Day-by-Day Response of Vaccinated Chimpanzees to Poliomyelitic Infection

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Because the experimental animals used here respond to poliomyelitis infection similarly to the response in man, the quantitative findings of the rise and fall of virus in relation to antibody in the vaccinated animals explain many of the phenomena observed in human vaccination. An important contribution.

This paper extends some preliminary observations that were presented to the American Public Health Association Annual Meeting in November, 1956; however, it must still be regarded as a progress note, since limitations of space restrict complete documentation of all the findings on which the conclusions are based. The observations deal with the alimentary excretion of Type I poliovirus in seven vaccinated and seven control chimpanzees following oral challenge with this virus. A particular effort has been made to quantitate the rise and decline of virus in relation to the level of serum neutralizing antibody.

The vaccinated animals received three or more 1 ml doses of commercial trivalent formol inactivated poliomyelitis vaccine supplied through the courtesy of the National Foundation for Infantile Paralysis. The experiments were begun in 1954 with the low potency preparations then available, but subsequently the animals received a booster dose on February 22, 1955, of a somewhat better preparation kindly supplied by Eli Lilly and Company. Since this time much better vaccines have become available, but these were the best obtainable at the time. On March 21, 1955, the chimpanzees received an oral challenge of Brunhilde cord virus (ca 25,000 PD50). Throat swabs and sera were collected daily for the first 10 days following exposure and somewhat less frequently for the following two weeks. Two swabs were obtained and stored individually from the tonsillar areas of each animal, while stools of approximately alternate days were pooled as weekly specimens (collections on Monday, Wednesday, and Friday).

All the materials for virus assay were suspended in a menstruum composed essentially of balanced salt solution, to which had been added 0.5 per cent lactalbumen hydrolysate and 0.12 per cent crystalline bovalbumen.[1] In some cases 2 per cent calf serum was also included. Stools were diluted at 1:3 before clarification in the Spinco centrifuge at 34,000 x gravity for one hour; swabs were placed in 2 ml of balanced salt solution at collection to be eluted later and similarly clarified without further treatment. All specimens were tumbled overnight at 4° C with 20 per cent anaesthetic ether. After settling, the top

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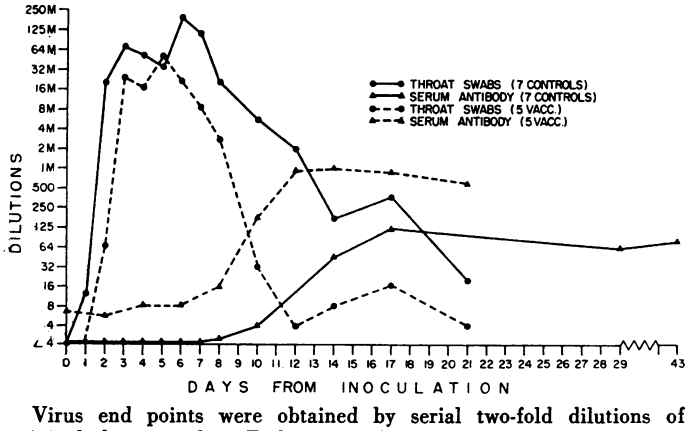
layer was removed and discarded, while the balance was freed from ether by evaporation at 4° C and used for inoculation. Blood serum was obtained at approximately the same times as the other materials in the routine manner. Everything was then stored at — 40° C until use. Virus isolations and neutralization tests were subsequently carried out in cultures of rhesus monkey kidney cells (monolayers for stools and the growth inhibition test for throat swabs and sera).

Starting at 1:9 to avoid toxicity, serial twofold dilutions of the clarified stools (in the saline medium) were inoculated into two monolayer kidney cell cultures for each dilution. The eluate from throat swabs was inoculated without further dilution, 0.25 ml being added to 0.5 ml of kidney cell suspension. Neutralization tests were carried out against 100 ID 50 of Connaught Standard Type I virus with twofold serum dilutions and two tubes per dilution. The experiments were terminated in seven to 10 days, depending upon the rate of cell growth in the uninoculated controls. End points of the cell inhibition test were checked microscopically and cell monolayers were studied daily for cytopathogenic changes. With few exceptions the virus assays were repeated two or three times. Passage and typing of the highest dilutions which produced cell destruction were carried out in most virus isolations.

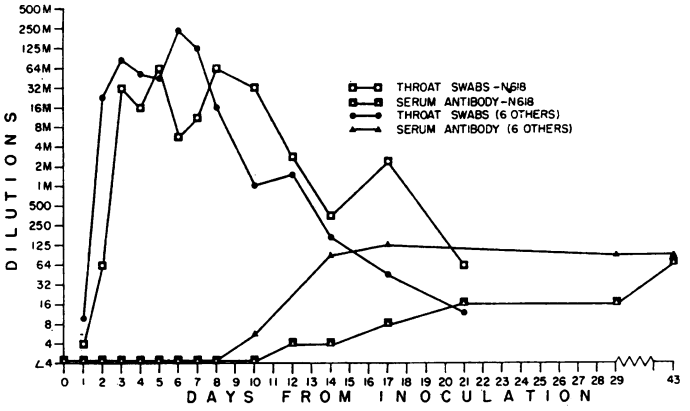
Following booster vaccination the maximal Type I serum neutralizing antibody shown by the animals ranged from 1:4 to 1:1,000 with a median of 1:8. (All were without demonstrable antibody initially.) At the time of challenge one month later there was little change.

As the data accumulated it was clear beyond doubt that five vaccinated animals with serum antibody levels averaging as low as 1:6 at challenge acquired alimentary infections, but carried virus in relatively high titer a much shorter time than did the controls. It was also evident that the highest titers reached by the vaccinated animals were, with the exception of one day, clearly lower than those observed in the controls. Conversely, two animals in the series with prechallenge titers of 1:64 and 1:1,000 failed to show demonstrable



Virus end points were obtained by serial two-fold dilutions of original throat swabs. End points of serum antibody were derived from similar dilutions of serum against approximately 100 ID 50 of Connaught Standard Type I virus.

Figure 1—Average Titers of Type I Poliovirus in Throats and of Homologous Serum Antibody in Vaccinated and Control Chimpanzees After Feeding of Type I Virus



This chart is a partial breakdown of the seven control animals averaged in Figure 1.

Figure 2—Throat Swab and Serum Antibody Titer in Seven Previously Uninoculated Chimpanzees (N-618—A Slow Reactor, Compared with the Average of Six Others)

alimentary infection. These were consequently omitted from further calculations.

These differences are shown in Figure 1. They are particularly striking in the throat swabs on the 10th day following challenge. Here the average titer of the vaccinated animals dropped to less than 1:4, while the average of the controls was nearly 1:6,000. By the 14th day the average titer of the controls was depressed to 1:200, but still stood in contrast to the control titer of 1:8 ($P = <0.0001$).

It came as a surprise that the findings of the 10th day, as cited above, did not completely rule out chance, despite their apparent disparity ($P = 0.17$). The reason for this may be seen in Figure 2, which compares the rise and fall of virus titer in the throat of one animal (N-618), as compared with the average of the other six control chimpanzees. N-618 maintained a peak titer of 1:32,000 to 1:64,000 for 10 days, although toward the end of this period all of the others had declined to about 1:1,000. This case is particularly instructive and will be discussed later. When it is omitted, however, although the average control level for the 10th day was reduced to 1:1,000, it was still vastly different from the average of about 1:4 shown by the vaccinated group ($P = <0.0001$).

For the first seven days (with the exception of the fifth) the average titers of the vaccinated animals were definitely lower than those of the controls (Figure 1), but because of great variability in the time at which the individual animals reached peak titers, the standard errors of the means were quite large. Consequently the values of P ranged from 0.2 to <0.0001 with the median at 0.043. While sampling errors are not ruled out in all of the individual determinations, the fact that control averages were strikingly lower on 10 of 11 days constitutes strong corroborative evidence that the differences between the two groups were real ($P = <0.0001$).

Fecal pools containing a week's collection (three specimens) showed the same type of difference between the vaccinated animals and the controls, viz., lower virus titer and abbreviation of the period of virus excretion in the former. These are shown in the bar diagram of Figure 3. As with the daily averages

of the throat swabs, the weekly averages were consistently lower in the vaccinated for four weeks following feeding of virus, despite considerable individual variation. (P is 0.027 for a pool of the second and fourth days, 0.020 for days seven to 11 and <0.0001 for the 14th to 18th days.) Figure 3 dramatically reflects the more rapid disappearance of virus from the feces of the vaccinated animals. No virus was detected in the latter after the 18th day, although it was still demonstrable in 5/7 of the controls in a pool of the 21st to 25th days and could still be isolated from two control animals on the 29th to 31st days.

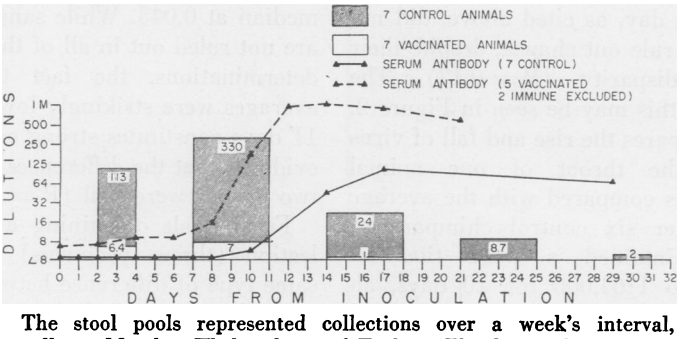
An apparent relationship between the decline of virus titer in the alimentary tract and the rise of neutralizing antibody in the blood serum is shown in all the figures. There are numerous reasons for believing that there exists a true inverse correlation between the two. These are detailed below:

¶ The serum antibody titer rose more rapidly in the vaccinated animals than in the controls and coincided with the fall of virus titer in the alimentary tract (both throat swabs and stool pools).

¶ The availability of daily specimens from the throat made it possible to show that the curve of declining virus titer crossed that of rising serum antibody at the same level (between 1:50 and 1:100) in both vaccinated and control animals, thus suggesting some fundamental equilibrium between virus and antibody. Furthermore, the curves crossed between the 14th and 17th days in the controls and on the 10th day in the vaccinated (Figure 2), coinciding with the more rapid antibody response in the latter.

¶ In the control animal (N-618) the excretion of virus from the throat was prolonged by comparison with the six other animals of the series. Thus, on the 10th day she showed a titer of 1:32,000, while the average of the others was but 1:1,000. Similarly on the 17th and 21st days N-618 showed titers of 1:2,500 and 1:64, respectively, in contrast to 1:50 and 1:13 for the remaining ones. No specimen was available beyond the 21st day. Although the curves of virus titer and serum antibody cross at approximately 1:48 on the 14th to 17th days for six controls, those of N-618 were not observed to cross, since in all probability the collection of throat swabs was not continued long enough to record it.

¶ The series contains two vaccinated animals that had acquired low levels of Type I antibody, but had none demonstrable at challenge. The limitation of



The stool pools represented collections over a week's interval, usually on Monday, Wednesday, and Friday. The first and last pools, however, contain only two alternate days.

Figure 3—Average Titers of Type I Poliovirus in Stool Pools of Chimps

space does not permit including them beyond the statement that these animals showed some characteristics of both vaccinated and controls. Both developed maximal titers of virus in the throat (1:250,000) and showed a somewhat delayed rise of antibody characteristic of controls. Virus and antibody curves crossed at 1:48 between the 12th and 14th days.

¶ The only two animals in the series of 16 that did not become infected had serum antibody titers of 1:64 and 1:1,000, respectively at challenge. All of the 14 infected animals had serum titers ranging from <1:4 to 1:16 ($P = 0.004$).

It remains to mention one more finding which suggests the eventual attainment of an equilibrium between virus and antibody. In Figures 1 and 2 it may be seen that following the dramatic drop in virus titer between the eighth and 12th days virus did not entirely disappear and showed a small rise, even in the presence of high serum antibody levels (see particularly the vaccinated animals in Figure 1). Should these findings be corroborated by more extensive observations they will indicate an equilibrium between virus and antibody, thus providing an acceptable explanation for the persistence of immunity throughout life.

Summary and Conclusions

The presence of serum antibody induced by commercial vaccines clearly limits the duration and amplitude (titer) of alimentary infection with Type I poliovirus in chimpanzees. This animal appears to react to poliovirus in a manner identical with that observed in man.[2-9] At the same time it permits a more detailed and well controlled analysis of the rise and decline of poliomyelitic infection than is usually possible in a human population; however, it should be feasible to relate scattered observations on man to the more complete findings in chimpanzees.

The observations here described might be extrapolated to suggest that mass vaccination will appreciably affect the epidemiology of poliomyelitis, at least in certain segments of the population. The groups most likely to show a reduction in the risk of exposure (and consequent loss of booster effects through natural channels) are the upper income brackets of the temperate zones, and sparse, remote populations all over the world. But this chapter is still to be written.

REFERENCES

1. Howe, H. A., and Bodian, D. Poliomyelitis in the Chimpanzee: A Clinical Pathological Study. Bull. Johns Hopkins Hosp. 69:149–181, 1941.
2. Howe, H. A. Studies of Active Immunogenesis in Poliomyelitis I. Persistence and Recall by Homotypic or Heterotypic Superinfection of Neutralizing Antibody Originally Induced in Chimpanzees by Vaccination or Infection. Am. J. Hyg. 60:371–391, 1954.
3. Bodian, D., and Paffenbarger, R. S., Jr. Poliomyelitis Infection in Households. Frequency of Viremia and Specific Antibody Response. Am. J. Hyg. 60:83–98, 1954.
4. Howe, H. A., and Bodian, D. Neural Mechanisms in Poliomyelitis. New York: Commonwealth Fund, 1942.
5. ———. Poliomyelitis by Accidental Contagion in the Chimpanzee. J. Exper. Med. 80:383–390, 1944.
6. Bodian, D., and Howe, H. A. Non-Paralytic Poliomyelitis in the Chimpanzee. Ibid. 81:255–274, 1945.
7. Howe, H. A.; Bodian, D.; and Morgan, I. M. Subclinical Poliomyelitis in the Chimpanzee and Its Relation to Alimentary Reinfection. Am. J. Hyg. 51:85–108, 1950.
8. Bodian, D. Experimental Studies on Passive Immunization Against Poliomyelitis. I. Protection with Human Gamma Globulin Against Intramuscular Inoculation, and Combined Passive and Active Immunization. Ibid. 54:132–143, No. 1 (July), 1951.
9. Howe, H. A. Antibody Response of Chimpanzees and Human Beings to Formalin-Inactivated Trivalent Poliomyelitis Vaccine. Ibid. 56:265–286, 1952.