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Excretion of Vacuolating SV-40 Virus (Papova Virus Group) After Ingestion as a Contaminant of Oral Poliovaccine.* (27392)

JOSEPH L. MELNICK AND SARA STINEBAUGH
Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas

The vacuolating SV-40 virus(1), the most recently discovered member of the papova virus group(2), became the object of immediate and widespread interest when it was discovered as a viable contaminant of killed and live virus vaccines grown in rhesus and cynomolgus kidney cell cultures(1,3,4). It appears that the virus has been injected as a live contaminant of formalinized polio- and adenovaccines into hundreds of thousands, if not millions, of persons, and that it has been fed in active form together with live poliovaccine to groups equally as large.

American(1) and British(5) investigators reported that 2 to 3 injections of some formalinized adeno- and poliovaccines were sufficient to produce vacuolating virus antibodies in man, though whether from simultaneous injection of living or inactivated vacuolating virus could not be established retrospectively. Intranasal administration of vacuolating virus has been found to lead to viral multiplication and antibody formation(6). In this instance the vacuolating virus was present as a live contaminant of another virus preparation being studied in human volunteers. It should be emphasized that in spite of the large numbers of persons injected or fed the virus, not a single human illness has been attributed to this agent.

Studies on the isolation of vacuolating virus from children who had inadvertently received contaminated lots of oral poliovaccine are reported here. Some negative studies on such individuals have been reported, but they have been very few in number(5,7).

Materials and methods. Tissue cultures. Monkey kidney (MK) monolayer cultures were grown and maintained in our lactalbumin medium, as described previously(8). Cultures were grown from African green monkeys (*Cercopithecus aethiops*). The animals were all tested for SV-40 antibody and only those free of antibody were selected for culturing. In all experiments a number of uninoculated cultures were observed to insure that vacuolating virus was not a spontaneous contaminant of the test cultures.

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Isolations. These were carried out by inoculation of 0.1 ml of fecal suspension into at least 3 Cercopithecus cultures, which for this slow growing virus(2) were then observed for 21 and preferably 28 days for cytopathic changes. The medium of the inoculated and control cultures was changed at weekly intervals. Retest of a number of specimens by subculturing negative cultures at 14 days did not increase the number of positive isolations. Positive specimens were passed and typed as SV-40.

Neutralization of poliovirus. Stool suspensions and vaccines in which vacuolating virus was sought often contained poliovirus. This was neutralized with antiserum prepared in rabbits against HeLa-grown poliovirus from strains never propagated in MK cells and consequently free of simian agents. Inoculum in this case was 0.2 ml of a mixture of equal parts of specimen and antiserum.

Detection of antibodies. In antibody determinations carried out by plaque reduction technics(9), virus stock was diluted to contain 50-75 PFU/0.1 ml and incubated with an equal volume of serum (diluted 1:4 or 1:10) for 2 hours at 37°C. Inoculum was 0.2 ml/bottle, and bottles were overlaid as described previously(9).

A faster, but less sensitive method used to test monkey sera was to incubate the sera 2 hours at 37° with an equal amount of virus diluted to contain 10^4 or 10^5 TCD_{50}/0.1 ml if read on the 21st day. However, only 100 TCD_{50} were manifest after 5-6 days at which time the tube neutralization tests were read. The inoculum of the virus-serum mixture was 0.2 ml per tube culture containing 1 ml of maintenance medium.

The vacuolating virus used in antibody determinations was a strain which we isolated in green monkey kidney cultures from the lot of Type 2 oral poliovaccine prepared for Sabin in 1956(10) and used in a number of field trials in this country and abroad(11). It was found to be antigenically identical with the strains we isolated from the other 2 types of oral poliovaccine, with a strain obtained from Sweet and Hilleman(1) and with a number of strains isolated from uninoculated MK cultures in our laboratory.

TABLE I. Polio and Vacuolating Virus Excretion.

Vaccinated children	Days after feeding 7	14	21	28
I-S-20	--	—	—	V
II-S-17	P	P	V	—
II-32	V	—	—	V
III-4	—	—	—	V
III-10	P	P	V	—
III-11	P	—	V	—
III-13	—	P	V	—
III-33	P	P	V	P
IV-S-27	—	—	—	V

V indicates vacuolating virus isolated; P, poliovirus; negative indicates failure to isolate any agent.

TABLE II. Apparent Inhibition of Poliovirus Excretion by Vacuolating Virus Carriers.

SV-40 carrier	% of children excreting poliovirus on days after feeding 7	14	21	28
Positive	44	44	0	11
Negative	83	80	71	35

Results. Concentration of vacuolating virus contaminant in oral vaccine used. Aliquots of Sabin's original large lot of vaccine (10) were treated with polio antiserum referred to above and assayed by counting the plaques, which for SV-40 appear between the 11th and 14th days(9). The concentrations of SV-40 found in the vaccines stored at –20° to –40° for 5 years were $10^{4.6}$, $10^{3.6}$, and $10^{4.8}$ PFU/ml for Types 1, 2, and 3, respectively. Aliquots of the same lots tested about 2 years earlier by Sweet and Hilleman(1) gave approximately the same titers in tube cultures.

Houston trial. Sabin's oral poliovaccine (10) was diluted so that a dose contained $10^{5.5}$ TCD_{50} of each poliovirus type. Later it was found that each child had also been given 100 to 1000 PFU of vacuolating virus as a contaminant. The children were 3 to 6 months old at the time of the virus feeding. Stools were selected for this study from 47 children whose response to the poliovaccine was relatively poor, *i.e.,* the poliovaccine strains either had failed to multiply or had multiplied for a shorter period than in the other children in the study. Of these, 9 were found to excrete vacuolating virus chiefly in the samples collected 21 or 28 days after administration of vaccine (Table I). It is note-

worthy that of the 9 children only one child yielded positive samples twice, on the 7th and 28th day. This child failed to become a poliovirus excretor. Three other children who were negative for poliovirus excretion in the month after the feeding yielded samples positive for vacuolating virus on the 28th day.

Of the 5 children in the group who excreted both viruses, 4 excreted poliovirus during the first 2 weeks, to be followed by vacuolating virus in the 3rd week. One child yielded samples positive for poliovirus in weeks 1, 2, and 4, with a sample positive for vacuolating virus in week 3.

The pattern of these 9 children seemed to be one of early excretion of polio in the first fortnight to be followed by vacuolating virus excretion in the second fortnight. The excretion of poliovirus seemed to be influenced by whether or not the child also became a vacuolating virus carrier. The rate of poliovirus carriage was less each week and terminated much earlier in the 9 children who excreted SV-40 than in the much larger number of children in the total study with whom they are compared in Table II.

New Orleans trial(12).† The same lot of Sabin's vaccine was used but fed to newborn children at a level up to 100 times the above dose as reported by Gelfand *et al.*(12). About 10^4 to 10^5 PFU of vacuolating virus was inadvertently ingested by each child.

The 80 children selected were typical of those in whom poliovirus multiplied. Only aliquots of samples proven positive for poliovirus in Gelfand's laboratory were submitted to us for study. Twelve of the 80 were found to have excreted vacuolating virus as well as poliovirus. The pattern of excretion shown in Fig. 1 was different from that found in the Houston study, where older infants had ingested only 1% of the dose of vacuolating virus.

Five of the 12 positive children excreted vacuolating virus only on the 1st and 2nd day after the feeding; this can probably be accounted for on the basis of passive transit through the alimentary tract. Seven children excreted vacuolating virus between the 6th and 35th days. Four infants yielded multiple positive samples, one on as many as 4 occasions (days 1, 5, 28, and 35).

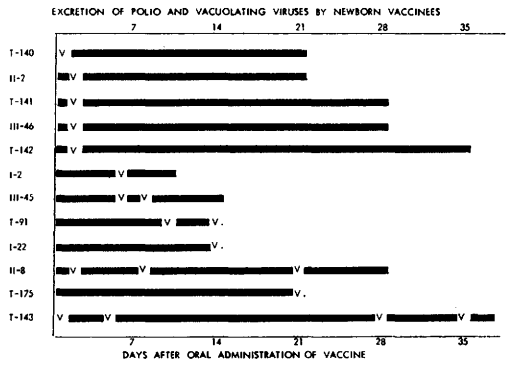

FIG. 1. Excretion pattern for each child is represented by a bar, the length of which represents duration of poliovirus excretion. V is inserted at the time when vacuolating virus was found in stool.

Cleveland trial(13).‡ Of 59 stools collected from 22 infants fed at birth or at 3 months of age with the same lot of Type 1 vaccine and at the same dose as in New Orleans, none yielded vacuolating virus.

Discussion. The present study indicates that vacuolating virus when fed to children may sometimes produce a low grade infection. Even though the virus has been recovered as long as 35 days after ingestion, the amounts excreted were very low indeed. Sabin(7) failed to recover SV-40 from 42 persons who received contaminated vaccine but he held his test cultures only 14 days. Had we done the same we would have missed 26 of the 29 positive specimens. Magrath *et al.* (5) were also unable to find evidence of SV-40 multiplication in 19 children, but they did not state how long their cultures were held. We have no real explanation for the negative findings in the Cleveland babies. However, all of the Cleveland specimens were collected within 2 weeks of feeding the virus, and it should be noted that in the Houston babies, of the 10 positive specimens only one was collected in this period.

Morris *et al.*(6) observed that SV-40 given

† This trial was planned and carried out by Drs. Henry Gelfand and John Fox, and their associates, to whom we are indebted for making these samples available to us.

‡ This trial was planned and reported by Dr. Frederick C. Robbins and his colleagues(13) who kindly sent us some of their specimens.

by the intranasal route could multiply in human beings, and produce a low level antibody response. Our sampling studies on a few of the positive Houston children failed to indicate an antibody response in sera collected one month and one year after exposure.

The problem of vacuolating virus-contaminated vaccine has been controlled in this country by using clean poliovirus seed and preparing vaccine only in cultures proven free of SV-40. Another method is to take advantage of the rapid destruction of SV-40 by divalent cations under conditions which stabilize the poliovirus in the vaccine(14). Oral poliovaccine, after heating in high concentrations of Mg ions for one to 2 hours at 50°C, has been found to be as effective in producing polio antibodies in man as the unheated vaccine. Such treatment so far has stabilized only those viruses which exhibit cubic symmetry, contain RNA, and are free of essential lipids(15). Viruses inactivated by this treatment include members of the following groups: myxovirus, adenovirus, herpesvirus, poxvirus, arborvirus, and papova virus. As no knowledge of the SV-40 contaminant in the vaccine existed until a new test system was introduced, consideration might be given to the cationic treatment of poliovaccines to remove other potentially present contaminants.

Summary. Children ingesting vacuolating SV-40 virus, a member of the papova group, may excrete virus in their stools. When 100 to 1000 PFU of vacuolating virus present as a contaminant of oral poliovaccine, were fed to 3- to 6-month-old infants, some of them excreted vacuolating virus for 4 weeks. Vacuolating virus seemed to interfere to some degree with the multiplication of poliovirus, for SV-40 carriers excreted less poliovirus and for shorter periods than did those children who did not become infected by the vacuolating virus. Two groups of newborn infants receiving large doses of SV-40 were also studied, and in one group vacuolating virus was found to be excreted for as long as 5 weeks. No disease attributable to SV-40 occurred in any of these children, nor has illness been recorded for any of the hundreds of thousands or millions who have either been injected or been fed with this agent.

Consideration should be given to the cationic treatment of oral poliovaccines. Such treatment stabilizes the vaccine virus and at the same time can remove SV-40 and other hypothetical agents which may be present but which have not been detected by present testing methods.

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