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POLIOMYELITIS: PROBLEMS IN PATHOGENESIS AND IMMUNIZATION**†

The pathogenesis of poliomyelitis has been studied intensively during the past fifty years by many investigators, yet our understanding of the course of events in the human infection is far from perfect. There are still many controversial issues, in general the same issues with which the early investigators were also concerned: viz., is poliomyelitis an entirely neural disease, or is there a systemic, extraneural phase? In what tissues does virus first multiply? How does it invade the central nervous system—from the blood or exclusively by neural pathways? What are the immune barriers against infection and paralysis and where do they operate?

In the discussion today, I should like first to review the current status of some of these problems, and subsequently to describe observations on humans infected with attenuated strains of poliovirus as they relate to certain aspects of the pathogenesis and immune mechanisms.

Current concepts of the nature of poliomyelitis lean toward an interpretation of the infection as primarily an extraneural one in which significant CNS invasion occurs only rarely. This is not a new idea. It was in fact the point of view expressed by Peabody, Draper, and Dochez in 1912[40] and by other investigators at that time. But it was lost sight of in the enthusiasm for neurotropic aspects of the infection which were studied so extensively in the ensuing 25 years, largely by inoculating monkeys either intranasally or directly into nervous tissue and employing almost exclusively the MV strain, which had become highly neurotropic by many neural passages. The return to a more natural perspective in terms of the human infection came in the early 1940's, when through the work of Harmon,[21] Trask, Vignec, and Paul,[49] Sabin and Ward,[46] and others, it became apparent that infection of the alimentary tract is an important phase in the pathogenesis of poliomyelitis. Renewed interest in the pathogenesis of poliomyelitis was stimulated by the discovery beginning in 1951 that a phase of viremia occurs in

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orally infected monkeys and chimpanzees[1, 28] and also in the natural human infection.[5, 28] Subsequently, the use of quantitative tissue culture methods of virus and antibody assay and the many new observations by Koprowski *et al.*[29–31] and Sabin[41, 42, 44] on humans infected with attenuated strains have considerably enlarged our understanding of the pathogenesis of poliomyelitis. There is now general agreement on the alimentary portal of entry, and it is known that the vulnerability of different parts of the alimentary tract to virus implantation and multiplication differs in different species. In man, the lower intestinal tract (ileum) is more susceptible than the throat, while the reverse is true in monkeys[31] and chimpanzees.[41] Sabin[41] has demonstrated that larger doses of virus are required to infect the throat than the intestinal tract of susceptible persons; we have found the same to be true in re-infection of naturally immune individuals.[27] The fact that the throat can be by-passed—as it was in children fed attenuated virus in capsules in a human trial to be described later—adds further evidence that multiplication of poliovirus in the throat is not an essential part of human infection.

SITES OF PRIMARY VIRAL MULTIPLICATION

Does virus multiply first in mucosal cells or in lymphoid tissues of the alimentary tract? Or does it avoid both of these and make its way to regional ganglia before multiplying?

The speed with which virus appears in the throat and feces[27, 41, 42] in humans after ingestion of attenuated strains—as short as 24 hours—would seem to narrow the field to a readily available cell, either lymphoid or epithelial. Although Faber *et al.*[18] have shown that virus inoculated directly into ganglia can multiply and be excreted into the throat and intestinal lumen, it would seem unlikely, as Bodian[4] has pointed out, that this cycle of invasion, multiplication, and excretion could occur as rapidly as within 24 hours.

The available evidence favors a primary extraneural phase of viral multiplication in the intestinal tract, but whether in epithelial mucosal cells or those of the lymphoid structures or both is not clear at the present time. The experimental evidence on early infection of the alimentary tract is meager, being based largely on a few chimpanzees sacrificed at various intervals after virus feeding.[5, 43] It is clear, however, that the sites with the largest amounts of virus, presumably due to local multiplication, are the throat and the ileum, both being areas rich in lymphatic structures. Tonsils and Peyer's patches dissected away from pharyngeal or intestinal wall have been assayed for virus, and in a few instances the washed wall of these areas has also been tested. High titers have been found in the tonsils by Bodian,[5]

but washed pharyngeal wall was not tested; Sabin,[45] on the other hand, found high titers in the pharyngeal wall but did not test the tonsils. It is difficult, therefore, to compare these two sets of results. Another problem is that the complete separation of the two tissues of the alimentary tract is virually impossible. There are many submucosal collections of lymphoid cells not necessarily as large as follicles distributed widely in the intestinal tract; virus isolated from "washed wall" might be associated with these cells rather than with the epithelial cells. On the other hand it is equally difficult to rule out multiplication in epithelial cells when assaying Peyer's patches and tonsils for virus. This is due to the fact that, as has recently been pointed out to me by Dr. Ernest Goodpasture,[18] both of these structures have a certain anatomical feature peculiar to them among lymphoid tissues. This consists of the presence of deep crypts extending far into the substance of the follicles—deep crypts which are lined with mucosal epithelial cells. In assaying for virus it would therefore be extremely difficult to separate the two types of tissue. One might even speculate that the localization in tonsils and Peyer's patches in the throat and ileum is due to the presence of these epithelial-lined recesses which form the most favorable sites for virus to lodge, adsorb, penetrate cells both epithelial and lymphoid and begin multiplying. In any event, here as elsewhere in the alimentary tract, in order to reach lymphoid cells, virus must penetrate through—or between—mucosal cells, another reason to suspect that both tissues might support virus growth.

The absence of lesions in either intestinal lymphoid or epithelial cells of orally infected monkeys and chimpanzees has been regarded by Faber[10] as strong evidence against their being sites of virus multiplication. This does not seem a valid argument, since it has been demonstrated that poliomyelitis virus[38] as well as others[43, 47] can multiply *in vitro* to high titers in cells without causing detectable alterations in cell morphology. Further, the isolation of virus in considerable amounts from lymph nodes and intestinal wall of chimpanzees[3, 43] indicates that multiplication *in vivo* can proceed without causing visible cell damage. From the point of view of the virus, this property of being able to live happily without disadvantage to the cells of its human host would seem essential to survival.

Although absence of lesions of lymphoid tissues is a feature of the incubation period in the experimental disease, this is not true of fatal human infections, for studies of lymphoid structures at autopsy have demonstrated, in addition to virus,[50] mild to marked histological alterations. Comments on the involvement of the reticulo-endothelial (R.E.) system were frequent in papers on poliomyelitis 25 years ago. Burrows[7] in 1931, on the basis of observations on cases dying early in the course, went so far as to consider

poliomyelitis a disease of the lymphatic system, which occasionally might involve the CNS. Landon and Smith[32] who reported 96 fatal cases in the 1931 New York City epidemic, probably the largest published series of complete human autopsies, noted that "the most exaggerated picture of the lymphatic structural involvement in poliomyelitis was that seen in the lymphoid tissues of the lower ileum and cecum." The changes included congested, hyperplastic follicles and Peyer's patches, and in some instances erosion and ulceration; all of these were much more striking in patients dying early in the course rather than later in convalescence. Similar alterations were found in the tonsils and spleen, but only minimal changes in the peripheral nodes. More recently, in 1951, these findings were corroborated by Sommers *et al.*,[48] who attempted to determine their specificity by examining lymphoid tissues from more than 50 autopsied cases of various acute childhood infections, both viral and bacterial. In only two of these controls were lesions detected comparable to those found in 82 per cent of poliomyelitis cases.

Although observations on fatal human cases do not indicate what happens in the early, primary phases of infection, yet the regular and often extensive involvement of the lymphatic structures, taken together with evidence of the early presence in them of virus in orally infected monkeys[25] and chimpanzees,[3,48] indicates that they may play an important role in the natural infection.

VIREMIA

Although viremia has been demonstrated to occur with some regularity in natural human infections,[5,28] there is now extensive evidence that in fully susceptible, antibody-negative individuals, viremia occurs very rarely following ingestion of laboratory attenuated strains.[9,29,43] There is also evidence of marked differences even between virulent strains in their capacity to invade the blood stream. Wenner and Komitsuka[51] demonstrated titers as high as 10^5 and 10^7 TCD_{50} per ml of blood in monkeys inoculated intramuscularly with a virulent Brunhilde strain of type I; in orally infected animals, Bodian[3] found the Mahoney strain at levels up to 10^5 TCD_{50} in the serum, while others, less paralytogenic, such as Y-SK (type II) and Saukett (type III) rarely exceeded 10^1 TCD_{50}.

Current problems associated with viremia include the meaning of this relationship between virulence and degree of blood stream invasion, the source of virus in the blood, and its significance in the pathogenesis of the CNS infection. As to the source of virus in the blood, the speed with which viremia can appear after oral infection with virulent strains in monkeys and

chimpanzees (in two or three days or even less) suggests that invasion of the blood stream occurs from sites of early multiplication either in the alimentary tract or other extraneural tissues. Sabin[48] has pointed out that it is not likely to be the alimentary tract, since attenuated strains which multiply well in the gut fail to invade the blood in significant amounts. Wenner and Komitsuka[51] demonstrated that high titers of virus can be found in the blood and in a variety of extraneural tissues (lymph nodes, GI tract, muscle) of cynomolgus monkeys inoculated intramuscularly with a Brunhilde strain of high paralytogenic capacity, and sacrificed early enough so that only trace amounts had appeared in the CNS. In orally infected chimpanzees sacrificed in the viremic period but before CNS invasion, Bodian[8] found virus in considerable amounts in the alimentary tract, the regional and distal lymph nodes, and the brown fat. The evidence suggests that with virulent, paralytogenic strains, multiplication occurs extensively in various extraneural sites and this multiplication results in viremia. With attenuated strains, on the other hand, as Sabin has shown,[48] infection is more restricted, involving chiefly the alimentary tract and regional nodes, only minimal multiplication (if any at all) occurring more distally in the R.E. system, spleen, peripheral nodes, and perhaps other tissues; and under these circumstances virus fails to appear in the blood stream.

A close parallel to this course of events has been demonstrated by Ørskov and Jensen (quoted by Madsen[35]) for an enteric infection of mice, induced by feeding Salmonella typhimurium. When fully virulent bacteria were fed, it was found that the organisms could be isolated first from the lymph follicles of the intestinal tract; this was followed rapidly by their appearance in regional lymph nodes, then in more distal parts of the R.E. system, the spleen, liver, and finally in the blood. In contrast, when mice were fed the same strain of bacteria which had been attenuated by serial passage at high temperatures, multiplication was demonstrated at considerable levels in intestinal lymph follicles, to a much lesser extent than with virulent strains in mesenteric nodes, spleen, and liver, and never was the organism cultured from the blood. Further decrease in virulence led to infections which involved only the intestinal lymph follicles.

A comparison of the findings on virus assay of chimpanzees infected with attenuated strains[48] and virulent strains[8] suggests that in poliomyelitis, as with the enteric infection of mice studied by Ørskov and his colleagues,[35] both attenuated and virulent strains multiply in the same sites but to different extents and under different genetic controls. The loss of "invasiveness" or "virulence" characteristic of attenuated strains is associated with a reduced capacity to multiply in extraneural sites, which in turn is associated with absence of viremia.[48]

MECHANISMS OF CNS INVASION

The significance of viremia in poliomyelitis is a part of the problem of how virus reaches the CNS. Does it invade directly from the blood? Or does it travel from the periphery via nervous pathways and is viremia merely an incidental occurrence? This also remains a controversial issue. Actually, there is a good deal to indicate that both mechanisms may play a role under certain circumstances. That poliovirus can travel and invade by way of nervous tissue once inside nerve cells is a well-established fact and has been thoroughly documented by many workers. But it does not follow that such is the required or even the usual course of events in the natural infection.

In summarizing the evidence favoring invasion from the blood, which seems to me the more likely explanation, the remarkably constant association of viremia and CNS invasiveness stands out strikingly. Although this association does not prove that CNS virus is blood-borne, it is difficult to relegate the correlation to a position of insignificance. Other findings which favor CNS invasion via the blood stream include: (i) the demonstration by German and Trask[17] of the regular occurrence of paralysis in monkeys inoculated cutaneously into a completely denervated limb; (ii) the experiments of Bodian[2] in which large doses of a virulent strain of virus introduced intravascularly resulted in paralysis with a short incubation period comparable to that following intracerebral inoculation; (iii) the fact that low levels of serum antibody produced by vaccination[15] or injection of gamma globulin[19] protect against the paralytic disease, most probably by blocking the viremic phase of infection.

Sabin,[43] who favors the view that invasion occurs by the neural route, believes that this blocking effect occurs not at the vascular level but at the cellular one, and that serum antibody prevents the progress of virus from extraneural sites of multiplication in the alimentary tract and elsewhere to peripheral nerve endings with which the cells are in close connection. Faber,[10] whose view is that primary multiplication takes place in ganglia and CNS invasion occurs from peripheral neural connections, visualizes the antibody present in mucus in the oropharynx as blocking primary neural entry. In both Sabin's and Faber's interpretation, antibody is considered to act at the cellular level rather than at the vascular one. If this is the case, however, one would expect that alimentary tract infection as well as CNS invasion would be prevented by the presence of circulating antibody. Instead, alimentary infection has been shown to occur in the presence of even relatively high titers of serum antibody.[27, 44]

The evidence favoring neural invasion rests largely on the demonstration of lesions and virus in peripheral ganglia early in the course of infection

induced by virus feeding. Faber *et al.* have shown this repeatedly in cynomolgus monkeys fed virulent strains of virus. That it is a somewhat irregular occurrence is suggested by the results of Faber and his coworkers[12] and by Bodian's findings that in only 2 of 8 chimpanzees fed virulent strains could trace amounts be detected in trigeminal and coeliac ganglia.[3] Sabin[49] has recently reported that in a chimpanzee infected with an attenuated strain, small amounts of virus were isolated from superior cervical and thoracic spinal sympathetic ganglia. The interpretation of these observations as indicative that CNS invasion necessarily occurs via neural pathways is not convincing. Actually if, as the results indicate, both virulent and attenuated strains may rapidly infect ganglia, this would argue that such peripheral invasion occurs regularly but it is not an important aspect of virulence. Obviously the data are not extensive enough to permit a final definitive interpretation.

INFECTION IN THE PRESENCE OF ANTIBODY

Studies by Brown and his associates,[6] by Fox *et al.*,[14] Horstmann *et al.*,[24] and others[20, 28] have shown that persons who already have specific antibodies as a result of previous natural infection do not readily become infected when exposed to a case of poliomyelitis or poliomyelitic infection in the family. In contrast, more than 90 per cent of susceptible, antibody-negative individuals become infected under similar circumstances. Recent field investigations have been concerned with the question, is there a qualitative difference in the immunity resulting from natural infection as compared to that which follows vaccination with Salk vaccine or inoculation with gamma globulin? Brown *et al.*[6] showed that gamma-globulin-inoculated children, when exposed to a case of poliomyelitis in their families, became infected and excreted virus as readily as did susceptible children who had not received gamma globulin. Similarly, several field studies carried out since 1954[8, 16, 24] indicate that vaccination with formalinized vaccine also fails to affect the incidence of alimentary infection among vaccinees on exposure to an infected person in the home.

This problem has also been investigated by observing the responses to ingestion of attenuated strains of poliovirus by individuals with naturally acquired and formalinized vaccine-induced antibodies. Koprowski *et al.*[29] found that none of 3 individuals with antibodies to type II naturally acquired showed antibody rises, but 2 of them excreted virus on single occasions 5 and 23 days after ingestion of the TN type II strain. Sabin has compared the responses of 8 "naturally immune" and 8 "vaccine immune" persons following feeding of attenuated strains, and found that low levels of naturally acquired antibody protected against alimentary infection,

whereas prefeeding vaccine-acquired antibody at levels up to 256 failed to inhibit multiplication in the alimentary tract in any of the volunteers tested.

We have been concerned with this problem, also, and have carried out two human trials with attenuated strains generously made available to us by Dr. Sabin. In the first one[27] a type III strain was fed to 5 individuals, 4 of whom became infected. The three whose prefeeding antibody was naturally acquired had brief periods of virus excretion in the feces 6 to 9 days, and

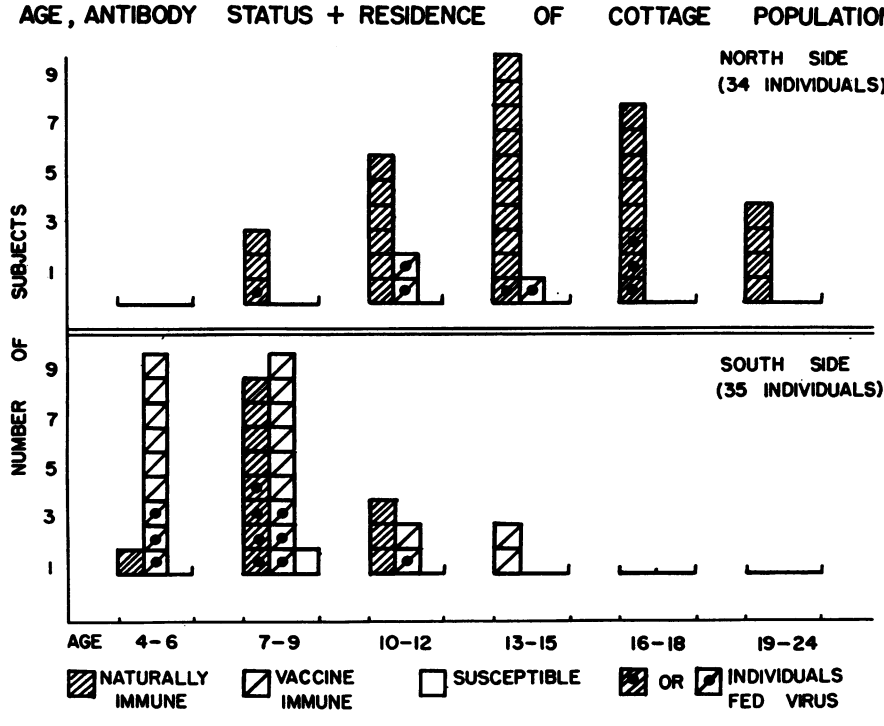

FIG. 1. Age and immune status of 69 persons forming total study population.

prompt neutralizing antibody rises; the fourth individual, a vaccinated child, excreted virus for 6 weeks, and developed CF as well as neutralizing antibody rises. Subsequently we have carried out a trial with an attenuated type I strain, also made available to us by Dr. Sabin who had used it previously to infect adult volunteeers. I should like to describe at this point the results of this second trial, a preliminary report of which has already been given. The observations represent work carried out by the Yale Poliomyelitis Study Unit[28] as a team and form part of a larger program of investigation of pathogenesis and immune mechanisms in poliomyelitis.

HUMAN TRIAL WITH ATTENUATED TYPE I POLIOVIRUS

The trial was carried out in an institution caring for mentally retarded children. The unit used consisted of a cottage housing 69 individuals whose ages ranged from 4 to 24 years. In general, the older individuals resided and had their play area in the north wing, while the younger children were largely in the south wing of the building. All of the children in the cottage either had naturally acquired type I antibodies, or had beeen vaccinated with commercially available Salk vaccine. Twenty were selected for virus feeding, 10 in the vaccine-immune (V) group, and 10 in the naturally immune (N) group—those in each group being matched for age as far as

TABLE 1.

INFECTION FOLLOWING VIRUS INGESTION, CORRELATED WITH NATURE OF IMMUNE STATUS.

RESIDENCE	IMMUNE STATUS			TOTAL INFECTED
	NATURAL	VACCINE	SUSCEPTIBLE	
NORTH	0/5	3/3	—	3/8
SOUTH	2/4	7/7	1/1	10/12
TOTAL	2/9 (20%)	10/10 (100%)	1/1	13/20 (65%)

possible. It turned out later that one child who was thought to have had low-level natural antibody was in fact fully susceptible and reacted with a primary infection.

The strain of virus used was the attenuated L-SC, type I, generously made available to us by Dr. Albert B. Sabin, who had previously used aliquots of the same lot to infect volunteers. The dosage fed to each of 20 test subjects was $10^{7.4}$ TCD_{50}. In an attempt to by-pass the throat, 7 individuals were fed this amount mixed with polyethylene glycol 400 in hard gelatin capsules;* the other 13 were given the same dose in liquid form in milk.

Figure 1 indicates the residence, whether north or south side, the age, and the antibody status of the 20 individuals fed virus, as well as of the rest of the cottage population who formed their close associates. The older indi-

* The capsules and the polyethylene glycol were kindly supplied to us by Dr. Hilary Koprowski, who had used the method previously in feeding virus to children.[81]

viduals living on the north side were chiefly naturally immune; 8 of these (5 N, 3 V) from 8-18 years of age, were fed virus. Of the south side population, 12 (5 N, 7 V, 1 susceptible), aged 4 to 10, were fed.

Of the 20 to whom virus was administered, 13 (65 per cent became infected (Table 1), as evidenced by virus excretion and significant antibody rise. Two of the 9 naturally immune (20 per cent) and all 10 (100 per cent) of the vaccine immunes thus became infected. None of the infected persons developed signs of illness at any time.

The effect of the method of virus administration, whether by capsule or in liquid form, is indicated in Table 2. The results confirm those of Koprowski

TABLE 2.

EFFECT OF CAPSULE OR LIQUID ADMINISTRATION OF VIRUS ON EXCRETION FROM THROAT AND FECES

IMMUNE STATUS	METHOD ADMINISTRATION	VIRUS EXCRETION		TOTAL INFECTED
		THROAT	FECES	
NATURAL	CAPSULE	0/4	1/4	1/4
	LIQUID	0/5	1/5	1/5
VACCINE	CAPSULE	0/3	3/3	3/3
	LIQUID	6/7	7/7	7/7

et al.[81] and indicate that if virus is fed in capsule form, infection of the throat can be by-passed. None of the 7 individuals who ingested $10^{7.4}$ TCD_{50} in capsule form excreted virus in the throat. Four of these were natural immunes, only one of whom became infected as evidenced by fecal excretion. Of the 3 capsule-fed vaccine immunes, none excreted virus in the throat, but all excreted it in the feces. Conversely, positive throat swabs were demonstrated in 6 of 7 vaccinees who were given virus in liquid form. In all in whom the throat was considered infected, throat swabs were negative 6 hours after virus ingestion, but became positive 1 to 3 days later and continued so for a matter of days.

The different patterns of virus excretion and antibody response in the infected individuals is illustrated in Figures 2 through 5. Figure 2 shows the results with subject #553—a 9-year-old boy, prefed naturally

acquired type I antibody at a level of 1 : 64.* Following ingestion of a large dose of attenuated type I virus in capsule form, he became infected, as evidenced by a gradual rise in specific antibody from 64 to 1024 over a period of 56 days. Virus excretion, however, was minimal, positive stool specimens being obtained on the 1st and 6th days only, and the throat being consistently negative. There was no significant CF response. This re-infection, then, in a naturally immune individual resulted in barely detectable amounts of virus in the intestinal tract, yet gave a significant (8 fold) antibody rise.

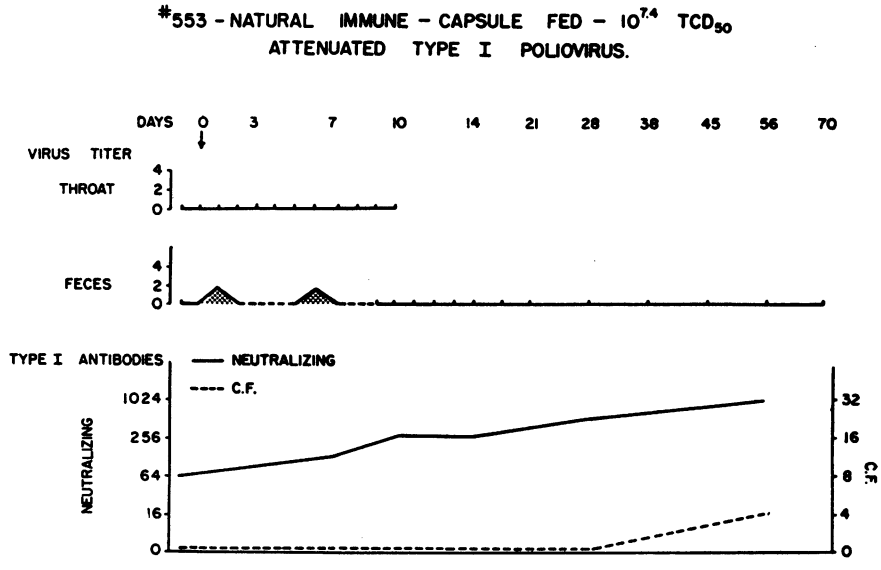

FIG. 2.

Figure 3 is also concerned with a naturally immune person who was fed virus in liquid form. In spite of the large dose, his throat was apparently not infected, and virus excretion in the stools was small in amount and strikingly intermittent. His antibody rise was slow and not very high; there was no significant CF response. The pattern is similar to that in the previous individual who also had naturally acquired antibody, and to the one other naturally immune person who became infected: all of these had minimal and intermittent excretion of virus in the feces, but definite rises in neutralizing antibody.

Turning now to "vaccine-immune" persons who became infected: #503

* The antibody levels of all individuals reported in this study were determined by the colorimetric neutralization test.[86]

(Figure 4) with a prefeeding neutralizing antibody level of 64, was fed virus in liquid form. He excreted virus in the throat from the 1st through the 9th day, in the feces steadily through the 11th day, and intermittently thereafter through the 38th day. He had a sharp rise in both neutralizing and CF antibodies, beginning on the 10th day.

In contrast, with respect to virus in the throat, is #540 (Figure 5), vaccine immune, but capsule fed. With a prefeeding titer of 16, this individual failed to excrete virus in the throat, but excreted it in the stools inter-

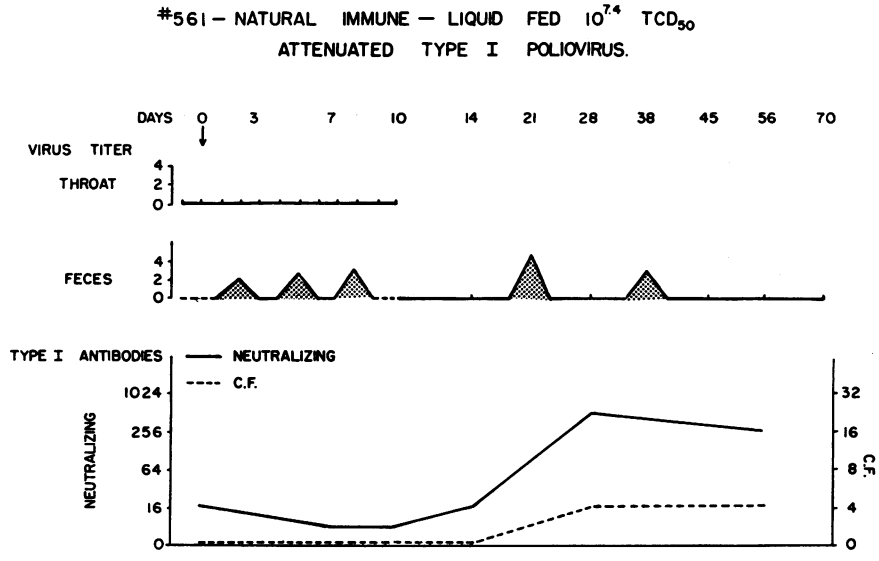

FIG. 3.

mittently from the 3d through the 21st day, in titers, usually of 3.5 to 4.5 logs per gram. Sharp rises in neutralizing and CF antibodies began on days 7 and 10. Thus, although the throat was not infected, this individual excreted virus and developed antibodies as promptly as did those liquid-fed individuals with positive throat swabs.

The patterns of response, then, of naturally immune and vaccinated persons with more or less comparable prefeeding antibody levels who became infected after ingestion of attenuated type I poliovirus, differed, as illustrated in the charts (Figs. 2-5); the 2 vaccinated individuals excreted relatively larger amounts of virus steadily during the first 2 weeks at least, and developed rises in both neutralizing and CF antibodies; the 2 naturally immune excreted little virus in the feces, excretion was intermittent, and

while they developed neutralizing antibody rise, there was no significant CF rise.

Spread of infection to associates. The next problem considered was the ease—or difficulty—with which an attenuated strain of poliovirus might spread to close associates of infected individuals. The population under study consisted of 69 individuals, all living in the same cottage, but roughly divided into two groups, the older in the North Wing and the younger in the South Wing. Essentially the conditions of living were analogous to those

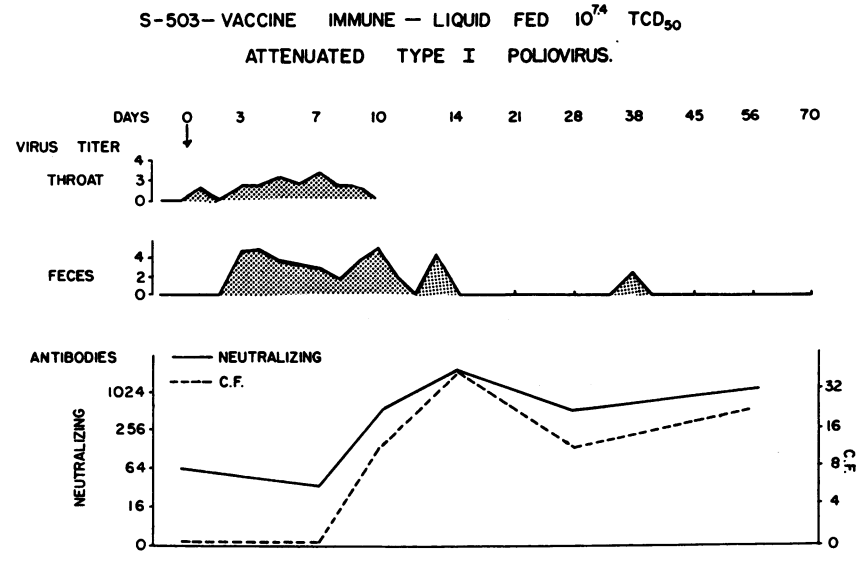

FIG. 4.

existing in family life, but the amount of fecal contamination of the environment was far greater because the subjects were low-grade mental defectives. In this situation, as reported above, 13 of 20 individuals fed virus became infected; and the degree of spread to the 49 associates was considerable (Table 3). Among the naturally immune contacts, 1 of 36 (3 per cent) became infected, while of the vaccine immunes the figure was 12 of 13 or 92 per cent. In all but 2 of the infected contacts, infection was evidenced by virus excretion and antibody rise; in the 2 there was serological evidence of infection but none of the 3 stool specimens collected at intervals of approximately 3, 5, and 7½ weeks after the trial began were positive for virus. These results and these rates of spread are not comparable to those in our previous trial[27] in which the type III strain used did not spread to any

of 7 naturally immune or, one susceptible individual, all of whom were adults, most of them living as ambulatory patients in a hospital ward. The situation is also different from those in which Sabin[43, 44] has used the same type I strain and failed to observe significant spread: his volunteers were all adults of normal mentality.

Incubation period. The speed with which infection with the attenuated type I virus spread to associates of individuals fed in our trial was of interest in terms of the duration of the incubation period in the natural human infection. Although stool specimens from the entire cottage were tested at

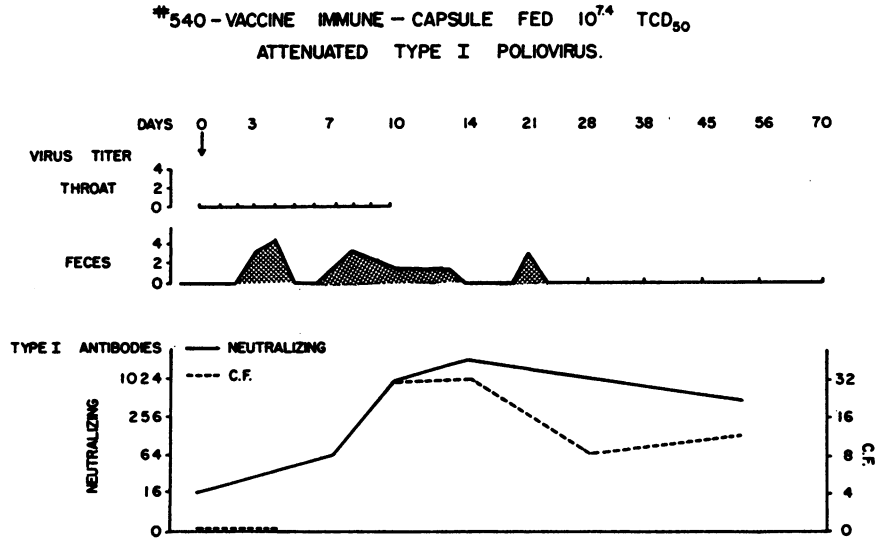

FIG. 5.

intervals, 14 persons (7 V, 7 N) had been selected as controls to be followed by means of more frequent stool and blood specimens. Of the 7 vaccinees in the group, all became infected and in 5 the first stool collected was already positive. This was on the 7th day after the trial had begun, i.e., 7 days after virus had been fed to the 20 test individuals. Thus the incubation period must have been very short—2-3 days in terms of establishment of alimentary infection since in 7 days or less 2 human passages had already occurred. Dick and Dane[9] in a recent report on a similar trial with an attenuated strain of virus collected earlier stool specimens from contacts, one of whom was found to be excreting virus on the 5th day after the test individual had been fed. These observations confirm earlier estimates of

the incubation period by Paul and others based on the occurrence of the "abortive" or minor illness form of poliomyelitis.[26, 39]

Role of antibody level in inhibiting alimentary infection. It was our aim in this human trial to compare infection in naturally immune and vaccine-immune individuals with comparable antibody levels, but because the responses to Salk vaccine were not very high, this was possible in only a limited number of instances. Table 4 correlates the occurrence of infection with the prefeeding type I antibody titers of the 20 individuals fed and their 49 associates, in both vaccine-immune and naturally immune individuals.

TABLE 3.

SPREAD OF INFECTION AMONG 49 ASSOCIATES OF 13 INFECTED PERSONS FED VIRUS. RELATION TO ANTIBODY STATUS.

	SOURCE OF INFECTION	INFECTION OF 49 ASSOCIATES	
	NUMBER OF INDIVID. FED WHO EXCRETED VIRUS IN FECES	IMMUNE STATUS "NATURAL"	"VACCINE"
NORTH	3	0/26	—
SOUTH	10	1/10	12/13
TOTAL	13	1/36 (3%)	12/13 (92%)

Those vaccinees with levels up to 16 all became infected. Unfortunately, there was none among the naturally immune with comparably low levels. Conversely, in the group with levels of 128 or more, there were 29 among the naturally immunes, one of whom became infected, but no comparable group among the vaccinees. Only in the range of pre-exposure levels of 32 to 64 can an adequate comparison be made. Here, 8 of 8 (100 per cent) of vaccinated individuals became infected, but only 2 of 15 (13 per cent) of those who had naturally acquired antibodies. This suggests, as has been pointed out before[27, 41] that a given level of circulating antibodies alone—at least at this 32-64 range—is not the whole story in resistance to infection; something else, conceivably local tissue immunity or conceivably previous high levels of antibody, alter the response.

The question of the stability of the attenuated strain of poliovirus used in this particular trial is being studied by testing the virulence of 1st and 2d

human passage virus isolated from infected individuals late in the course of infection. The results are as yet incomplete.

To summarize at this point the results of our trial with the L-SC attenuated strain of poliovirus in this "closed" cottage population: (i) Of the 69 persons in the group, 27 eventually became infected as evidenced by virus excretion and antibody rises. None of these persons became ill at any time with signs suggestive of poliomyelitis. (ii) Of the 20 test individuals fed virus, 2 of 9 or 20 per cent of the naturally immune and all 10 of the vaccine

TABLE 4.

RELATION BETWEEN ANTIBODY TITER AT TIME OF EXPOSURE AND INFECTION IN 69 INDIVIDUALS

TYPE I ANTIBODY LEVEL-PRE-EXPOSURE	VACCINE NUMBER	VACCINE IMMUNE %	NATURALLY NUMBER	NATURALLY IMMUNE %
0-<4	5/5	100	(1/1*)	-
4-16	9/9	100	-	-
32-64	8/8	100	2/15	13
128-256	0/1	-	1/20	5
512-1024	-	-	0/9	0

*SUSCEPTIBLE INDIVIDUAL

immunes became infected. The vaccine-immune group excreted relatively larger amounts of virus for longer periods of time and developed CF as well as neutralizing antibody rises. The two in the naturally immune group excreted minimal amounts of virus intermittently, exhibited slow rises in neutralizing antibody and no significant CF antibody response. (iii) The throat was bypassed successfully by feeding virus in hard gelatin capsules. Of the vaccinees, none of 3 capsule-fed individuals who became infected excreted virus in the throat, while throat swabs were positive in 6 of 7 who were fed the same dose in liquid form. Absence of virus in the throat did not delay its appearance in the stools, nor was there any significant difference in antibody response between throat-positive and throat-negative individuals. (iv) Spread of infection to close associates of individuals fed virus

occurred in 1 of 36 (3 per cent) naturally immune, and 12 of 13 (92 per cent) vaccine-immune contacts. (v) Considering the entire trial, it was possible to compare the ease with which infection was acquired by 15 naturally immune and 8 vaccine-immune persons with comparable type I antibody levels (32 to 64). Two of the naturally immune (13 per cent) and all of the vaccine immunes (100 per cent) became infected.

DISCUSSION

The results of this trial confirm certain earlier observations and provide additional data on human responses to poliomyelitis virus infection, the relative nature of immunity, and the existence of qualitative differences between naturally acquired and vaccine-induced immunity.

As in previous similar studies a difference was observed in these two groups in that individuals with *naturally* acquired antibody were far more resistant to infection than were those with *vaccine*-acquired antibody of approximately the same level. This difference might be explained by assuming that the first line of defense is a tissue barrier (? local immunity) based on one or more previous experiences of the alimentary tract with living, multiplying virus. The second line of defense then becomes the presence of circulating antibody. Another possible explanation is that "tissue immunity" does not enter the picture, but there are qualitative differences in the antibody produced by the two different mechanisms, and higher levels of vaccine-induced than of natural antibody are required to prevent infection of the alimentary tract. There is some evidence that such qualitative differences do exist.[44] Thus Sabin's so-called "low-avidity" neutralizing antibody follows vaccination, and appears also in the acute phase of poliomyelitic infection; in contrast is "high-avidity" antibody which is found in convalescence from natural infection or following infection with an attenuated strain.[21] As to whether very high levels, i.e. >1000, of vaccine-induced antibody will offer the same protection against intestinal infection afforded by lower levels of natural antibody, there is no direct evidence available. However, the experience of Sabin[44] who found that three volunteers with prefeeding titers of 1:256 were readily infected with an attenuated type I strain, indicates at least that moderately high levels of vaccine-induced antibody are not effective in this respect.

Of the many variables involved in determining responses on exposure to polioviruses, the immune barriers and the sites at which they operate are of primary importance in terms of immunization. The present state of knowledge does not allow more than general conclusions about this aspect. It is clear that circulating antibody and probably tissue barriers are involved

in limiting the infectious process, in keeping it localized to the alimentary tract and regional lymphoid structures, in preventing spread to the CNS either from the blood stream, or along neural pathways. The effectiveness of the blocking mechanisms is governed to a considerable extent by the virulence of the infecting strain, and the dosage of virus or degree of exposure. That naturally acquired immunity is more "solid" than vaccine-acquired immunity seems likely, and the probability is great that it is also more durable. Nevertheless, by enhancing the potency of the formalinized vaccine, its effectiveness may, in the future, compare more favorably with that achieved by natural infection. In the meantime, however, there is still much to be learned about human responses to polioviruses. For this reason, we believe that the pursuit of problems of active immunization with attenuated viruses is an important area of investigation in terms of the pathogenesis of poliomyelitis and immunization against the infection as well as the disease.

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