Serodifferentiation of Poliovirus Strains for Studies of Oral Vaccine

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THE USE of living, avirulent, oral poliovirus vaccines on a broad scale in the United States will inevitably be accompanied by the occurrence of some cases of paralytic disease in recently vaccinated persons, their families, and community associates. When these vaccines are used during the poliomyelitis season, which may be spring, summer, or autumn in different sections of the nation, and especially when they are used to combat epidemics already in progress, such instances of concurrent illness may be frequent. Despite evidence of the safety of oral poliovirus vaccines composed of the Sabin strains, every case of significant central nervous system disease which occurs in some epidemiologic relationship with oral vaccination must be investigated. If no evidence for a causal association between vaccination and illness is thereby adduced, the results of those investigations would have the useful effect of increasing still further scientific, medical, and public confidence in the safety of the oral method of poliomyelitis vaccination.

Evidence bearing on the relationship between oral poliovirus vaccine and cases of paralytic poliomyelitis is of two kinds—laboratory and epidemiologic. The overall problem is extensive and complex and cannot be discussed completely at this time. It would be greatly simplified, however, if there were available a means of identifying the vaccine poliovirus strains with a high level of probability and of distinguishing them from all other strains of the same virus type but of "wild" or extraneous origin.

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Background

During the past several years, a number of strain "markers" of polioviruses have been discovered. Some of the better known of these are the *d* marker which measures the differing capacity of attenuated and virulent strains to produce cellular destructive plaques in tissue cultures at different levels of $NaHCO_3$ concentration (*1*), the *rct* (or *T*) marker which measures the differing reproductive capacities of strains at different temperatures (*2*), the *MS* marker which measures the greater ability of virulent as compared with attenuated poliovirus strains to produce plaques on a stable line of monkey kidney cells (*3*), and *E* marker which measures differences in the ease with which different strains can be eluted following adsorption to cellulose resin (*4*) or $Al(OH)_3$ gel (*5*). Unfortunately, studies of these markers have revealed that they are, in general, related to virulence, rather than relatively stable genetic attributes of the strains tested (*6*). The vaccine viruses possess those markers characteristic of avirulent strains, but their progeny, following multiplication in man, often exhibit changes which may be related to some increase in monkey neurovirulence. If our problem is to determine whether a virus isolated from a paralyzed vaccinee or contact is of virulent "wild" origin or may be derived from a virulent mutant of the vaccine strain, tests for these markers are obviously not helpful.

The antigenic composition of a virus is a fundamental characteristic and is the basis for type designation. Lesser grades of antigenic difference also exist, and, as among the influenza viruses, may be useful in designating and identifying strains within the same virus type. Within the enterovirus group, the so-called prime strains represent related, but distinguishable, subtypic entities. These considerations suggested that relatively stable antigenic differences may exist among polioviruses which might serve as markers unrelated to virulence.

Intratypic antigenic differences among polioviruses were first definitely demonstrated by Wenner and associates for type 2 (*7*). By means of various serologic techniques, this finding was confirmed by other investigators and extended to all three virus types (*8–14*). On the other hand, some of these workers reported that the variant strains developed in the laboratory under varying conditions of pH and temperature, as well as in different tissue culture systems or in abnormal hosts, did not differ significantly from their parent strains (*7,10, 14*). Even after successive passage in tissue cultures containing progressively increased amounts of homologous antiserum, McBride (*15*) was able to select only a small number of particles serologically distinguishable from their ancestors, although under appropriate selective conditions mutants with other markers were readily recovered. Serologic comparisons of vaccine and progeny viruses after passage through human hosts have been made by several investigators working with various strains, including those contained in the Sabin vaccine, and using differing techniques (*11,13, 14*). In general, antigenic stability following human passage was demonstrated, although some exceptions were reported. It appeared that antigenic characterization of the poliovirus vaccine strains might make it possible to identify them after human passage, irrespective of any change in virulence which might have taken place.

Methods and Findings

In order to undertake such studies in our laboratory the technique proposed by Wecker (*12*) was modified and then adopted for routine use. Our procedures have been reported in detail (*16*). Briefly they are as follows:

For each virus to be tested, we use three groups of plastic petri dishes containing confluent monolayers of primary monkey kidney cells. Each dish is inoculated with 0.2 ml. of diluted virus suspension, pretitered to contain 20–30 plaque-forming units. Viruses are allowed to absorb at 36° C. for 1.5 hours, then one group of dishes is overlayered with plain agar medium as a control and two groups with agar medium containing different concentrations of a strain-specific antiserum prepared against type 1 Sabin vaccine. These concentrations were chosen to bracket the end point of complete vaccine virus plaque inhibition. The "mean percent plaque breakthrough" (MPB) is calculated on the basis of plaque counts made at the end of an appropriate incubation period as determined by the control dishes. Each test run also includes similar tests using the vaccine strain, a known vaccine-passage strain, and a known "wild" strain as controls. The MPB is the value used for the antigenic comparison of strains. Under the criteria established, strains with MPB values less than 10 percent are considered "vaccine-like," those of 10–20 percent are "intermediate," and those 20 percent or greater are "non-vaccine-like."

Table 1. Mean percent plaque breakthrough (MPB) values of known vaccine-derived and "wild" type 1 poliovirus strains, from a single test run with anti-LSc2ab serum

Strain	Origin	MPB
LSc2ab	Sabin vaccine, type 1	0. 3
1–015	1st human passage of LSc2ab	. 0
4851	1st human passage of LSc2ab	. 5
4853	2d human passage of LSc2ab; contact of 4851.	. 0
7201	1st human passage of LSc2ab	. 5
7216	2d human passage of LSc2ab; contact of 7201.	1. 0
Lwoff	Laboratory variant, virulent derivative of LSc2ab.	1. 0
Mahoney	Remote ancestor of LSc2ab	24. 0
2900	Presumed Mahoney derivative; from Salk vaccine-associated case, 1955.	26. 0
23379	Paralytic case, Louisiana, 1955	28. 0
3224	Healthy carrier, Louisiana, 1955; unassociated with 23379.	35. 0
946	Healthy carrier, California, 1960	31. 0
1299	Fatal case, Rhode Island, 1960	43. 0
282	Healthy carrier, Georgia, 1960	68. 0

Table 2. Modified Wecker and McBride serodifferentiation test results for serial type 1 poliovirus isolates from two vaccinated children using anti-LSc2ab serum, with test values of LSc2ab and Mahoney viruses for comparison

Days after vaccination and virus strain	Modified Wecker test results (MPB)	McBride test results (NK)	Classification
Child A			
2	2. 0	72	Vaccine-like.
4	3. 9	84	Do.
6	4. 3	87	Do.
8	5. 7	84	Do.
14	0	78	Do.
21	3. 8	87	Do.
Child B			
2	1. 4	108	Vaccine-like.
4	5. 7	100	Do.
6	4. 7	83	Do.
8	7. 5	86	Do.
10	19. 6	40	Non-vaccine-like.
14	31. 5	39	Do.
21	24. 7	31	Do.
28	37. 5	44	Do.
Virus strain			
LSc2ab	0. 5, 2. 1	100	Vaccine.
Mahoney	26, 39	24	Non-vaccine-like.

MPB—mean percent plaque breakthrough.
NK—ratio of values reflecting the kinetics of neutralization of an unknown strain and the homologous vaccine strain.

For the development of this modified technique and its evaluation, a collection of type 1 isolates was repeatedly tested. Included were a group of strains related to Sabin's oral poliovirus vaccine and a group with no known relationship. These strains, their origin, and their MPB values in a typical test are shown in table 1. Segregation of vaccine-like and non-vaccine-like isolates was clear cut. The only exceptional finding was that Mahoney (and its immediate derivative, 2900) fell into the vaccine-unrelated group, although strain LSc2ab was presumably derived from it after many passages in monkey testicular tissue culture, intradermally in monkeys, and finally after passage in monkey kidney cultures and plaque selection.

All of the vaccine-passage strains included in table 1 had been isolated within a short time after the donor had received oral vaccine. In order to determine whether continued intestinal multiplication would affect the antigenic character of progeny strains, tests were performed on several series of isolates obtained sequentially from vaccinated children. As recently reported by us (*17*), five of seven such series from different children showed no significant antigenic changes during a period of 3 or 4 weeks. In two series, however, an abrupt change occurred after about 1 week following vaccination, the isolates thereafter appearing non-vaccine-like. Table 2 shows one series of each sort. Each isolate was tested by McBride's technique based on studies of the kinetics of neutralization (*10*), and by the modified Wecker technique, with equivalent results in each instance.

The problem of antigenic stability was also approached on a larger scale but with less exactness by testing the random type 1 isolates from a large number of vaccinated children. Fecal specimens had been collected from these children at various times after oral vaccine feeding in a field study undertaken at a time when very few "wild" type 1 polioviruses were in natural circulation. The classification of the virus strains, based on the criteria given above for interpretation of the modified Wecker test, is shown in table 3. Viruses isolated during the first week after vaccination were vaccine-like in the vast majority of instances;

Table 3. Classification of type 1 poliovirus strains isolated at various intervals of time from persons who had received oral poliovirus vaccine, type 1

Classification	Number of isolates during—				
	1st week	2d week	3d week	4th week or later	Total
Vaccine-like	98	45	4	1	148
Intermediate	2	11	2	4	19
Non-vaccine-like	0	13	5	11	29
Total	100	69	11	16	196
Percent vaccine-like	98	65	36	6	76

none could be called non-vaccine-like. During successive weeks, the percentage of vaccine-like strains decreased. By the third week a substantial proportion could not be related to the vaccine by this test, and after the third week most of the isolates were indistinguishable from "wild," naturally occurring strains.

To determine whether all "wild" type 1 polioviruses can be classified with certainty as non-vaccine-like, we tested 128 isolates collected over a period of several years from patients and healthy carriers living in many different parts of the United States and having no contact with oral vaccine. Table 4 gives the results, using the same criteria for classification based on the modified Wecker technique. It is apparent that the vast majority of "wild" isolates can be identified as non-vaccine-like, and that this is not related to whether the donors were patients or carriers. However, a few strains were misclassified as vaccine-like, and, if their origin had been unknown and if they had been involved in an oral vaccine problem, they might have caused serious confusion.

It should be noted that tests on all of the isolates included in tables 3 and 4 were performed under code, and the source of each strain was recorded only after serologic classification.

We have referred to the phenomenon demonstrated above as "antigenic drift." Wasserman and Fox (*18*), using a modification of the McBride technique, and W. A. Woods and associates (personal communication), using a disk diffusion-plaque technique (*11*), have also described antigenic changes in type 1 viruses isolated from serially collected specimens from vaccinated persons. There appears to be a marked and general tendency for vaccine virus strains to drift antigenically during the course of intestinal multiplication.

Table 4. Classification of "wild" type 1 poliovirus strains isolated from persons with no known association with oral poliovirus vaccine

Classification	Number of isolates from—		
	Clinical cases	Asymptomatic infections	Total
Vaccine-like	2	0	2
Intermediate	6	2	8
Non-vaccine-like	100	18	118
Total	108	20	128
Percent non-vaccine-like	93	90	92

Conclusions

In view of the data presented, it is obvious that antigenic characterization of an individual isolate from a single poliomyelitis patient, who had been vaccinated with oral poliovirus vaccine or who had been a contact of a vaccinated person, may not provide clear-cut evidence for or against the causal association of type 1 oral vaccine virus and disease. The interpretation of serodifferentiation tests is dependent upon the interval of time between administration of vaccine (or contact with a vaccinated person) and collection of the specimens from which viruses had been isolated. Furthermore, information about the antigenic characteristics of known "wild" type 1 polioviruses, which may have been collected in the same area prior to a community vaccination program, would be helpful.

An additional problem which may lead to error in interpretation should be mentioned briefly. Interference between enteroviruses in the human host is an accepted phenomenon, although multiple infections do occur. If an individual infected with type x is susceptible to and is exposed to type y, one of three possible results will be observed: x and y may multiply side by side and be excreted together; x may prevent infection by y and continue to be excreted alone; or y may displace x as a single infection and then y may be excreted alone. Which of these alternatives occurs probably depends upon dose, intrinsic differences in strain infectivity, and the amount of time that has passed since the onset of infection with x. In practical terms, if an individual is infected with a wild poliovirus (or other enterovirus) and in the incubation period of disease which will result from that infection is vaccinated with oral poliovirus vaccine of a different viral type, the vaccine strain may displace the "wild" strain by the time illness develops. In such a circumstance, the poliovirus isolated from a fecal

specimen collected after the diagnosis of poliomyelitis has been made may be found to have the antigenic characteristics of the vaccine strain, which nevertheless may have played no role in the pathogenesis of the illness.

Despite the difficulties which have been discussed, antigenic serodifferentiation of poliovirus isolates from patients who have had contact with oral poliovirus vaccine should be performed whenever possible. If interpreted cautiously, these tests may provide valuable evidence to supplement other laboratory and epidemiologic data in studies of the possible role of oral vaccines as the etiologic agents of disease. When clusters of cases occur within circumscribed epidemiologic situations, the consistency of the evidence of these tests is a factor which may add impressive weight to overall analysis, as it did in studies of the epidemic of 1958–59 in Leopoldville, Congo (*19*), and that of 1960 in Berlin, Germany (*20*).

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Thalidomide

Unidentified tablets of thalidomide, the drug which caused numerous cases of birth deformities in Europe, may still be in family medicine cabinets in the United States, the Food and Drug Administration warned on August 23, 1962.

More than 2.5 million tablets and smaller quantities of liquids and powders containing thalidomide were distributed to 1,270 physicians for clinical trials prior to March 1962, when the trials were halted. In a survey by the FDA, 259 of 1,258 physicians interviewed (11 were deceased and 1 in a mental hospital) had taken inadequate steps to contact patients who were given the drug. Many of the 259 physicians felt that such action was unnecessary because of the length of time that had passed since the patient was given the drug; others had no records indicating which of their patients had received the drug.

The tablets distributed for clinical trial were in various colors and sizes and were usually given to patients in envelopes or other containers bearing only the directions for use. To help prevent accidental use of the drug, President Kennedy in August advised the public to check medicine cabinets and throw out drugs that are unidentified or left over from previous illnesses.

The 1,258 physicians interviewed in the FDA survey reported a total of 20,771 patients as having received thalidomide. Of this number, 3,879 were women of child-bearing age and 624 of them were pregnant. Most of the pregnant patients received the drug in the last trimester of pregnancy. Eleven have not yet delivered and are being closely observed by their doctors. Nine cases of abnormalities have been reported in offspring of patients who took thalidomide. Eight of the cases are still under investigation. In the ninth case the drug was taken only in the last trimester of pregnancy. The physician in the case concluded that the drug was not responsible, and the FDA concurred after reviewing the case.

Most of the physicians interviewed said they had received in March 1962 the manufacturer's advice to stop using the drug, but 85 said they were not warned of adverse reactions to the drug, and 42 said they did not get any message from the manufacturer. Followup telephone calls and visits were made by the manufacturer's representatives beginning in March and continuing through July 1962.

More than half of the physicians interviewed had no record of the quantities of the drug returned or destroyed pursuant to the manufacturer's instructions. When asked if they had signed a statement concerning their qualifications as required by FDA regulations for drug investigations, 640 physicians said that they had; 247 said they had not; the others either said they could not remember or did not answer the question. In response to a question about reporting the findings of their clinical trials, 276 physicians said they had made written reports; 102 said they had made verbal reports; the others either said they had made no reports or did not answer the question.

Among the more significant laboratory and clinical reports on thalidomide published during 1956–62 are the following:

In August 1956, the results of animal studies of "a new synthetic product with sedative properties," thalidomide, were reported by Kunz, Keller, and Mückter in *Arzneimittel-Forschung* 6: 426–430. In the same issue, H. Jung reported on clinical studies of thalidomide (pp. 430–432).

During December 1960 and January 1961, the *British Medical Journal* published three letters ascribing neuropathic side effects to thalidomide (5217: 1954, 5219: 130, 5221: 291). In May 1961, polyneuritic effects were described by Scheid and associates in *Deutsche Medizinische Wochenschrift* 86: 938–940. In the September 30, 1961, *British Medical Journal*, neuropathic side effects were the subject of an article by Fullerton and Kremer (5256: 855–858) and an editorial (5256: 876–877).

In September 1961, H. Wiedemann described an increase in hypoplastic and aplastic deformities in children born in Germany in late 1959 (*Medizinische Welt* 37: 1863–1866). In December 1961, W. Lenz implicated thalidomide (*Deutsche Medizinische Wochenschrift* 86: 2555–2556).

Five letters concerning thalidomide and congenital abnormalities were published in the issues of *Lancet* for December 16, 1961 (2: 1358), January 6, 1962 (1: 45–46), and February 10, 1962 (1: 326). In the February 10 issue, *Lancet* also published an article by A. Speirs (1: 303–305) and an editorial (1: 307–308) on thalidomide and birth deformities.