the animals regained consciousness 5 cc. of stool was placed in the mouth and the cheeks were lightly massaged. Contamination of the buccal surfaces and tongue was thus achieved. This elaboration was necessitated by the fact that the animals would not take the stool willingly or swallow it if fully conscious. The method was not entirely satisfactory since definite infection of the olfactory bulbs took place in one control animal and in one immunized animal, and a second immunized animal had questionable lesions in his olfactory bulbs.

Passive immunization was produced with homologous hyperimmune monkey serum capable of neutralizing 500 M.I.D. of Kotter virus when equal quantities of whole serum and virus were mixed and injected intracerebrally into *rhesus* monkeys in quantities of 0.8 cc. The test chimpanzees were given 50 cc. of this serum intraperitoneally 26 hours before inoculation. One hour before inoculation they again received 50 cc. of serum and this procedure was repeated before each inoculation on the 2 following days, although the amount of serum was reduced to 35 cc. Each of the 3 immunized animals thus received four injections of serum totaling 170 cc. and three inoculations of stool. Two of the chimpanzees were listless for an hour following the injections of serum but no serious reactions were encountered.

Neutralization tests were performed as follows: Whole serum and virus emulsion were mixed in appropriate quantities and allowed to stand at room temperature for 2 hours, followed by 2 hours in the ice box. Preparation of these mixtures was staggered so that approximately 4 hours elapsed before the inoculation of each. A total of 0.8 cc. of inoculum was employed, 0.4 cc. being injected through a trephine hole over the midline into each lateral thalamus in an effort to minimize backflow around the needle puncture and standardize the procedure. Final concentrations of virus of 10, 5, and 1 per cent were used. Since these did not show any significant differences, all the tests are pooled. Because during the past 2 years all intracerebral inoculations employing 1 to 10 per cent cord virus have showed a nearly uniform success rate of 90 per cent regardless of the strain of virus used, 99 inoculations have been accumulated as controls. Of these ten were unsuccessful, but this series of controls furnishes an adequate statistical basis for the interpretation of the two or more individual tests carried out with each specimen of serum. It was thus possible to calculate the probabilities of encountering by chance alone any of the results obtained. These probabilities have been included in the tables showing neutralization tests.

#### *Character of the Infection in the Control Chimpanzees*

All of the control chimpanzees had 1–2° of fever following inoculation, but the time of its appearance varied from the 5th to the 16th day following the first inoculation (Table I). Its duration was 1 to 2 days. Chimpanzee A4-36 which had a temperature of 101.2° on the 5th day was the only animal in the series with a definite olfactory infection.

None of the controls developed frank, paralytic poliomyelitis, although chimpanzee A4-34 had depression of tendon reflexes in both the right leg and arm and showed striking lesions in the brain and in the spinal cord. The other 2 animals exhibited no signs whatever of paralysis and had very mild lesions in the CNS which will be pictured and discussed in the paper immediately following (21, Figs. 4 to 8). Had it not been possible to isolate virus from the

stools of all the animals, the diagnosis of poliomyelitis would have been considered impossible to establish in chimpanzee A4-35. Table II shows the dates on which virus was demonstrated in the stools of the animals under consideration. It can be seen that it was isolated from them all by the use of a small number of monkeys.

TABLE I  
*Résumé of Findings*  
*Chimpanzees Inoculated with Poliomyelitis Virus*

Animal	Immunization	Paralysis	Temperature*	Lesions in CNS	Virus in stools 19th-28th days	Blood antibody	
						At inoculation	30 days
A4-34 (c)	None	None	102°, 13th-14th day	+++	+	-	
A4-35 (c)	None	None	101.2°, 16th day	+	+	-	
A4-36 (c)	None	None	101.2°, 5th day	++	+	-	
A4-47	170 cc. serum	None	101.4°, 15th-16th day	+	+	+	+
A4-48	170 cc. serum	None	101.8°, 11th day†	++	+	+	-
A5-01	170 cc. serum	None	Spikes 101-2° 7, 14, 23rd day‡	+	+	+	+

c, control animal.

\* Rectal temperature of normal chimpanzees 99-100°F.

† Mild diarrhea during this period.

TABLE II  
*Virus in Chimpanzee Stools*

Animal	Days from inoculation							
	6	13	19	21	22	26	27	28
A4-34 (c)	++	---	+--					
A4-35 (c)	+--	---			+--	---		
A4-36 (c)				+				
A4-47			++					+--
A4-48			+---					--
A5-01			+---				--	

c, control animal.

Each individual test indicated by plus or minus sign.

In summary it can be said that while the 3 control animals were shown to have virus in the stools and lesions in the CNS, the disease was extremely mild and thus quite different from that seen in the *rhesus* monkeys inoculated intranasally with the same material.

*Character of the Infection in Passively Immunized Animals*

The 3 animals which received 170 cc. of the homologous hyperimmune serum distinguished themselves in no important degree from the similarly inoculated controls. All had at least 1 day of fever (Table I), all showed mild lesions in the CNS, and all had virus in the stools (Tables I and II). While it appeared that the lesions were milder than in the controls and that more animals were required to demonstrate virus in the stools,<sup>1</sup> the series is admittedly small and these differences may not be significant (21, Figs. 9 to 14).

TABLE III  
*Neutralization Tests with Chimpanzee Sera against the Virus Used for Inoculation*

Animal	Sera obtained on		
	Admission	Last day of immunization	27-28 days after immunization
A4-34	PP		
A4-35	PP		
A4-36	PP		
A4-47	PPPP	NNNNN (0.002 per cent)	PPPPNN (9.4 per cent)
A4-48	PPPP	NNNNN (0.002 per cent)	PPPP
A5-01	PPPN	PNNNNN (0.008 per cent)	PNNN (0.4 per cent)
Controls	89P 10N		

P, paralysis (each letter represents an individual test).

N, no paralysis (neutralization).

Figures in parenthesis represent the percentage likelihood that these results would be obtained by chance alone. Values less than 3 per cent are considered significant.

*Relationship of Antibody to Infection with Virus*

Of great interest were the serum antibody levels of the entire series of animals. These are summarized in Table I but given in full detail in Table III. The control animals which had not received hyperimmune serum had no antibody against the concentrations of virus used in the test.<sup>2</sup> The sera of the 3 experimental animals which received hyperimmune serum all neutralized 10 per cent virus (500 M.I.D.) at the end of the immunization period. Thirty days later, however, only one serum had definite protective properties, while one probably showed some antibody, and the other none against the same doses of virus. It thus appeared that antibody was disappearing at differing rates in the animals, but no correlation could be made out between this and the severity of infection.

<sup>1</sup> The method of multiple inoculation has been found quite effective for demonstrating virus in stools which on the first tests appeared to be negative (22).

<sup>2</sup> The virus used in the tests titrated out as far as  $10^{-4}$  in 0.8 cc. inocula, so that each serum sample which showed protective power was capable of neutralizing at least 50 to 500 M.I.D.

## DISCUSSION

Since the two groups uniformly presented the same picture, the small number of animals used in the experiment does not seriously detract from the conclusions. It will be recalled also that all the animals, both control and test, had lesions in the CNS and virus in the stools. One animal in each group had suffered accidental olfactory contamination and a second of the test animals had two cuffed vessels in the outer layers of its olfactory bulbs. This mischance had to some degree vitiated the attempt to employ the alimentary portal exclusively, but it is questionable whether it materially alters the interpretation of the experiments. Infection took place in the remaining immunized animal by an extraolfactory portal and we have as yet no information indicating that there are any important differences in the ability of the humoral mechanism to operate upon these two portals.

The infections were all extremely mild so that it cannot be maintained that the protective mechanism was overwhelmed. While the lesions of the test animals were on the whole less severe than those of the controls, it would be hazardous on the basis of this experience to suggest that the serum had significantly modified the invasion of the CNS. A much larger series of animals would be required to reach such a conclusion.

Although our results are not in accord with those of Kramer (14) there are certain similarities and differences between the two studies which illustrate the difficulties encountered in experiments of this type. Kramer used 0.2 cc. of immune serum administered intraperitoneally to a mouse. This is a quantity much greater relatively than that involved in our experiments and one which could be given to human beings only under exceptional circumstances. Also he employed the intracerebral route for the challenge dose. From one point of view this may be considered a more drastic test than inoculation by a peripheral portal but its highly artificial nature makes evaluation difficult. One could argue that the hemorrhage attending the inoculation simulates an *in vivo* neutralization test and provides little information regarding the normal mechanisms of the body. Both Kramer's experiments and ours were performed with sera from heterologous animal species and since our results were negative, it may be argued that monkey serum was not effective in the chimpanzee. Occasional instances in which sera that were protective in the same species were not capable of protecting other species have been recorded (23). While these situations are unpredictable as regards the species which may be incompatible, they are also extremely rare in comparison to the numerous instances in which at least the first courses of sera from heterologous species are effective. Nevertheless, we cannot entirely rule out the possibility that monkey serum might be eliminated more rapidly than would a strictly homologous serum. This could produce a situation in which the blood antibody fell to an ineffective level during the relatively long incubation period which is characteristic of

poliomyelitis. Since there are no facts available regarding this point, further experimentation will be necessary.

This much remains certain, that the presence of blood antibody at the time of inoculation did not prevent infection. It is thus implied that, should passive immunization be possible in this disease, a high level of antibody must be maintained for a considerable period if it is to be effective. While the case for humoral immunity in poliomyelitis still is by no means a closed one, experiments of this type suggest that the conditions for maintaining passive immunity even to very mild infection by more or less "normal" portals are difficult to achieve. This fact alone has an important bearing upon what might be expected from field trials in human populations.

#### SUMMARY

A series of 6 chimpanzees was inoculated orally with stool from a poliomyelitis patient.

Three of these animals had been given 170 cc. of homologous, hyperimmune monkey serum prior to inoculation, while three were unprotected.

All 6 of the animals were subsequently shown to have poliomyelitis virus in their stools and characteristic lesions in their central nervous systems although none of them developed paralytic poliomyelitis.

All of the immunized animals had demonstrable blood antibody at the time of inoculation although it was present in only one case 30 days later.

#### BIBLIOGRAPHY

1. Toomey, J. A., *Am. J. Dis. Child.*, 1936, **52**, 802.
2. Flexner, S., *J. Exp. Med.*, 1937, **65**, 497.
3. Kessel, J. F., and Stimpert, F. D., *J. Immunol.*, 1941, **40**, 61.
4. Howe, H. A., and Bodian, D., *J. Exp. Med.*, 1941, **74**, 145.
5. Howe, H. A., and Bodian, D., *Neural mechanisms in poliomyelitis*, New York, The Commonwealth Fund, 1942.
6. Brodie, M., *J. Immunol.*, 1935, **28**, 1.
7. Kramer, S. D., *J. Immunol.*, 1936, **31**, 167.
8. Olitsky, P. K., and Cox, H. R., *J. Exp. Med.*, 1936, **63**, 109.
9. Rhoads, C. P., *J. Exp. Med.*, 1931, **53**, 123.
10. Schultz, E. W., and Gebhardt, L. P., *California and Western Med.*, 1935, **43**, 111.
11. Kolmer, J. A., *J. Immunol.*, 1936, **31**, 119.
12. Gordon, F. B., Hudson, N. P., and Harrison, J. A., *J. Infect. Dis.*, 1939, **64**, 241.
13. Weyer, E. R., Park, W. H., and Banzhof, E. J., *J. Exp. Med.*, 1931, **53**, 553.
14. Kramer, S. D., *J. Immunol.*, 1943, **47**, 67.
15. Stokes, J., Wolman, I., Carpenter, H. C., and Margolis, J., *Am. J. Dis. Child.*, 1935, **50**, 581.
16. Waltner, K., *Arch. Kinderheilk.*, 1933, **100**, 147.
17. Davide, H., *Off. Internat. Hyg. Pub., Bull. Mensuel.*, 1928, **20**, 74.

18. Howe, H. A., and Bodian, D., *J. Exp. Med.*, 1944, **80**, 383.
19. Howe, H. A., and Bodian, D., *Bull. Johns Hopkins Hosp.*, 1941, **69**, 149.
20. Howe, H. A., and Bodian, D., *J. Infect. Dis.*, 1940, **66**, 198.
21. Bodian, D., and Howe, H. A., *J. Exp. Med.*, 1945, **81**, 255.
22. Howe, H. A., and Bodian, D., *Am. J. Hyg.*, 1944, **40**, 224.
23. Tillett, W. S., *J. Exp. Med.*, 1928, **48**, 791.