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Received December 16, 1952. P.S.E.B.M., 1953, v82.

Further Studies on Oral Administration of Living Poliomyelitis Virus to Human Subjects. (20091)

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In a previous study(1) the results of oral administration of living poliomyelitis virus to 20 human volunteers were reported. In most of the subjects the virus was recovered from the stool; 16 subjects who were not immune prior to the feeding developed Lansing type neutralizing antibodies in their blood; none of the subjects showed any apparent untoward effects from the administration of the virus nor was any febrile response noted.

In the present communication, the results are reported of the effects of oral administration of the same type of virus to 61 human subjects, all of whom had no Lansing antibodies prior to the experiment. It will be seen that the results obtained in the first trial have been confirmed and to a certain extent amplified.

Materials and methods. The subjects for experiment were selected in the following way: 181 mentally defective children ranging in age from 8 months to 8 years were bled in April, 1952, and the sera tested for neutralizing antibody content against the MEFl strain, Type II poliomyelitis virus (see below). The children were inmates of a State institution for mental defectives. Of the children who had no antibodies, 61 were selected for the trial after permission of each child's parents had been legally obtained. Each child was tested again for presence of neutralizing antibodies during the last week of June, 1952, and fed the virus on July 1. During the following period of 21 days the children were placed in an isolation unit under constant medical observation and nursing care. Rectal temperatures were taken every 8 hours and search was made daily for development of somatic disturbances with particular attention to the central nervous system. Stools for virus isolation were collected from every treated child on the 5th, 8th, 12th, 15th, 18th and 21st days after feeding. Blood for virus isolation was obtained from each subject by venous puncture. The patients were divided into two groups and members of each group were bled on alternate days for 12 days, starting on the first day after feeding. Thirty days following virus feeding, all patients were bled and the sera submitted to

* In cooperation with the California State Departments of Mental Hygiene and Public Health, and the George Williams Hooper Foundation, University of California, San Francisco.

neutralization test. *Virus strains.* The TN strain(1,2) of rodent-adapted poliomyelitis virus was used for feeding purposes. It was administered in the form of a 20% suspension of infected cotton rat brain and spinal cord tissues prepared exactly as described elsewhere(1). Two pools of infectious material were used: One (No. 24) represented the 35th cotton rat passage, and the other (No. 31) represented the 2nd cotton rat passage of the virus(1). Each feeding dose consisted of 5 ml of the suspension diluted with 2 oz of chocolate milk for children, or feeding formula for infants. The MEFl strain of poliomyelitis virus(3) was used for the neutralization test.

Methods for examination of stool and blood. Stool specimens were processed according to the method described before(1) and were assayed for the presence of virus by intracerebral inoculation of 10 Swiss albino mice per specimen. If signs of paralysis were noted in any of the inoculated animals, the affected mice were sacrificed and the brain and spinal cord tissues subinoculated into another group of 8 mice. In every case the identity of the agent isolated by the mouse passage technique was confirmed by neutralization test against Lansing type-specific hyperimmune serum obtained from a rhesus monkey recovered from paralytic infection(1). Blood specimens were injected intracerebrally into mice within 4 hours after withdrawal. Each sample was injected into 10 mice. When none of the inoculated animals showed signs of illness, one or two animals were sacrificed on the 7th and 14th day after inoculation and their brain and spinal cord tissues subinoculated into other groups of mice. Following this, another "blind" passage was made and the virus was considered to be absent in the patient's blood if neither the inoculated nor the subinoculated groups of mice showed signs of illness. *Neutralization test.* The technic of the test followed that described previously (1), except that in most cases only one dilution of virus was used against serial dilutions of serum.

Results. Distribution of antibodies against Type II poliomyelitis virus. Out of 181 patients bled in April, 1952, undiluted sera of 106 failed to neutralize the MEFl strain of poliomyelitis virus. Sera of 10 did neutralize the virus if tested in undiluted form but were devoid of neutralizing power when diluted 1:3; sera of 21 gave partial protection in undiluted form and in 1:3 dilution. Thus, 31 patients were classified in the "doubtful" category. Sera from the remaining 44 patients neutralized the virus when tested both in undiluted form and in 1:3 dilution. Of the 106 inmates whose sera showed no neutralizing power against Type II virus, 61 were ultimately selected for the trial and were fed the virus.

Lack of clinical manifestations of poliomyelitic infection. None of the 61 children showed any apparent signs or symptoms of illness which could be attributed to the ingestion of the virus. A few children developed elevated temperature which lasted only a few hours. The cause was promptly recognized in some trivial condition such as constipation, mild tonsillitis, nasopharyngitis, etc., and prompt response was obtained with adequate therapy. In 3 cases, diarrhea developed and stool culture demonstrated the presence of a Shigella organism. In no patient was there any evidence of meningeal irritation or of paralytic changes.

Absence of viremia. No virus was isolated from any of the blood specimens obtained from the patients during the 12 days following oral administration of the virus.

Antibody response. The results are summarized in the accompanying table. It may be observed that a virus identified as TN strain was isolated from 53 stool specimens obtained from 29 of the 61 patients. In 16 of these patients, TN virus was isolated from only one stool specimen, in 6 patients from 2 stools, in 4 patients from 3 stools, in 2 patients from 4 stools and in 1 patient from 5 stools. It may also be observed that the antibody rise against Type II poliomyelitis virus was significant in most of the cases. No neutralizing antibodies were present in the sera of 6 patients (Nos. 8, 16, 43, 51, 54, 57) 30 days after feeding with virus, and only one of these patients (No. 8) excreted virus in his stool, *i.e.*, that which was collected on the 5th day of the trial. No sig-

TABLE I. Occurrence of Intestinal Carriage and Serological Evidence of Immunity in 61 Human Subjects after Oral Administration of TN Strain of Poliomyelitis Virus.

Virus isolated from stools (days postfeeding)	50% protective serum titer§ Prefeeding	Postfeeding	Virus isolated from stools (days postfeeding)	50% protective serum titer§ Prefeeding	Postfeeding
*8	0†	>1:600	15	0‡	1:126
5, 8, 15	1:4‡	1:200	18	0	1:294
*0	1:2‡	1:174	0	0	1:119
5, 8	1:10	1:398	20	0	1:165
15	0	1:210	12, 15	0	1:18
5	0	1:1030	5	0‡	1:105
*8	1:4‡	1:236	0	0‡	1:27
5	1:4‡	0	0	1:3	1:204
*5, 8	1:5‡	1:318	20	0	1:13
0	0	1:161	12, 15, 18, 20	1:3	1:105
12	0	1:822	0	1:2	1:24
0	0	1:344	0	0	0
0	0‡	1:80	8, 18	0‡	1:33
12, 18	0	1:250	0	0	1:39
0	0	1:20	0	0‡	1:127
0	0‡	0	8	0	1:18
18	0	1:31	0	1:4	1:127
0	0	1:22	18	0	1:328
0	0	1:340	0	0	1:468
0	1:4	1:131	0	0‡	0
*0	0‡	1:16	0	0	1:154
0	1:4	1:250	0	1:10‡	1:12
0	0	1:586	0	0‡	0
*0	1:27‡	1:22	5, 8, 12, 15, 18	0‡	1:362
5, 18	0‡	1:318	0	0	1:50
0	0	1:58	0	0‡	0
*5, 8, 15, 18	0‡	1:248	18	0	1:21
5, 8, 12	0‡	>1:250	0	1:26‡	1:71
0	0‡	1:145	12, 15, 18	0‡	1:48
*0	0‡	1:26	5, 8, 15	0	1:39
15	0	1:80			

* These individuals were fed with virus pool #24; all others received pool #31.
† 0 = <1:2 titer.
‡ Repetition of neutralization test gave similar results.
§ Based on the cumulation titer of the virus pool; 76 LD_{50} were used in neutralization test.

nificant rise in the antibody level was observed in the blood of 3 patients (Nos. 24, 53, 59) after administration of the virus, although the sera of these 3 patients seemed to show some neutralizing power when collected before ingestion of the virus. The antibody rise in the remaining 52 patients was significant enough to consider their immunological response adequate.

Comments. The results of the present study indicate that no clinical signs of illness were elicited by feeding the living TN strain of poliomyelitis virus to 61 children and thus confirm and amplify findings described in the previous paper(1) in which a similar lack of clinical manifestations was observed in 20 human volunteers fed the same virus. It is interesting to note that 30 of the present subjects fed the virus disclosed evidence of pre-existing severe brain damage, a condition which in the past has been considered to be a predisposing factor to the paralytic form of poliomyelitis(4). Yet none of the patients developed new neurological manifestations following feeding. In this respect it is pertinent to observe that although epidemic form of poliomyelitis had been present in the immediate vicinity of the institution for many years, no cases of paralytic poliomyelitis had ever been noted among the institutionalized children(5). That this fact can be explained on the assumption of a high incidence of the subclinical form of the disease among the inmates is not supported by the finding of a high percentage (60%) of non-immune individuals among the 181 tested prior to the trial. It should be noted also that 19 children were affected by mongolism, a condition

which has been considered to hinder formation of antibodies(6). Yet the immune responses in mongolians were not significantly different from those of the other children.

The finding that viremia was not observed in any of the 61 subjects should be emphasized in view of the recently advanced theory of Bodian that the absence of virus in the circulating blood precludes the development of paralysis(7).

Although the presence of virus in the stool could be demonstrated in 29 out of 61 patients, the antibody response would indicate that infection was established in many more cases, since the sera of only 6 patients had no neutralizing antibodies on the 30th day after ingestion of virus. One may possibly assume that in 5 out of these 6 patients the virus was destroyed before reaching the intestinal tract since no virus was isolated from their stool specimens. There was either insignificant or no rise in antibody response in 3 other patients whose blood did show some neutralizing power when drawn prior to feeding with virus. However, if one may draw a theoretical parallel with results obtained in cynomolgus monkeys(8), the level of antibodies observed in the latter 3 patients should be considered high enough to prevent paralysis after a peripheral exposure to virulent Lansing type virus. In any event, perhaps another oral administration of the TN strain to the 9 patients would result in multiplication of the virus in the intestinal tract and subsequent adequate serological response. On the other hand, a study of the genetic background of these 9 patients may throw additional light on their "non-reactivity" to the infectious process. The recent accumulation of data on the relationship between susceptibility to the paralytic form of poliomyelitis and the genetic constitution of the individual (9-11) may warrant investigation of the familial background of the 9 patients.

Summary. Sixty-one children who had no antibodies against Type II (Lansing) poliomyelitis virus were fed the TN strain of virus. None showed any clinical signs of illness. Viremia was absent in every case. In 29 individuals the virus was isolated from the stool from the 5th to the 20th day after feeding. Specific antibody rise was observed in most of the patients.

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Received December 1, 1952. P.S.E.B.M., 1953, v82.

Heparin-Like Anticoagulants from Mollusca. (20092)

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In the present investigation a study of a wide variety of aquatic life forms such as carp, whitefish, scallops, clams, and oysters revealed that 2 species of clams, *Mactra spissula* (sea surf clam) and *Artica islandica* (ocean quahog clam), contain unusually large amounts of heparin-like substances which exhibit high anticoagulant potency and low