American Journal of

PUBLIC HEALTH

and the Nation's Health

Official Monthly Publication of the American Public Health Association, Inc.

Volume 47 January, 1957 Number 1

Poliomyelitis Vaccination in the Fall of 1956

JONAS E. SALK, M.D., F.A.P.H.A.

In the minds of many public health administrators must be the question: what are the next steps in the poliomyelitis prophylaxis program? The recommendations listed here should supply the answers in so far as the answers can be found in the current studies.

In our report, a year ago, on the status of poliomyelitis vaccination in the fall of 1955,[1] emphasis was principally on the importance of production-consistency, in relation to vaccine safety. This year, emphasis is on degree of vaccine effectiveness and persistence of immunity. These, and other questions, are considered in this review of the status of vaccination in the fall of 1956.

The Basic Question

Basically, principal interest centers around the long-standing theoretical question as to whether or not the development of effective and durable immunity to a virus disease is acquired only through the experience of infection, or whether such immunity can be induced by a nonliving antigen. Some believe it to be a self-evident truth that it is not possible to reproduce the immunizing effect of natural infection without the infection-experience provided by a living virus. Others, who do not share this view, question this axiom. It is this axiom that is under test, and more specifically, as it applies to poliomyelitis. Whether or not a killed-virus vaccine, properly prepared and properly used, prevents the paralysis of poliomyelitis, will soon have an answer.

It is already clear that (1) epidemics of paralytic poliomyelitis can be controlled and that (2) effectiveness is of a high order of magnitude, even among individuals who received but one dose of vaccine. The questions that remain are (1) whether or not it is possible to protect all who are vaccinated and (2) whether or not immunity so induced needs to be reinforced periodically. Although the answers to these questions can be arrived at empirically, it is also

Dr. Salk is Commonwealth Professor of Preventive Medicine and Director of the Virus Research Laboratory, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.

This paper was presented before a Joint Session of the Epidemiology and Laboratory Sections of the American Public Health Association at the Eighty-Fourth Annual Meeting in Atlantic City, N. J., November 13, 1956.

Studies referred to were aided by a grant from the National Foundation for Infantile Paralysis.

possible to do so by examining the rationale for a mechanism of immunity, since it is in the nature of the mechanism that the answer will be found.

Mechanism of Immunity

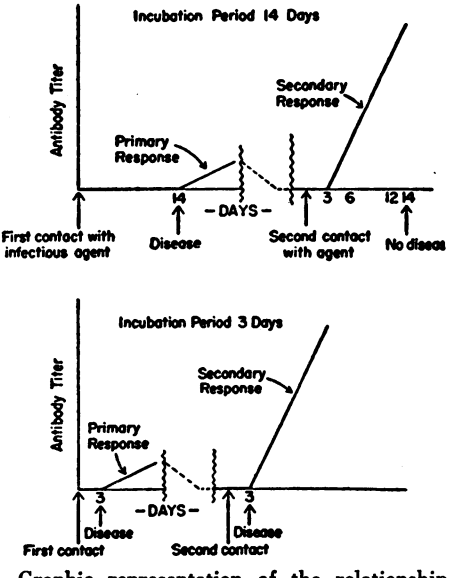
Role of Antibody—There is now general agreement that rarely, if at all, does virus reach the central nervous system other than via the blood stream; and that the presence of virus-neutralizing antibody in the circulating blood effectively intercepts invasion of the central nervous system by virus. Therefore, if immunity to paralytic poliomyelitis depends upon the continued presence of antibody in the circulating blood, then the factors that influence the regularity with which it is possible to induce antibody formation, and to maintain its presence, are of prime importance.

It has long been known that individuals vary in degree of immunologic responsiveness, both to infection and to vaccination; but it is known, also, that a critical factor affecting regularity of vaccination response is the adequacy of the mass of antigen administered or, expressed in practical terms, vaccine potency. It is known, also, that the degree of response to vaccination can be enhanced, and the period of time over which antibody continues to be demonstrable can be extended, by the use of multiple inoculations at suitably spaced intervals.

Role of Immunologic Hyperreactivity—If no more could be said of the mechanism of immunity to poliomyelitis than that paralysis is prevented by circulating antibody, then the answer to the question of duration of immunity, or need for reinoculation, would have to await the outcome of studies over an extended period of time. It is fortunate, perhaps, that a number of unexpected observations have been made,[2] each interesting and significant in itself, but together, of even greater interest for their significance in relation to the question of mechanism of immunity. The indications are not only that antibody is effective in preventing paralysis, if demonstrably present in the bloodstream at the time of exposure to natural infection, but that, under some circumstances, antibody can effectively reappear through the operation of a hyperreactive immunologic mechanism, primed by the earlier immunologic experience, and then later set off by exposure to the living virus in nature. Thus, under circumstances where antibody concentrations, after infection or vaccination, have declined to nondetectable levels, it appears that invasion of the central nervous system is prevented, even though infection of more superficial tissues is not prevented.

It has long been known for other antigens (Figure 1),[3] and has now been demonstrated for poliomyelitis, that the speed and degree of antibody response to vaccination is considerably increased

Figure 1



Graphic representation of the relationship of the secondary antibody response and the incubation period to permanent immunity. (C. M. MacLeod, J. Immunol., 1953.)

Figure 2

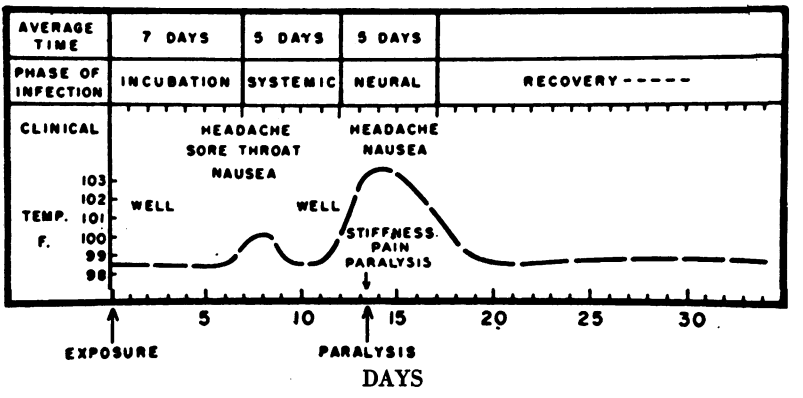
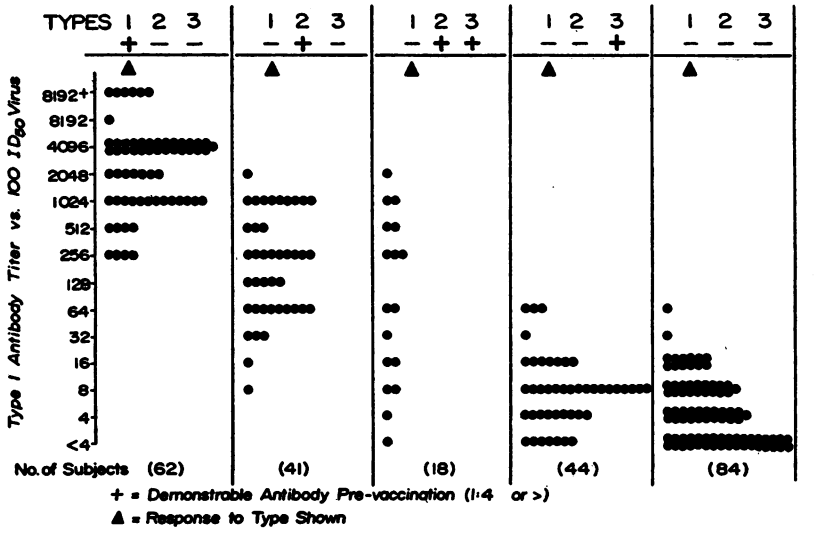


Diagram showing time relations of clinical and pathogenic events in paralytic poliomyelitis infections. Many of the data on which the illustration is based come from studies of the human disease, but quantitative details are supported by experimental studies in other primates. D. Bodian, 3rd Inter. Polio. Conf., 1954.

in persons previously sensitized either by infection or by vaccination; in such persons, antibody rise is evident in from three to four days.[4,5] It has been demonstrated (Figure 2) [6] that an interval of about 7–12 days elapses between the time of entrance of virus into the host and the time when it has multiplied sufficiently to reach the secondary site in the central nervous system. These

Figure 3



Type 1 Antibody Levels after One Dose of Vaccine (Lot 309) in Persons with Different Prevaccination Antibody Patterns

facts, when considered together, indicate the way in which a hyperreactive immunologic mechanism could cause antibody to re-emerge early enough and in sufficient concentration to prevent the virus, that is multiplying superficially, from invading the CNS. That such a mechanism may be operative is suggested by two incidental observations made independently of one another.[2]

Shared Antigens Between Types—The first observation is that the type I antibody response, to the initial dose of vaccine, in persons who prior to vaccination possess type II antibody, is distinctly greater than is the type I response in persons who possess type III antibody, or in persons who have no detectable antibody for any of the three types (Figure 3).[7] It is of further interest that, of the group who have both type II and type III antibody from prior natural infection, only some react to vaccination with a type I response characteristic for those who have had only a prior type II infection, while others react as if they have had a type III infection only. It seems likely that the difference in reactivity between those who have type II antibody *only*, and those who have *both* type II and type III antibody, may be because in some instances the type II antibody is present, not because of a previous type II infection, but because an heterotypic response accompanied a type III infection. All of the data together[2,7] indicate that there is sharing of antigens between types I and II, and between II and III, but rarely, if at all, between I and III.

Crossing of Paralysis-Immunity Between Types—The second observation is related to the frequency of occurrence of paralytic poliomyelitis in persons with different prior experience with the viruses of each type. In recent years it has been observed that approximately 80 per cent of paralytic cases are caused by type I viruses. From comparative serologic studies in persons who have had paralytic infections, it appears that a type I infection, with paralysis, occurs much less often than might be expected by chance alone, in those who have had a prior type II infection, and that paralyses caused by type I viruses occur predominantly in persons who have never had a previous poliovirus infection, and in persons who may have had a prior type III infection. The mechanism for this effect could be based upon the fact that the type II viruses that have been prevalent in recent years possess a small amount of antigenic substance that is characteristic for the type I viruses and, therefore, persons who have had a prior type II infection could conceivably possess type I antibody, residual from the initial heterotypic response, at a level too low to be detected but effective nevertheless, or they possess an immunologic mechanism that was rendered sufficiently hyperreactive to prevent the development of paralysis upon subsequent contact with type I virus.

Indications of Persistence of Immunity Induced by Killed-Virus Vaccine—The possibility that such a mechanism is operative following effective vaccination with a killed-virus vaccine is indicated by two observations. The first is that higher levels of antibody have been observed after natural infection in individuals previously vaccinated as compared with those not vaccinated (or those ineffectively vaccinated).[8] This suggests that previously vaccinated individuals hyperreact immunologically when exposed to natural infection.

The second observation is that there seems to have been no obvious trend toward a greater incidence of paralytic polio, as the interval after vaccination increased, either in the course of the 1954 Field Trial season, when vaccine of poor potency was used, and antibody titers tended to decline to nondetectable levels,[9] or in the subsequent two seasons, especially among those given one dose.

This could be interpreted to mean that once a protective level of antibody was induced by sufficient antigenic stimulation to induce hyperreactivity as well, then, with the disappearance of antibody, the prevention of paralysis is effectively mediated through the operation of the phenomenon of immunologic hyperreactivity.

If this reasoning is sound, then it should follow that vaccination would result in persistent immunity, if there is continued presence of circulating antibody or if there is persistence of immunologic hyperreactivity induced by vaccination. If the character of antibody response to the booster dose, or to subsequent inoculations, is used as a measure of the state of hyperreactivity induced by the previous inoculations, then it would appear that even a single dose of vaccine, of adequate potency, could induce long-lasting immunity, just as does a single infection-experience.[7, 8, 10, 11] Although it appears that this theoretical possibility may be realized in a high proportion of instances,[11] it would not be wise, from the practical viewpoint, to rely upon but one dose of vaccine. Not only is the immunologic effect of two doses, spaced by several weeks, greater than the effect of a double dose given at one time, but the chance failure of a one-dose procedure, due to vaccine of relatively low potency or to a low level of responsiveness of certain individuals, would be minimized by the two-dose procedure. By providing for a third opportunity to establish a primary effect, there is a further safeguard against the chance of failure to induce some degree of immunologic reactivity by vaccination.

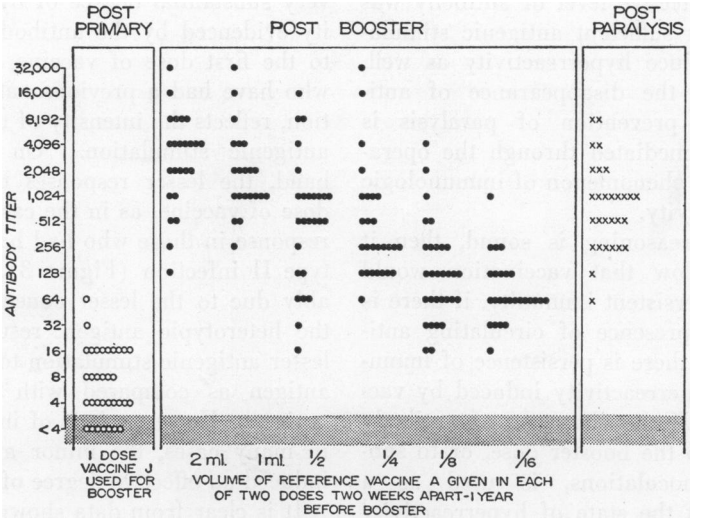
It is now clearly evident that the larger the antigenic mass involved in the primary immunization experience, the greater will be the resultant degree of hyperreactivity.[11] It is of interest that similar effects are observed where hyperreactivity is induced by infection rather than by vaccination.[10] Thus, the very substantial degree of hyperreactivity evidenced by the antibody response to the first dose of vaccine, in persons who have had a previous natural infection, reflects the intensity of the original antigenic stimulation. On the other hand, the lesser responses to the first dose of vaccine, as in the case of type I response in those who had had previous type II infection (Figure 3), are probably due to the lesser concentration of the heterotypic antigen, resulting in a lesser antigenic stimulation to the minor antigen as compared with the major antigen. However, it is of interest that, in many cases, the minor antigen had induced an effective degree of immunity.

It is clear from data shown in Figure 4[11] that a combination of adequate dosage and the lapse of sufficient time can result in immunologic effects that appear to be equal to or greater than that induced by natural infection. With responses of an order of magnitude here illustrated, it would not be unreasonable to expect that the hyperreactive state, induced by the administration of a killed-virus vaccine, would be as effective for immunity as that resulting from a natural infection. The validity of the analysis that has been made and the reasonableness of these expectations must, of course, stand the test of time.

From the immunologic data presented last spring[11] it was concluded that it should be possible to achieve the theoretical ideal, except in those with an agammaglobulinemia-type of defective immunologic mechanism; and, that the practical limitation was merely one of vaccine potency, and the proper use of potent vaccine.

This brings to the fore the question of interpretation of vaccine failures occurring at longer or shorter intervals after completion of a reasonable course of inoculations. Are such failures due to waning of immunity or are such failures due to the combination of min-

Figure 4



Type 1 Antibody Response after "Booster" in Relation to Potency of Vaccine Used for Primary Inoculation and as Compared with Antibody Titer Following Natural Infection

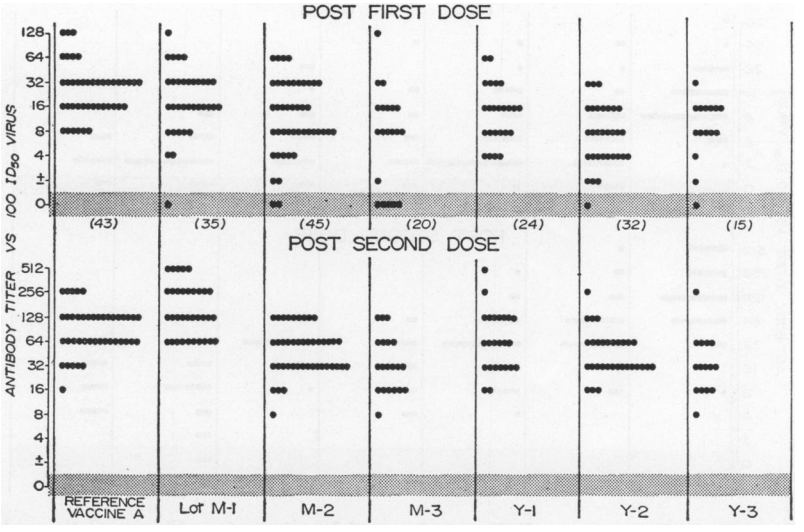
imal level of vaccine potency together with relatively low level of responsiveness in these particular individuals? It will be necessary, of course, to exclude the possibility that an entirely different etiologic agent has been the cause of the paralysis. If cases of paralytic poliomyelitis occur within the first season after completion of immunization, the more reasonable interpretation would be initial failure to respond satisfactorily rather than waning immunity. The effect of initial failure to respond satisfactorily should be expected to be evident in subsequent seasons, with the occurrence of a similar proportion of paralytic cases. However, if, in subsequent seasons, the proportion of cases does not increase significantly, it may then be presumed that immunity does not wane. Particular caution will be required in the interpretation of trends, because of the difficulty of distinguishing the occasional individual who may not have responded initially from one in whom immunity may have waned.

The goal to be achieved is to provide sufficiently potent vaccine, or a sufficient number of inoculations, to satisfy the need of all who have no absolute defect in their immunologic mechanism—that is to say, all except the rare persons with agammaglobulinemia. It appears that this may already be realized with the type II component of the vaccine. It should be possible to do the same with the types I and III components.

Vaccine Administration

From experience gathered thus far, it would appear that the problem of paralytic poliomyelitis can be solved upon the basis of principles already established and through application of our present technical knowledge. However, there are several technical questions that merit some discussion. One of these has to do with the schedule of vaccine administration; particularly, with the effect induced when a third dose of vaccine is given approximately three

Figure 5



Type 1 Antibody Response to Different Lots of Vaccine after First and Second Doses Given Four Weeks Apart in Children Without Prevaccination Antibody to Any Type

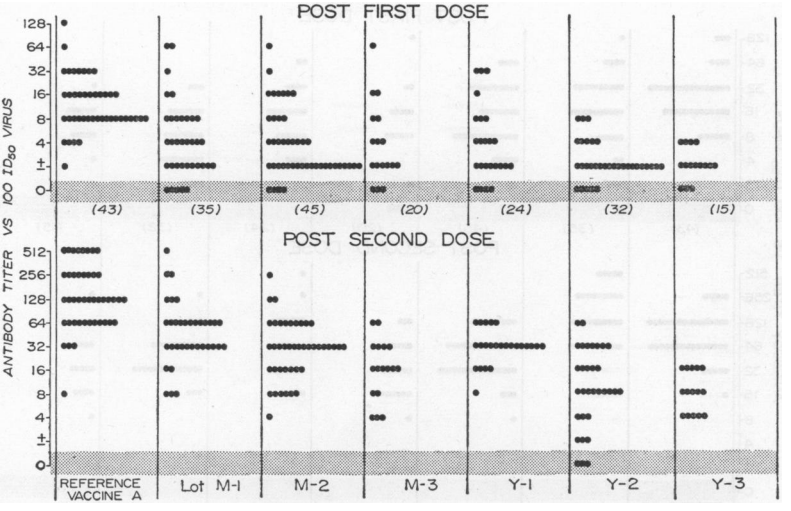
months after a second inoculation. Others concern certain technical problems of vaccine manufacture including the problem of the source of virus for preparation of vaccine.

It has already been shown that much higher levels of antibody are induced if a given amount of virus antigen is administered in divided doses rather than as a single inoculum, and with spacing at intervals that are not too close together. These were the reasons for recommending an interval of from two to six weeks between the first two doses and seven months between the second and third doses. The total length of this period would permit the administration of three doses between one season and the next. Since there are times when it might be desirable to reduce further the interval during which an incompletely vaccinated individual is exposed to risk, the problem needing attention is the determination of the shortest interval that takes full advantage of spaced inoculations without keeping the individual at risk too long while he is undergoing vaccine treatment for immunization.

Effect of Third Dose Approximately Three Months After Second—Studies were undertaken to see what effect would be observed if a third injection was given approximately three months after the second dose. The decision to do this study was made when we were confronted with the question of the advisability of allowing children who had two doses of vaccine to go through the poliomyelitis season under circumstances where it was known, or it was suspected, that two doses may not have induced the formation either of sufficient antibody or of a sufficient degree of immunologic hyperreactivity.

In this study, which involved children in the kindergarten and first grade, two inoculations were given using, for primary immunization, six lots of manufactured vaccine available for general use in 1955 and 1956. For comparison, Reference Vaccine A was also used. The two doses were separated by an interval of four weeks, and each consisted of

Figure 6



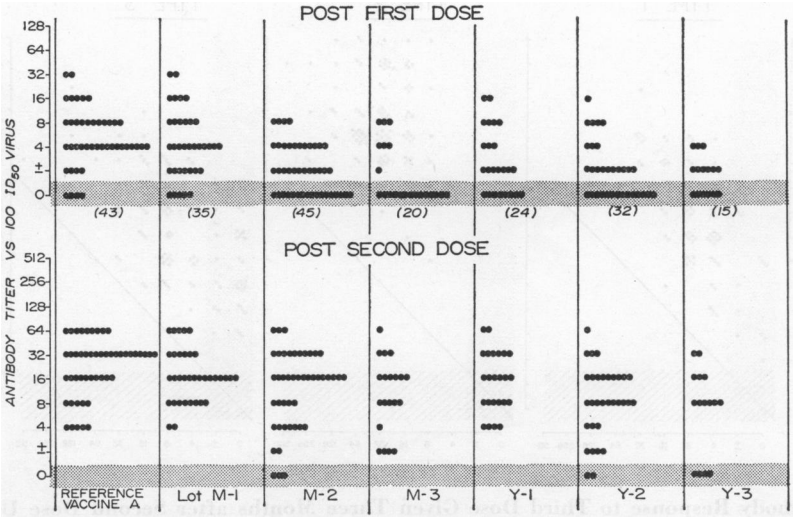
Type 2 Antibody Response to Different Lots of Vaccine after First and Second Doses Given Four Weeks Apart in Children Without Prevaccination Antibody to Any Type

1 ml, administered intramuscularly. Venous blood was drawn at the time of each inoculation, and two weeks after the second. The decision was made, on the basis of the results of the serologic tests, to reinoculate all children who, after the second dose, had no demonstrable antibody for any one of the three types, or in whom antibody levels were relatively low. For the third dose a lot of vaccine, different from the first two, was used. This was done because, under practical circumstances, the third dose would likely be given from a different vaccine lot, while the first two might be from the same lot. For the third dose, the same lot of vaccine was used in all groups; it was one prepared in our laboratory, of known performance in children, and approximately of the same order of potency as Reference Vaccine A. This lot (Vaccine J) was selected to permit comparison with certain other experiments, using this same lot of vaccine to test the effect of dosage and time.[11]

The results of the post-first, and post-second dose responses to the six lots of commercial vaccine under test, as compared with Reference Vaccine A, have been reported recently [11] but the data are presented here again (Figures 5, 6, 7). However, the results of the response to the third dose are reported here for the first time and are shown in Figures 8 and 9. It is clear that after administration of combinations of the particular vaccine lots employed, there was none among the 100 individuals selected for this study who did not develop clearly demonstrable antibody following the third dose which was given approximately three months after the second. However, it is to be noted that there are some in whom the response, at the time of the third dose, could be interpreted as having the characteristics of a primary response, rather than of the booster type; the latter would imply existence of a hyperreactive immunologic state.

Antibody titer in Figure 8 is expressed also as the least amount of serum containing antibody activity. In the system

Figure 7



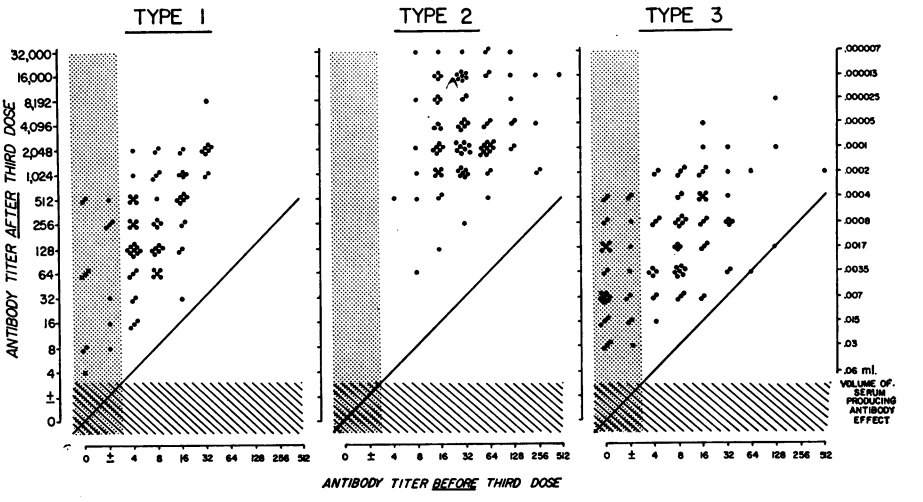
Type 3 Antibody Response to Different Lots of Vaccine After First and Second Doses Given Four Weeks Apart in Children Without Prevaccination Antibody to Any Type

employed to measure antibody in these studies, 0.25 ml of each of a series of dilutions of serum is allowed to react with approximately 100 $TCID_{50}$ of virus. The latter indicates that 100 times more than the minimal amount of virus required to infect 50 per cent of the inoculated tissue culture tubes is used to react with the serum. The virus is added to each dilution of serum beginning with a 1:4 dilution; accordingly, each culture tube containing a 1:4 dilution contains 0.0625 ml of serum together with 100 $TCID_{50}$ of virus. Thus, for antibody to be present at a level of 1:4, there must be sufficient antibody in 0.06 ml of serum to neutralize 100 $TCID_{50}$ of virus.

A single reason to explain the difference in individual reactivity is difficult to give without further study. Some individuals may have responded poorly because they require, for response, greater amounts of antigen than was administered and, in some, the effect could have been due, in part, to the relative shortness of the interval between the second and third doses. The extent to which the latter is a factor cannot be concluded from this study alone. Therefore, it is intended to give the third inoculation, at a longer interval after the second dose, to another comparably treated group of children, and, in that way, compare the relative efficiency of the shorter and longer interval.

Effect of 0.1 ml Inoculations—That the degree of antibody response, both primary and booster, is related to the quantity of antigen administered, was clearly shown in previous studies.[10, 11] This is further illustrated by another study in which 0.1 ml of vaccine had been given intradermally, for the first two doses, and was followed by 1.0 ml, given intramuscularly, for the third dose. This study came about because of the interest of a physician who, in his practice, had used two doses of 0.1 ml each, intradermally, two weeks apart, and who desired to know the effect of this procedure upon antibody response to the vaccine and the procedure he employed. He also wished to know whether or not to use 0.1 ml for the third dose.

Figure 8



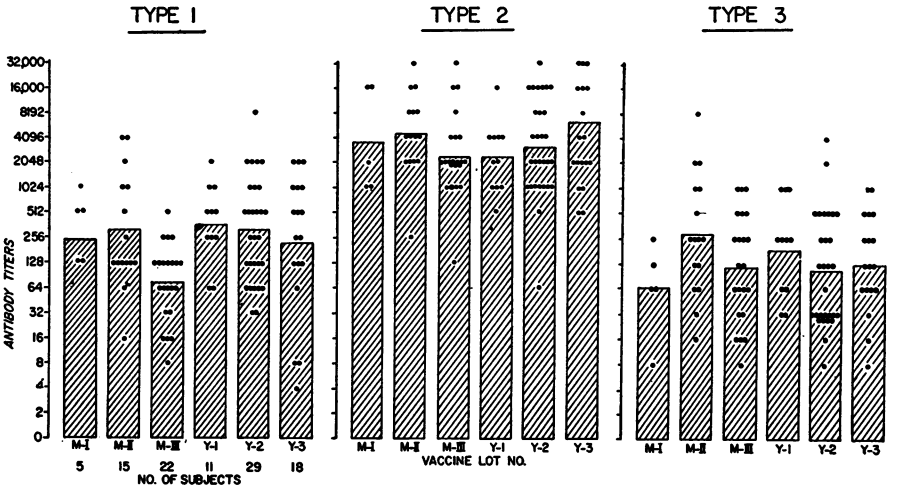
Antibody Response to Third Dose Given Three Months after Second Dose Using 1 ml Intramuscularly for Each Dose—Composite of Results for 100 Subjects in Six Groups Given Different Lots of Commercially Prepared Vaccine for First and Second Dose and Vaccine J for Third Dose

On the basis of the results of previous studies, it was advised that 1.0 ml be used, rather than 0.1 ml, and that venous blood be obtained before and two weeks after to answer the questions concerning the effect of his procedure. The first two doses were given either from one lot or from two lots during the period from October 29, 1955, to February 29, 1956; in almost all instances two weeks apart. The third dose, from a third lot of vaccine, was given to all on May 19, 1956; thus, the interval between the second and third doses varied between three and six months.

Of the 160 children in the study, from whom paired blood samples were available, it was possible to select 91 in whom it could be assumed, with reasonable certainty, that they possessed no antibody for any of the three types before their first dose of vaccine. This selection was based largely on the level of antibody after the second dose. If there had been prevaccination antibody from a prior natural infection, then the titer of antibody for one or more types could be expected to be well above 1:64. Therefore, an analysis was made of pre- and post-third dose antibody levels only in those instances where the pre-third dose titer was 1:64 or less. These results are shown in Figure 10. It is clear from these data that the general level of response was lower, as compared with the experience shown in Figure 8, and that in some instances the response was so poor as to suggest that there had been little or no effect induced by the first two doses.

It is possible, of course, that the differences just noted could have been due to different potencies of the two lots of vaccine used for the third dose in the respective studies. That this is not likely to provide a full explanation for the difference between the data in Figure 8 and Figure 10 is indicated by a comparison of the levels of antibody before administration of the third dose. That the difference is due to the size of the inocu-

Figure 9



Difference in Response to Third Dose Using Vaccine J in Groups Given Different Lots of Commercial Vaccine for First and Second Doses—Third Dose Administered Three Months after Second Dose 1.0 ml i.m. for Each Dose

lum used for the first two doses, and not to chance variation in potency, is suggested by the degree of uniformity (Figure 9) that was demonstrated for six randomly selected lots of commercially prepared vaccine, three of which were the products of the same manufacturer who produced the vaccines used in the study involving the small volume given intradermally.

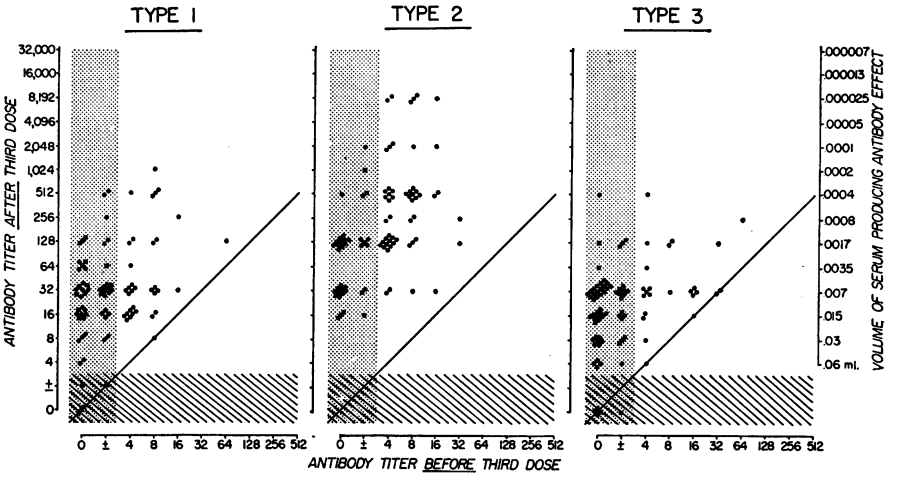
It is clear from the foregoing that the intradermal route does not increase the efficiency of response sufficiently, if at all, to permit reduction in quantity of vaccine administered, without sacrifice of effectiveness for those persons who require more than minimal quantities of vaccine for response.

Technical Problems Related to Vaccine Production

A year ago emphasis was principally on the technical details concerned with the production of a safe vaccine. This year emphasis is on technical details concerned with effectiveness—or potency. To state an objective, a desirable and practicable one would be the achievement of full protection in all, or almost all, after the administration of the first two doses of vaccine. That this can be accomplished is suggested by the serologic effects induced with all three components of Reference Vaccine A [11] and by the experience with the type II component of commercially prepared vaccine (Figures 8, 10). It would seem likely that an analysis of the factors responsible for the difference in effects between the three types could lead to the introduction of appropriate modifications. Several questions related to the problem of potency and to other problems of vaccine preparation and testing, will be considered individually.

Strain Composition in Relation to Vaccine Potency—The question of strain differences has been under study for some time, and from various viewpoints. The problem becomes more interesting as more studies are done. This is a time-

Figure 10



Antibody Response to Third Dose—0.1 ml Intradermally for First and Second Doses Followed by 1.0 ml Intramuscularly for Third Dose. Interval of Two Weeks Between First and Second and Three to Six Months Between Second and Third Doses. Commercial Vaccine for All Three Doses

consuming activity because of the large numbers of animals needed and because of the many variables requiring study; the work has been interrupted many times because of the pressure of more urgent questions.

It is now clear, from accumulated experience, that a change of the type I strain, to one less virulent than Mahoney, is not essential for safety. Such a change would not necessarily improve the vaccine; but, a change of strain to one that is more potent antigenically could aid in accomplishing this objective, if a suitable strain could be found. The pattern of activity of the type II antigen suggests the goal. Regardless of strain employed, attention still must be given to the quantitative factors of importance in providing and retaining an adequate antigenic mass, and to the production problems related thereto.

Production Factors Related to Antigenic Potency—(1) The first factor of importance is growth of virus in a tissue culture system that will yield, in a unit volume of fluid, a sufficient concentration of antigen. The critical factor here is the number of susceptible cells available. The yield is affected also by the treatment of the tissue before cultivation, by the medium employed, and by the stage at which virus is introduced. Later in this discussion, consideration will be given to a substitute for monkey-kidney tissue for propagation of virus.

(2) The second factor of importance is filtration. This is crucial for safety and should be carried out in a way that will result in little or no loss of antigenic substance. This problem has been solved on a manufacturing scale by the uniform adoption of filtration methods employing the Seitz-type filter and by balancing suitably the volume of fluid processed against the tendency of the filter to adsorb and to release virus. Now that fritted glass filters are no longer used for the critical filtration step, it is probably not necessary to employ the double filtration which was introduced as a requirement a year ago when fritted glass filters were also in use.

(3) The third factor concerns the

possibility of destruction of antigenicity by overinactivation or by the effect of a preservative for sterility. However, there is a wide margin between the point of destruction of infectivity and loss of antigenic activity at 37° C, 1:4,000 formalin, and pH 7.0.

(4) Finally, the effect of processing on potency can be tested by comparing the immunizing activity of the manufactured vaccine with that of a reference standard of known performance in human subjects. Refinements in the degree of precision of performance of comparative tests for antigenicity will, in time, permit greater refinement in the control of factors that contribute to variation in potency.

Technical Aspects of Safety Testing—Dependence upon the safety test for confidence in the safety of vaccine released for use is now relegated to its proper role, with increased attention to those factors in manufacturing that are of importance in the elimination of infective virus.[1] The uniformity in consistency of production of vaccine, free of living virus, by the time it is brought to the stage of testing, has reduced the problem of safety testing from one concerned with sensitivity [12] to one in which the technical performance of the test constitutes the remaining problem.

Reference here is to the problem caused by viruses present in the monkey-kidney tissue used in cultures for safety-testing.[18] Sometimes these agents render cultures unsatisfactory for observations for safety-test purposes, and the test then has to be repeated. At other times, a test can be completed, but a virus that is not poliovirus is found in one or more cultures. Although a positive identification can now be made, in most cases, and it can be said, with assurance, that the agent that emerged was present in the culture, and was not introduced with the vaccine under test, such findings demand so much work to prove the origin of these viruses, or to exclude their connection with the vaccine under test, that there is sufficient reason to want to eliminate this problem, if it is possible to do so. This hazard has been one of the principal causes of delays in release of vaccine in accordance with a preplanned schedule. This problem could be solved by the adoption, for safety-test purposes, of a cell that grows in continuous culture, and that provides at least the same degree of sensitivity for detection of traces of poliomyelitis virus as do cultures of monkey-kidney tissue. A number of such cell-lines are now available and it is merely a matter of testing each and adapting one to the problem at hand. Such studies are now in progress.

The Question of "Duplicate" Safety-Testing in the Light of the Record of Consistency in Manufacturing—On the basis of experience now accumulated, it should be in order to reappraise the value, for safety, of duplicate testing by the manufacturer and by the National Institutes of Health. It is now agreed that a large factor for safety is provided by the record of consistency of the manufacturing process. The critical tests of the manufacturing process are those performed upon samples removed in the course of inactivation of the monovalent pools, particularly those samples removed from the reaction mixture close to the earliest time when the tests should be negative if the process was proceeding satisfactorily. Therefore, there is a need to reevaluate duplicate testing of the trivalent vaccine, which is a test made at a stage of manufacture well beyond the point at which the critical tests are shown to be consistently negative. If duplicate testing is not needed, valuable facilities and personnel, as well as financial resources, could be directed into more constructive activities.

To illustrate the degree to which consistency in manufacturing has been achieved, one manufacturer has very kindly provided information that 107,-

699,000 ml of vaccine material has been processed consecutively; of this, 611,000 ml were tested in tissue culture, at various stages, and 11,000 ml were tested in monkeys, with no evidence of infectious virus.

For the same reasons, the value of the safety test in monkeys might be reappraised. It might be said, however, that the test in monkeys, which is made on trivalent vaccine in filled containers, provides reassurance against contamination in pooling and in filling of vials. If this be so, then such tests can be made in tissue culture. It might also be said that the test in monkeys is a control against the contingency of the presence of an agent that may have originated in the monkeys that contributed the kidneys for the cultures used for propagation of virus for vaccine. In reply to the latter, the suggestion might be made that this question could become of less importance if a substitute is found, in the form of a continuously-propagating cell-line, instead of monkey-kidney cell cultures, to be used for producing virus for vaccine. There is reason to believe that this may soon be possible.

A Continuously Propagating Cell-Line as a Source of Virus for Vaccine—In cultures of trypsinized monkey-heart tissue, being observed for other purposes, Miss Elsie Ward noticed a cluster of cells, different from the rest. It was found that this cell could be maintained in continuous cultivation, in relatively simple medium and that poliomyelitis viruses multiplied as well in these cells as they do in monkey-kidney cultures. Filtration of virus, inactivation, and antigenic potency of vaccine so prepared, appear to be satisfactory.

Since this cell is self-propagating, as are certain cells that are known to have originated from neoplastic tissue, an objection to the use of a cell of this kind might be that it may have unknown neoplastic properties. However, it is possible to test directly for the capacity to induce neoplasia since the cell can be tested in the donor species. Thus far we have seen no evidence of neoplasia after inoculation of monkeys by a variety of routes. That this cell is of simian and not of human origin provides further reassurance regarding unknown neoplastic factors that might be of concern in using continuous cell lines of human origin. Furthermore, fluids derived from such cultures would be treated with formaldehyde in a way that tends to destroy agents involved in living processes; and it is possible to make direct tests to establish this for any property possessed by these cells, or fluids derived from them.

For want of more satisfactory nomenclature, the cell is referred to as monkey-heart cell. The cell is epithelial-like in character and on a glass surface forms continuous sheets, and is readily maintained in continuous culture in the presence of Mixture 199 containing 10 per cent calf serum. Multiplication is at the rate of approximately six- to eightfold in a week. This degree of increase can be enhanced by the use of greater concentrations of serum. At the end of one week the cells are removed from the glass surface by gentle treatment with trypsin. They are separated from the trypsin solution, resuspended in fresh medium, and inoculated into new flasks, either for maintenance of the cell-line or for preparation of flasks or tubes for virus inoculation.

At the time that virus is inoculated, medium is removed, the inside of the container is washed to remove the calf serum medium, and Mixture 199 alone is introduced. Under these circumstances, as is true also with cultures of trypsinized monkey-kidney cells, virus is released into a medium that is essentially protein-free, except for the protein introduced by cell rupture. Virus yields are at least as good as they are from

monkey-kidney tissue cultures; the optimal dilution of virus to be used, the time of inoculation and of harvesting are, at present, being investigated. The characteristic cellular changes are somewhat different from those seen in monkey-kidney cultures, both macroscopically and microscopically; however, ultimately, the cell-sheet is destroyed. It has been possible to adapt these cells for usefulness in many of the ways in which monkey-kidney cells are used, both for studies with poliomyelitis viruses and with a number of other viruses as well.

The availability of a cell such as the monkey-heart cell, would help circumvent certain technical problems to which reference has been made. Moreover, it would reduce dependence upon a source of the raw materials from which vaccines are made.

Recommendations

Children in 1954 Field Trial—Recently, attention was called [14] to the desirability of revaccinating all children who participated in the 1954 Field Trial. This recommendation was made because much of the vaccine used in 1954 was of poor antigenicity and the intervals between inoculations were too short. It is likely that some of these children have not been revaccinated as suggested; there are then some who have a false sense of security.

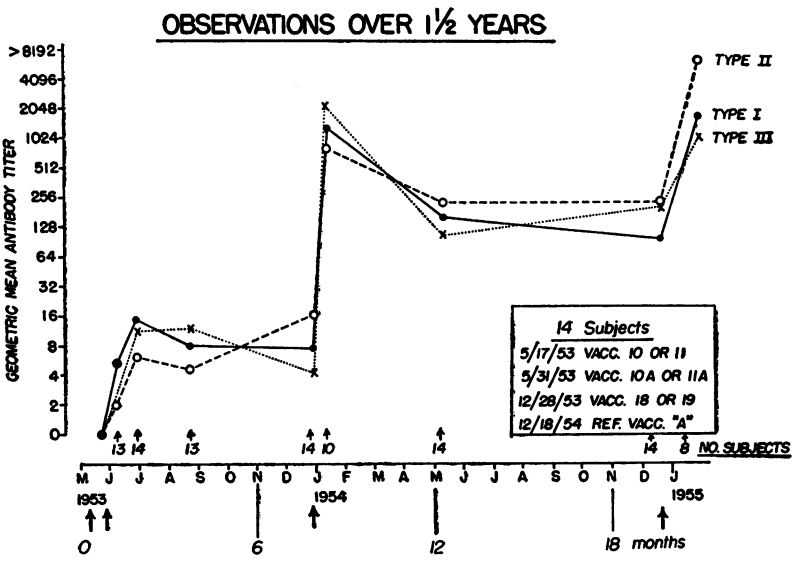
Children Given 0.1 ml Intradermally—It would be advisable to follow the same principles and suggestions as were made for the Field Trial group, in supplementing the immunization of children who, in 1955 or 1956, may have received smaller quantities of vaccine than is believed to be desirable, i.e., those given 0.1 ml rather than 1.0 ml, in any variation that resulted in the administration of less than 3.0 ml of vaccine at properly spaced intervals.

Too Short Intervals Between Inoculations—There are instances in which children received three doses within an interval of five weeks, and other instances of treatment on some other schedule in which less than two weeks had elapsed between the first two doses, or less than seven months between the second and third dose. Under such circumstances, it would be well to consider additional inoculations, thereby giving the benefit of any doubt to the individual who was so treated. It may well be that the additional inoculations are not necessary, but in the absence of a readily available test for immunity, doubt can be resolved by additional treatment before the ensuing polio season.

Test for Immunity—As for a readily available test for immunity, one has been developed that has been useful.[11] This test,[15] using a drop of finger-blood, is dependent upon the demonstration that antibody, above a certain level, is induced, after administration of the third dose. If this occurs it is presumed that a sufficiently hyperreactive state exists. The level selected in the early tests, in part because this was the limit of sensitivity of the technic employed, was an antibody titer of 1:64. However, this level is excessive, and a level of 1:16 can now be measured, using the same sized drop of capillary blood, and is more reasonable, even though, this, too, is undoubtedly well inside the limit of the range of effectiveness. A summary of findings, after application of this test, has been reported elsewhere,[11] and leads to the conclusion that this test will be more useful for research purposes, or for survey in selected groups, than for general application. As in the case of the Schick test, it would seem to be less bother to reinoculate, than to do a test for immunity.

Need for Further Inoculations—There has not yet been time to accumulate sufficient experience on protection against paralytic polio after three doses

Figure 11



Antibody Persistence after Primary and Secondary Antigenic Stimulation in Children with No Preantibody to Any Type

of vaccine, as presently prepared and administered. Until this is known and some judgment made upon the basis of such knowledge, it is difficult to make a firm recommendation in regard to the question of further inoculations. It would seem, from the theoretical discussion in this report, that this should not be necessary, but the practical limitations—at least at the beginning of introduction of this procedure—have also been noted. It is to be expected that just as vaccine is prepared with a high degree of consistency for safety, a similar degree in consistency for effectiveness will ultimately be achieved, with the inoculation schedule now in use.

From scattered and incomplete reports it seems that the effect of three properly spaced inoculations is approximating the ultimate goal. The effect of a fourth dose was tested in groups of children in our early studies and a chart (Figure 11) previously published [10] is reproduced here to illustrate the effect observed. The problem of reinoculation is not in relation to the large majority of individuals, of which this small group is representative, but the problem is in relation to the rare individual for whom the amount of antigen administered was not sufficient to induce the minimal effect necessary for immunity to paralysis.

Age Groups to Be Vaccinated—From a consideration of many factors that might be applied for the control of paralytic polio in the shortest possible time, it would seem that three injections of 1 ml each should be given to all potentially susceptible individuals. A potentially susceptible individual is one within the age groups in which polio has occurred in the country in question. In the United States, paralytic polio has occurred in persons in the sixth and seven decades of life, although such cases are rare. However, it is not unusual for persons in the third, fourth, and fifth decades to become severely stricken. In fact, in recent

years, approximately one-fourth of the total incidence has been in persons in this age group. This group will be the most difficult to vaccinate because they are not part of an organized program of preventive medicine as are children, particularly those who are brought into life under the influence of modern pediatric practice.

Polio Vaccination in Pediatric Practice—It would seem that vaccination against poliomyelitis should be added to the procedures now employed for the prevention of diphtheria, tetanus, and pertussis. Administration of the polio virus antigens at the same time as the others should, theoretically, not affect adversely the response to either. Such studies are being done by a number of groups and are soon to be reported. In some studies, polio vaccine and the vaccine for diptheria, pertussis, and tetanus have been combined. The striking difference in local and systemic reactions induced by the two vaccines is noteworthy. To keep the issue clear, most physicians perfer to administer the two vaccines either at different times or into separate sites.

The schedule for pediatric use might well begin by vaccination of the mother, preferably before her first pregnancy. In women vaccinated against polio in childhood or early adulthood, a single dose of vaccine before first pregnancy will markedly enhance antibody levels and confer upon the newborn a high level of passive immunity, effective for the first several months of life. Subsequent active immunization will provide the longer-term protection. At present, it is recommended for all that three doses of 1 ml each be given with an interval of from two to six weeks or longer between the first two doses, and seven months or longer between the second and third doses. If the first two doses are given in the spring and it is desired to administer a third dose before the ensuing polio season, and therefore within an interval of less than seven months since the second dose, this can be done; for further assurance, however, an interval of seven months or longer should elapse before the administration of another dose prior to the following season.

Whether or not it will be necesary to reinoculate later in childhood, or at the age of entrance into school, or later, is a question that can be answered more readily when the degree of disparity that may exist between theory and practice becomes more clearly apparent.

Summary

The question of degree and duration of vaccine effectiveness has been considered by directing attention to the mechanism of immunity to paralytic poliomyelitis and to the factors that are of importance for the preparation of effective vaccine, and for effective use. It appears that immunity to paralysis is mediated either through the presence of antibody in the circulating blood, or through the rapid reappearance of antibody triggered by exposure of a hyperreactive immunologic mechanism. A summary of recommendations has been made on the basis of this concept, together with a consideration of available data in support of it.

In relation to the question of vaccine effectiveness, it has been suggested that a realizable goal is the achievement of a level of potency, such that two doses will induce the desired effect. This has been achieved, under laboratory conditions; under manufacturing conditions this seems to be true for the type II component. When this effect is achieved, it would still be desirable to continue the administration of three doses to provide the extra margin of assurance to overcome variation in response among individuals. Technical means whereby this might be accomplished have been suggested, and other

technical modifications that would facilitate vaccine production and testing have been discussed. Most significant of these would be the elimination of the need for monkeys either for the production of virus for vaccine or for testing. That this can be accomplished has been suggested by reference to work in progress.

It would appear from this review that responsibility for the problem of eliminating paralytic poliomyelitis rests with each individual for whom there is a need for vaccine, either for himself or for those for whom he is responsible; this responsibility is shared by those who are in a position to bring this knowledge to him and to help him avail himself of the necessary treatment. Little need be said about this other than to emphasize that the indications provided in this review suggest that there need be little, if any, paralytic poliomyelitis in the United States in 1957 if all who are potentially susceptible are treated with vaccine that is now available.

REFERENCES

1. Salk, Jonas E. Poliomyelitis Vaccine in the Fall of 1955. A.J.P.H. 46, 1:1, 1956.
2. ————. A Concept of the Mechanism of Immunity for Preventing Paralysis in Poliomyelitis. Ann. New York Acad. Sc. 61, 4:1023, 1955.
3. MacLeod, Colin. Relation of the Incubation Period and the Secondary Immue Response to Lasting Immunity to Infectious Diseases. J. Immunol. 70:421, 1953.
4. Salk, Jonas E., et al. Studies in Human Subjects on Active Immunization Against Poliomyelitis. II. A Practical Means for Inducing and Maintaining Antibody Formation. A.J.P.H. 44, 8:994, 1954.
5. ————. Present Status of the Problem of Vaccination Against Poliomyelitis. A.J.P.H. 45, 3:285, 1955.
6. Bodian, David. Poliomyelitis. Papers and Discussions Presented at the Third International Poliomyelitis Congress, Discussion Panel. Philadelphia, Pa.: Lippincott, 1955, p. 200.
7. Salk, Jonas E. Requirements for Persistent Immunity to Poliomyelitis. Am. J. M. Sc. 232, 4:369, 1956.
8. ————. Vaccination Against Paralytic Poliomyelitis—Performance and Prospects. A.J.P.H. 45, 5:575, 1955.
9. Francis, Thomas Jr., et al. An Evaluation of 1954 Field Trials of Poliomyelitis Vaccine (Summary Report). A.J.P.H. 45:Part 2 (May), 1955.
10. Salk, Jonas E. Considerations in the Preparation and Use of Poliomyelitis Virus Vaccine. J.A.M.A. 158:1239, 1955.
11. ————. Influence of Antigenic Potency on Degree and Duration of Effectiveness of Poliomyelitis Vaccine. J.A.M.A. 162, 16:1451 (Dec. 15), 1956.
12. Cornfield, Jerome, et al. Some Statistical Aspects of Safety Testing for the Salk Poliomyelitis Vaccine. Pub. Health Rep. 71, 10:1045, 1956.
13. Hull, Robert N., et al. Investigations of New Viral Agents Recovered from Tissue Cultures of Monkey Kidney Cells. I. The Origin and Properties of Cytopathogenic Agents S.V. 1, 2, 4, 5, 6, 11, 12, and 15. Am. J. Hyg. 63:204, 1956.
14. Salk, Jonas E. Reinoculation of Poliomyelitis Vaccine. J.A.M.A. 161, 4:392, 1956.
15. Salk, Jonas E., and Laurent, Angela. To be published.