November 23, 1955

Dr. Albert B. Sabin
Children's Hospital Research Foundation
University of Cincinnati
Cincinnati, Ohio

Dear Dr. Sabin:

Dr. Shannon has asked me to send you the enclosed Interim Report of the Public Health Service's Technical Committee on Poliomyelitis Vaccine.

This report was released in connection with a poliomyelitis symposium at the American Public Health Association in Kansas City on November 17, and will be published in an early issue of the Journal of the American Medical Association.

You will recall that this permanent Committee emerged from the larger ad hoc groups on which you served in late April and early May, and to which you made many important contributions.

I am also enclosing, as a matter of possible interest to you, a copy of a paper on poliomyelitis epidemiology by Dr. Alexander Langmuir of the Public Health Service's Communicable Disease Center in Atlanta, Georgia.

Sincerely yours,

John E. Fletcher, Chief
Scientific Reports Branch
Interim Report
of the
Public Health Service's
TECHNICAL COMMITTEE ON POLIOMYELITIS VACCINE

November 11, 1955

David Bodian
Thomas Francis, Jr.
Carl Larson

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James A. Shannon, Chairman

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Committee Objectives

On May 23, 1955, the Surgeon General of the Public Health Service appointed a Technical Committee for Poliomyelitis Vaccine to conduct a continuing review of production and testing data on lots of vaccine submitted by manufacturers and to make recommendations concerning the release of lots of vaccine. As a result of an earlier review, it was evident that it would also be necessary for this Committee to conduct developmental studies of the details of manufacturing and testing procedure and to recommend modification of existing requirements for production which would further ensure uniformly safe and potent vaccine.

Release of Vaccine

The significance of a negative test for infective virus on a given lot of vaccine is greatly enhanced if the particular lot is one of a series in which tests for infective virus are negative. This principle was confirmed by observations in the poliomyelitis vaccine field trial. Accordingly, recommendations for release of vaccine by the Committee have been influenced as much by the plant record for consistency of performance as by the negative results of tests on the individual lots considered. A combination of consistency of performance, together with a negative test on a particular lot, provides the greatest measure of assurance of safety. Observations on field performance of poliomyelitis vaccine in the United States, Canada, and Denmark during 1955 have fully supported this concept.

Investigation of Vaccine Manufacturing and Testing Methods

The Committee has been in operation for more than five months. During this time it has met repeatedly with manufacturers' technical representatives. With their cooperation the Committee has investigated the important aspects of vaccine processing and testing. This report contains a summary account of the information obtained and the decisions reached. Although it is not possible here to present the voluminous data that have become available or to evaluate such information in detail, this summary may be useful in informing the medical profession and the public of the present state of the problems with which the Committee has been concerned. Fuller technical accounts dealing with the separate subjects to which reference is made will be submitted for publication in Public Health Reports or other scientific journals.

Explanation of the Problem of Consistency in Manufacture

The Committee sought information bearing on the question of lack of consistency in production of vaccine by some manufacturers. Since the observed inconsistencies were not of the same order of magnitude in the case of all manufacturers--in fact, it could be said that the operation of the inactivation process was highly consistent in some laboratories--it was clear that the establishment of an equally consistent record of negative tests by all manufacturers would necessitate an analysis of differences in manufacturing details.

In the case of those manufacturers whose process lacked the desired degree of consistency, it appeared that in the early phase of
inactivation, virus was being destroyed as rapidly and as uniformly as was expected. In some instances, however, there were traces of infectious virus that resisted treatment which theoretically was capable of inactivating much greater amounts of virus. This conclusion was evident from the results of the testing practices that were in effect before as well as after May 26, when the requirement for volume of sampling was changed. Moreover, such traces of active virus, in many instances, were not eliminated by the application of still further treatment. This was true for all three types of virus.

In considering reasons for failure of complete inactivation, it seemed that such failure could be due either to protection of virus from direct contact with formaldehyde or to the existence of virus particles that are inherently more resistant to formaldehyde. There is no evidence for the latter possibility. Rather, the facts support the concept that a physical barrier interfered with the contract required for chemical interaction between formaldehyde and the virus. The fact that failure of complete inactivation was not an industry-wide problem could also be interpreted to mean that details of manufacturing were at fault rather than the ineffectiveness of formaldehyde as a means for destroying virus infectivity in suitably performed routine vaccine manufacture.

In searching for the significant technical factors that might be operative, plant processes were reviewed and an examination was made of representative samples of virus-containing fluids prepared for the inactivation step by the different manufacturers. Although differences in details of processing were recognized in the review of plant procedures
and certain conclusions could be reached therefrom, the significance of these differences became more apparent when studies were made of virus fluids prepared for inactivation by each of the manufacturers. The striking differences observed were in the degree of clarity of the different fluids, and in the character of the sediments that were present in some. It was noted, also, that fluids which contained sediments failed to inactivate under conditions applied simultaneously and effectively to other fluids not having such sediments. This observation pointed strongly to a solid phase as the source of the physical barrier against complete interaction between virus and formaldehyde. Virus incorporated within a solid phase should not be expected to be inactivated in the same way as virus free in the fluid phase; in fact, it was for this reason that filtration was required from the beginning as a step in the procedure for vaccine manufacture. Unfortunately, the importance of uniform standards of filtration in large scale production could not be fully appreciated until the difficulties resulting from departures from former production methods became known to the controlling agency.

In searching further, it was found that fluids that failed to be inactivated had often not been filtered just before addition of formaldehyde, but had been filtered a number of months earlier. This made it difficult to decide, in some instances, whether the solid phase was formed on standing, whether it was due to material that passed through a defective filtration process, or whether it was due to both factors. It was evident, nevertheless, that such practices as would permit the existence of a solid phase could account for the occurrence of inconsistency
of inactivation. It was not possible in most cases to evaluate the relative importance of inadequacy of filtration as compared with storage after filtration.

The idea that inadequacy of filtration, even without the influence of storage, might be responsible for the existence of "protected virus" was suggested by several observations. First, inconsistencies encountered seemed to be associated with the use of fritted glass filters. However, it was also evident, from a considerable body of experience, that fritted glass filters could be used satisfactorily. Second, the kind of inconsistency observed was not encountered by laboratories when Seitz-type filters were used, nor was this encountered in the preparation of vaccine for the field trial when Seitz-type filters for the most part were used.

Current Filtration and Inactivation Requirements

Physical homogeneity as a necessary prerequisite for satisfactory inactivation can be approximated by requiring the application of proper filtration procedures in fixed time relationships to the inactivation process. This is being done (1) by requiring that the pre-inactivation filtration be carried out not longer than 72 hours prior to initiating the formaldehyde treatment; and (2) by requiring that a second filtration be carried out during the inactivation process, at the time when infectivity is reduced to a point at or below the minimally detectable level, and several days prior to the taking of the first 500-ml safety-test sample. By these practices, the degree of physical homogeneity required for a consistently reproducible process is maintained. Precipitates or aggregates above a certain size are eliminated both before the
formaldehyde treatment is to be begun, and again during the process and prior to termination of the inactivation procedure.

The type of filter to be employed, the number required, and the maximal volumes to be passed in order to provide the kind of assurance desired have been specified in the current requirements. The purpose of these steps is to provide assurance of effective contact between the virus particles in suspension and formaldehyde throughout the inactivation process. Approximately a one million-fold reduction in infectivity titer is effected in any two- to three-day period, and filtration in the course of processing is to be performed sometime between the third and sixth day of formaldehyde treatment. Therefore, any virus released from crystalline or amorphous precipitates, or disaggregated during the last filtration, will still be exposed to an inactivating force of at least three days of formaldehyde treatment. The absence of particles greater in size than can pass the prescribed filtration procedure is considered to be the key to consistently complete inactivation.

If any manufacturer wishes to introduce additional filtration steps beyond those prescribed, or additional treatments that have virucidal effects, these too may be employed. But it should not be expected that such additional treatments would create greater safety, since complete inactivation is probably more dependent on the absence of protected virus in aggregates than on the particular inactivating method employed.
Tissue Culture Safety Tests

Subsequent to the last filtration, and at a point in time when it may be presumed that virus infectivity is destroyed—usually six to nine days—a sample is removed from each container in which virus is being inactivated, and 500 ml is tested in tissue culture. It is necessary not only that this sample be free of demonstrable infective virus, but that this also be true of a second similar sample which is removed after continued exposure to formaldehyde for an additional three days. With the elimination of particles of the size that will be retained by the filtration procedure prescribed, it may be presumed that during the additional three-day period, the inactivating effect of the formaldehyde will be the same as that during the first three-day period of treatment when the rate of inactivation is determined for each lot being tested.

If any sample is found to contain live virus after the application of formaldehyde for a period of time equal to three times the period required to reduce infectivity to just perceptible levels, the prescribed filtration is repeated. Subsequently, there must be negative tests for virus on two 500-ml samples removed three days apart in the course of further processing. If the additional treatment is not successful, a review of the manufacturing process is indicated.

After the two negative tests on 500-ml samples of the monovalent pools, a trivalent mixture is made, and this is also tested in tissue culture, using a 1500-ml sample. In addition to the specific information required on each lot of vaccine, a continuous record is kept of the outcome of each monovalent strain pool started in each plant, and of each
trivalent vaccine pool, to ascertain consistency of performance. A continuing record of the sensitivity of tissue culture tests for the detection of minimal amounts of virus is also maintained. The release of vaccine is contingent upon an analysis of information, not only from each individual lot but from the plant record as well.

**Monkey Safety Test**

A further test of safety is made by inoculation of monkeys. The monkey safety test has been shifted from its previous position, where it was applied to the trivalent pool, and has now been adapted for use as the final container test. It is required that a minimum of five monkeys be inoculated with samplings of each filling lot, and that there be a minimum of twenty monkeys for each manufacturing lot from which these fillings are derived.

Tests made on these animals include tests for potential susceptibility, which requires the demonstration of the absence of circulating antibody in the serum prior to inoculation. In addition, samples of spinal cord segments are removed after eighteen days, not only for histologic study, but also to permit attempts at virus isolation in those cases that are suggestively positive histologically. The need for material for virus isolation attempts arises from the observation that in a number of instances a vaccine lot was considered positive for poliomyelitis virus on the basis of questionable histologic findings. The evidence now indicates that such lesions may have been due to a recent
or remote encephalitis infection acquired before the test procedure, and resulted in discarding of some vaccine which would have been safe for use.

It is now evident that under usual circumstances the monkey safety test is less sensitive than the tissue culture tests prescribed--because the volume that can be tested in tissue culture is greater, and because the strains of virus included in the vaccine are less infective in monkeys than in tissue culture. However, an effort has been made to increase the sensitivity of the monkey test by increasing the susceptibility of the test animal, by increasing the number of monkeys used, and by injecting each animal with vaccine not only intracerebrally, but intraspinally and intramuscularly as well.

As an additional safety test, conducted to establish the effect of vaccine administration via the route usually used in man, twelve monkeys are inoculated intramuscularly, each with 3 ml of undiluted vaccine given in divided doses and at weekly intervals. These monkeys were formerly considered only as part of the potency test, but can now be considered as an integral part of the monkey safety testing scheme. They now receive an additional intracerebral injection and are studied histologically for evidence of lesions in the spinal cord.

**Potency Tests**

Although the present potency tests and criteria are satisfactory for practical purposes, studies are being made to determine the best methods for increasing the discriminatory power of the test. However, the principle will remain the same in that a reference vaccine will
continue to be used. The potency of this reference standard has been established by tests in children who have no antibodies for any of the three types.

A number of suggestions have been made for using different animals or different test plans for establishing potency. The most rational approach is to use a reference vaccine standardized for its performance in children, as is now in effect. Therefore, the problem is one of level of acceptability and of technique for establishing the relationship to the standard of reference. These questions are still under study and will be evaluated as comparisons can be made of the relative potencies of the vaccines used this past summer, and their performance in the heavily epidemic areas.

**Strain Composition**

It is the opinion of the Committee that vaccine properly made by the present methods with the Mehoney strain provides an entirely safe immunizing agent. Nevertheless, a change in the type I strain is being considered in the hope of finding a less virulent strain with equal or better immunizing stability, filterability, and other characteristics of importance in manufacturing.

This subject has been under investigation in a number of laboratories, and it is now evident that there have not as yet been found any type I strain that is truly "avirulent" for monkeys when given to adequate numbers of monkeys in the form of undiluted tissue culture fluid, and by all routes, including the intramuscular. Twelve strains of type I virus, furnished by investigators particularly interested in
this problem, have been shown now to be of relatively low virulence. However, it still remains to be demonstrated whether any type I strains can be considered as completely avirulent when tested in a stringent manner. The present types II and III strains are in the category of strains of lesser virulence, and seem to be less active in producing disease in monkeys than a number of selected type I strains that are in the category referred to as "avirulent" or "attenuated."

A study that has required more than a year for completion has revealed that antigenic capacity of equivalent degree is found in a number of the type I strains characterized by low virulence for the monkey. The problems that now remain to be resolved are those related to suitability for use in production, such as filterability, and especially the question of stability in the presence of the preservative used.

In addition, information will be required on the resistance of monkeys vaccinated with any new strain to challenge with Mahoney, since field experience has been with a virus containing the latter strain. Information obtained from these studies will permit a decision on the advisability of substituting another type I strain for the present Mahoney strain. If another strain is established as superior to Mahoney, it should not be expected that it can be included in commercially distributed vaccine before the 1956 poliomyelitis season. The time when this will be possible cannot be determined at the present time.

**Summary**

In summary, the Committee is of the opinion that the principal factors which were involved in manufacturing difficulties
have been identified and corrective measures have been taken.

Among these factors is the absolute need for removal of particles within which virus may be protected from inactivation by formaldehyde. Provisions have been made to ensure as far as possible the removal of such protected particles by suitably spaced filtration procedures.

In addition, the safety test program has been strengthened by improved sampling procedures in the tissue culture tests and by increasing the sensitivity of the monkey safety tests. These measures, together with continuous review of plant production records, assure the safety of released vaccine and should make possible an increased availability of vaccine.