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BEHAVIOUR OF CHIMPANZEE-AVIRULENT POLIOMYELITIS VIRUSES IN EXPERIMENTALLY INFECTED HUMAN VOLUNTEERS*

BY

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Poliomyelitis is generally more severe in adults than in very young children. Any method of immunization must avoid the possibility of creating a large adult population without resistance to paralytic poliomyelitis. Natural inapparent infection in early childhood, the process by which the vast majority of the world population acquires immunity to poliomyelitis, is known to provide lifelong protection. The remarkable observations of Paul *et al.* (1951) on Eskimos indicate that even in the absence of reinfection the body continues to produce specific antibodies for as long as 40 years after exposure to the virus. Natural infection with poliomyelitis viruses thus seems to provide the body with a "built-in" booster mechanism.

Studies on experimentally produced and naturally occurring attenuated strains of poliomyelitis virus are designed to provide information which would indicate whether or not the natural process of immunization can be duplicated without incurring the varying risk of paralysis encountered in nature. The first exploratory qualitative experiments on human beings were performed by Koprowski *et al.* (1952, 1954, 1955) with a rodent-adapted Type 2 poliomyelitis virus of diminished virulence for monkeys. In 1953, utilizing tissue culture techniques, we succeeded in segregating from highly virulent strains of each of the three immunological types of poliomyelitis virus, variants which are avirulent for monkeys by the intracerebral, oral, and parenteral routes (Sabin *et al.*, 1954). Further studies showed that these strains possessed a varying and limited capacity for producing paralysis after direct injection into the spinal cord of monkeys, but similar inoculations into the spinal cord of chimpanzees produced neither paralysis nor

lesions. Yet when these strains were fed to chimpanzees they produced clinically inapparent immunogenic alimentary infections, unassociated with the viraemia which is so regularly encountered in infections with virulent viruses (Sabin, 1954, 1955a, 1955b).

The purpose of the present study was to determine by quantitative methods the comparative behaviour in human beings of the same culture fluids which had been extensively studied for a period of 18 months in monkeys and chimpanzees. The volunteers, 21–30-year-old inmates of a Federal reformatory, were selected on the basis of previous tests which showed that they lacked demonstrable antibody for one or more types of poliomyelitis virus. The three types of virus in the form of monkey kidney tissue culture fluid were administered in doses of 0.001, 0.1, 0.5, or 1 ml. in a teaspoonful of milk to 26 men—8 men for the Type 1 virus, and 9 each for Types 2 and 3. Four men who on initial testing had no demonstrable antibody for any of the three types were inoculated intramuscularly with the Type 3 virus—two with 0.001 ml. and two with 0.1 ml. Quantitative tests for virus were performed at frequent intervals on the stools and blood and on swabs from the mouth and tongue, and from the posterior pharyngeal wall. Antibodies were determined quantitatively over a period of three months.

Results

The results of this study, which will be reported in detail elsewhere, may be summarized as follows:

1. Human beings are more susceptible than chimpanzees to infection by the oral route. The smallest dose used—that is, 0.001 ml. of culture fluid containing $10^{4.2}$ to $10^{4.5}$ TCD₅₀ of virus—sufficed to produce an immunogenic alimentary infection in the volunteers (Table I).

TABLE I.—Comparative Susceptibility of Human and Chimpanzee Alimentary Tracts to Infection with Chimpanzee-avirulent Poliomyelitis Viruses

Type and Strain of Virus	Dose		No. Infected	
	ml.	TCD ₅₀	Humans	Chimpanzees
1 "Mah." KP33	0.5	$10^{7.2}$	2/2	4/5
	0.1	$10^{6.5}$	3/3	—
	0.001	$10^{4.5}$	3/3	1/3
2 YSK, KP51	1.0	$10^{7.2}$	3/3	5/8
	0.1	$10^{6.2}$	3/3	1/3
	0.001	$10^{4.2}$	1/1*	0/2
3 Leon, KP34	1.0	$10^{7.4}$	3/3	5/5
	0.1	$10^{6.4}$	3/3	—
	0.001	$10^{4.4}$	3/3	0/3

* Two other volunteers who had irregularly demonstrable traces of Type 2 antibody showed no evidence of infection after ingestion of this dose of virus

2. There was no multiplication in the mouth and tongue, nor was virus present there except in traces on rare occasions, even when it was recovered from the throat in amounts as high as $10^{4.5}$ TCD₅₀ per swab (Fig. 1).

3. Whether or not the virus localized and multiplied in the throat was determined by the dose that was swallowed.

TABLE II.—Influence of Amount of Poliomyelitis Virus Swallowed on Localization of Infection in Throat of Human Volunteers

Type of Virus Ingested	No. of Individuals with Virus in Throat or Stools 5 Days or Longer after Swallowing Indicated Amount (ml.) of Culture Fluid					
	Throat			Stools		
	0.5–1	0.1	0.001	0.5–1.0	0.1	0.001
1	2/2	2/3	0/3	2/2	3/3	2/3
2	3/3	3/3	0/1	3/3	3/3	1/1
3	3/3	2/3	0/3	3/3	3/3	3/3

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It occurred in all who ingested $10^{7.2}$ to $10^{7.5}$ TCD₅₀, in 7 of 9 receiving $10^{6.2}$ to $10^{6.5}$ TCD₅₀, and in none of those who swallowed $10^{4.2}$ to $10^{4.5}$ TCD₅₀, although infection of the lower alimentary tract, evidenced by continued excretion of virus in the faeces, was demonstrated in all but one of the latter group (Table II, Fig. 2).

4. There is suggestive evidence of virus multiplication as early as 24 hours after ingestion. The duration of virus excretion in the stools was as short as 7 to 10 days, and as long as 12 weeks. The largest amounts of virus, as high as 10^5 to 10^6 TCD₅₀ per gramme of faeces, were excreted by the Type 1 group (Figs. 1 and 2).

5. Despite the extensive multiplication of these viruses somewhere in the alimentary tract, none was detected in the blood of any volunteer, and the infections were entirely asymptomatic. This makes one wonder whether the viraemia and minor illnesses encountered in intimate associates of clinically diagnosed cases of poliomyelitis may not be due to multiplication of the virulent viruses in special cells or tissues which are not affected by the chimpanzee-avirulent strains.

6. The Type 3 virus failed to produce an immunogenic infection after intramuscular injection, except in one individual in whom some of the virus quickly localized in the lower alimentary tract (but not in the throat) and was excreted in the faeces in amounts of 10^3 to 10^5 TCD₅₀ per gramme from the 5th to the 28th days (Table III). This localization in the alimentary tract after intramuscular injection was not observed in chimpanzees inoculated with attenuated strains.

7. The antibody response (Figs. 3, 4, and 5) was of a higher order in the volunteers with extensive multiplication of virus in the throat than in those who exhibited little or no multiplication in the throat (Figs. 1 and 2). This finding suggests that a more highly immunogenic, and perhaps more localized, infection could be produced by simply swabbing the throat with virus, and experiments on this mode of infection are now in progress.

8. In several volunteers who ingested the Type 3 virus, the antibody dropped to very low or undetectable levels in a few weeks, despite the fact that extensive viral multiplication had occurred in their lower alimentary tract (Fig. 5).

TABLE III.—Comparative Effect of Chimpanzee-avirulent Type 3 Poliomyelitis Virus (Leon, KP34) by Oral and Intramuscular Routes

Route	Dose		Volunteer No.	Antibody Before Virus			Result	
	ml.	TCD ₅₀		1	2	3	Enteric Infection	Antibody
Oral	0.1	$10^{6.4}$	1 2 3	+	+	0	+	+
				0	+	0	+	+
				0	+	0	+	+
	0.001	$10^{4.4}$	4 5 6	+	+	0	+	+
				+	0	0	+	+
				+	+	0	+	+
Intra-muscular	0.1	$10^{6.4}$	7 8	0	0	0	+	+
				0	0	0	+	+
	0.001	$10^{4.4}$	9 10*	0	0	0	0	0
						Trace	0	+
								Accelerated booster

* This volunteer had only a trace of irregularly demonstrable antibody for Type 3 and none for Types 1 and 2 prior to inoculation of virus.

9. Observations on four volunteers with low levels of naturally acquired antibody prior to ingestion of large amounts of virus indicated that the multiplication of virus which occurred in their alimentary tract did not lead to an accelerated appearance of antibody such as is observed after the intramuscular injection of minute amounts of pre-

Fig. 1.—Type 1 chimpanzee-avirulent poliomyelitis virus in human volunteers. Propagation of ingested virus in mouth, throat, and lower alimentary tract.

Fig. 2.—Type 3 chimpanzee-avirulent poliomyelitis virus in human volunteers. Extent of propagation in throat and lower alimentary tract. (a) Effect of dose on localization in throat. (b) Effect of propagation in throat on antibody response.

formed antigen. The ultimate rise in antibody occurred at about the same time as in human beings or chimpanzees with a primary infection. In two volunteers with only traces of antibody, the ingestion of $10^{4.2}$ TCD₅₀ of virus was followed neither by alimentary infection nor by a rise in antibody, suggesting that immune individuals may not be reinfected when the dose of virus is not excessive.

Discussion

Studies of the genetic aspects and of the mutational patterns of the characters relating to virulence of the poliomyelitis viruses indicated that intracerebral virulence for monkeys and paralytic activity for monkeys by extra-neural routes and for chimpanzees by the spinal route are determined by distinct genetic factors (Sabin, 1955b). Since chimpanzees are not available in sufficiently large numbers to permit extensive spinal tests for the appearance of potentially dangerous mutants in the human alimentary tract, intracerebral tests in monkeys with original stool extracts and stool cultures were carried out to provide an index of mutability of a given strain. These tests showed that the Type 3 virus came out as avirulent as it went in, while the Type 1 and Type 2 viruses yielded a certain proportion of virus particles with increased virulence for the brain-stem neurones of monkeys. It is noteworthy, however, that a study of such mutants derived from the chimpanzee and

human alimentary tracts revealed that they were not paralytogenic after extraneural inoculation in monkeys or after direct spinal inoculation in chimpanzees.

Similar studies are now in progress with naturally occurring monkey intracerebrally avirulent Types 1, 2, and 3 strains recovered from healthy children, to determine their relative immunogenic and mutagenic properties. When such comparative studies have indicated the best strains to use it will be possible to determine whether or not the optimum attenuated poliomyelitis viruses, either as a drop of culture fluid in a teaspoonful of milk or simply swabbed on the

throat, can be employed for primary immunization or for the production of an infection type of immunity in individuals temporarily protected by maternal antibody or non-infective vaccines.

Summary

Strains of each of the three types of poliomyelitis virus, which were experimentally segregated in monkey kidney tissue culture and shown to be avirulent for monkeys by intracerebral and extraneural routes and for chimpanzees even after inoculation into the grey matter of the spinal cord, were administered to 30 human volunteers. These men were 21 to 30 years old and possessed no demonstrable antibody for the poliomyelitis viruses they received. Twenty-six men received Types 1, 2, or 3 virus in doses varying from 0.001 to 1 ml. in a teaspoonful of milk by mouth. The others who were without antibody for all three types received the Type 3 virus intramuscularly. There was no virus multiplication in the mouth or anterior portion of the tongue. With the larger doses the viruses multiplied in the throat as well as in the lower alimentary tract, while after ingestion of the smaller doses no virus was found in the throat even when it continued to be excreted in the faeces for four to six weeks. The smallest amount, 0.001 ml. containing approximately 10,000 tissue-culture doses of virus, initiated an immunogenic alimentary infection in all human volunteers.

These data indicated that the human alimentary tract is more readily infected than that of chimpanzees. Chimpanzees are more readily infected by mouth than monkeys, while the reverse obtains for the nervous system—monkeys being much more susceptible than chimpanzees. No viraemia was found in any of the volunteers, and there was no evidence even of minor illness. Amounts of Type 3 virus which readily produced infection after ingestion failed to do so after intramuscular injection except in one man, in whom the virus localized in the lower alimentary tract. The antibody response, which occurred in varying degree in different men, was of a higher order in those with extensive viral multiplication in the throat. No harmful mutants were found after multiplication in the alimentary tract. Similar studies are now in progress with naturally occurring, intracerebrally avirulent Types 1, 2, and 3 poliomyelitis viruses which were recovered from healthy children who had no contact with clinical cases of poliomyelitis. Studies on immunization of larger numbers of human beings are being postponed until comparative studies have indicated the best attenuated strains to use for this purpose.

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FIG. 3.—Type 1 poliomyelitis virus in human volunteers. Neutralizing antibody after single feeding.

FIG. 4.—Type 2 poliomyelitis virus in human volunteers. Neutralizing antibody after single feeding.

FIG. 5.—Type 3 poliomyelitis virus in human volunteers. Neutralizing antibody after single feeding.