Antigenic Activity of Poliomyelitis Vaccines Undergoing Field Test

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So many questions have been asked regarding methods of testing polio vaccine for safety and for antigenicity that this report of Dr. Salk's studies will be specially welcome.

Investigators in the field of poliomyelitis appear to be divided between two theoretical approaches to the practical question of immunization of man against this disease. There are those who believe that it may be possible to induce and maintain immunity throughout life with a vaccine consisting of virus in a noninfectious form. Others are of the opinion that lifelong immunity can best be provided through the use of a living attenuated virus for each of the three immunologic types and administered, preferably, by a natural route. If this is not a precise expression of the opinions of all whose predominant orientation is one way or the other, we believe it does convey the essential features of the two points of view and there are reasonable reasons for believing that either may lead to the solution of the practical problem of immunization of man.

We have accepted the assumption that immunity is mediated principally, or entirely, through the action of antibody. It might then be expected, in accordance with well established immunologic principles, that the presence of antibody either in the circulating blood or within fluids bathing neural tissue—or the existence of a hyperreactive state of the antibody forming system, resulting from either natural or artificial immunization—might provide the modus operandi for effective immunity. The question then becomes whether or not a noninfectious vaccine can produce these effects, or do these effects result exclusively from contact with living virus?

This question has been answered in part. It is now amply evident that the injection of noninfectious virus can simulate at least some of the effects resulting from infection with the living virus. The question that now remains is whether or not the kind of immunity that accompanies the serologic response to infection with living virus is the same or different from that which results from the injection of a noninfectious antigen. While it is true that the immune response induced by the infectious process results from multiplication of the living virus, the immune response to a noninfectious virus requires the administration of a sufficient quantity of effective antigen, given in such a way that the

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The substance of this report is abstracted, in part, from a paper, under the title "Studies on Non-Infectious Poliomyelitis Virus Vaccines," presented at the Third International Poliomyelitis Conference held in Rome, Italy, September 8, 1954.

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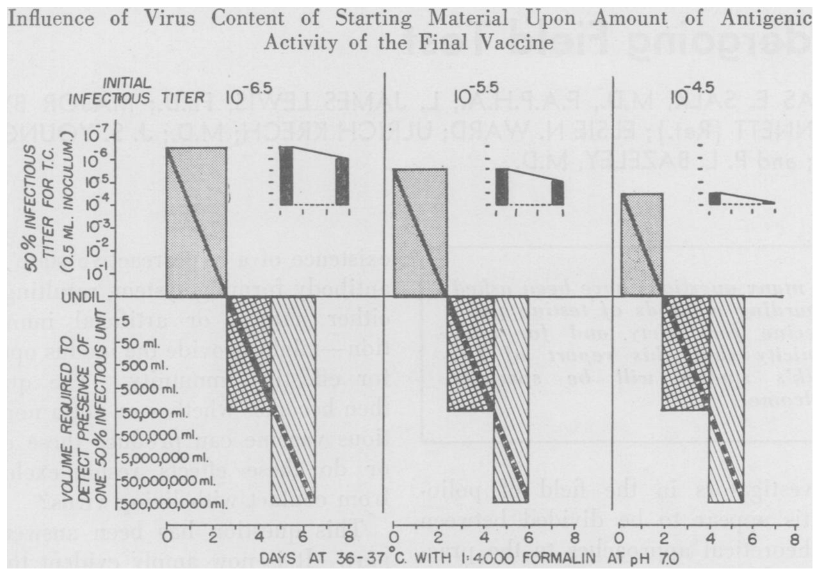
Figure 1

Dissociation of Infectious and Antigenic Activity of Poliomyelitis Virus in the Course of Converting 50 Liters of Tissue Culture Fluid into Vaccine



sequence of events, serologically speaking, that follows infection is reproduced by the noninfectious antigen. Work in our laboratory on attempts to develop an attenuated living-virus vaccine was continued up to the time when it was observed in human subjects that injection of a noninfectious virus preparation could simulate the serologic response of infection. Even though the actual determination as to whether or not a noninfectious vaccine could prevent paralysis in children was yet to be made, our studies on the development of an attenuated virus vaccine have been postponed.

The substance of this paper deals with the presentation of early data on certain characteristics of the noninfectious vaccine preparations employed in the large-scale tests in man initiated in the spring of 1954.

Principle Underlying Method Employed for Destruction of Virus Infectivity—In the course of developing methods for preparing noninfectious vaccines it has become apparent that virus concentration is the most critical factor for the preparation of a potent antigen. An attempt is made to illustrate this graphically in Figure 1. In this figure are shown three examples of tissue culture fluids possessing virus titers of $10^{-6.5}$, $10^{-5.5}$, and $10^{-4.5}$, respectively. As may be seen, with 1:4,000 formalin at 35–37° C. and at pH 7.0, virus infectivity appears to decline at a constant rate. During the first two and one-half days infectivity is readily measurable by titration. At two and one-half days to three days virus can be detected by inoculation into tissue culture tubes of 0.5 ml. samples of undiluted fluid. At later intervals the size of the sampling would have to be increased for the detection of residual virus. As the time of inactivation is prolonged, the size of the sample required has to be increased logarithmically. Beyond a certain point in time, the sample required for detecting residual virus activity would exceed the

Figure 2

Infectivity Titer for Tissue Culture of the Three Strain Components of Each of the 18 Lots of Poliomyelitis Virus Vaccine Used in Field Studies in the Summer of 1954

Arranged in Descending Order of Titer to Show Degree of Consistency as Well as the Degree of Variation Among the Different Batches of Virus of Each Type

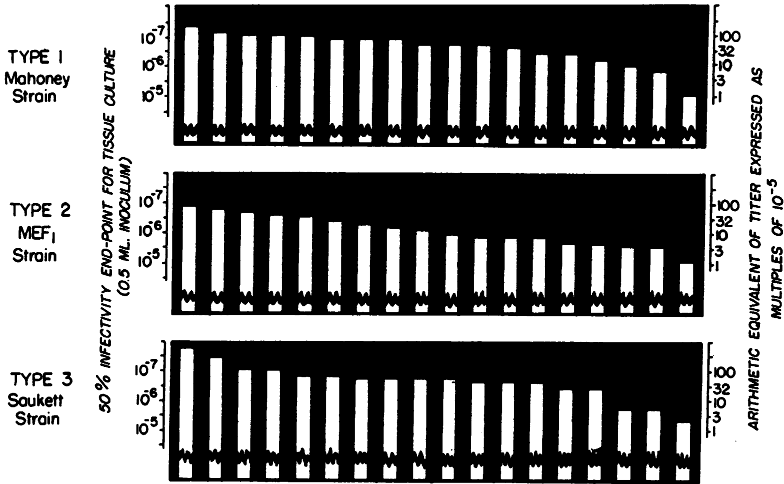
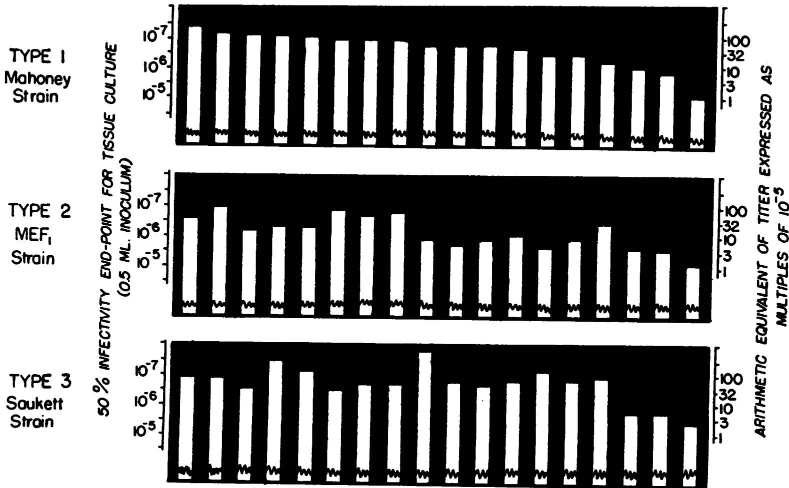


Figure 3

Arranged to Show Variation in Titer of Different Components of Each Batch of Vaccine



volume of fluid being converted into vaccine. Thus, if the chemical treatment is allowed to continue further, there is little likelihood, indeed, that any infectious particles would be present within the finite contents of a vessel in which the reaction proceeds in the manner illustrated in Figure 1. Effects different

from those here described might be produced if precautions are not taken against the introduction of virus by contamination, or if virus is protected within tissue particles, or adsorbed upon a precipitate, or possibly if other strains of virus or other media are employed.

After virus infectivity is destroyed, it is then possible to measure the antigenic effect of the noninfectious virus. In this way it has been found, as could have been assumed a priori, that there exists a minimal quantity of noninfectious antigen that is required to effect an immune response. If, for the moment, we consider that multiple inoculations into monkeys of vaccine produced from a fluid possessing an infectious titer of 10^{-4} will be ineffective antigenically, then we may indicate schematically the relative potencies of vaccines prepared from fluids possessing infectious titers of $10^{-6.5}$, $10^{-5.5}$, and $10^{-4.5}$ by the solid black columns shown in Figure 1. If treatment with formaldehyde is allowed to proceed longer than the time required to destroy infectivity, antigenicity will then decline at a rate shown by the slope of the line connecting each pair of solid black bars. Thus, it may be seen from a comparison of the slopes of the two lines, one showing loss of infectivity and the other showing loss of antigenicity, that the infectious and antigenic properties of the virus can be dissociated readily if the concentration of virus in the fluid used for preparation of the vaccine is well above a critical level. Starting with fluids rich in virus, the margin of safety between the point of destruction of infectivity and of significant loss of antigenicity is such as to make it difficult to destroy antigenicity completely unless a prolonged or a drastic treatment is used for destroying virus infectivity.

Data on the Preparation of Vaccine for Large-Scale Tests in Man—We would like now to present some data derived from the application of these principles for the preparation of a quantity of vaccine being used in field studies initiated in the spring of 1954. The purpose of these studies is to determine whether or not paralysis in children can be prevented under natural conditions of exposure. In these studies 18 different lots of vaccine, each of approximately 100–150 liters, were prepared in a similar manner. The kinds of analyses to be presented here will furnish information, when all of the correlations are completed, concerning the minimal requirements for a vaccine that can be expected to induce antibody formation in man and do so as consistently as is possible within the normal limits of biologic variation. Such in-

Table 1—Mean of Infectious Titers Before and After Filtration of Tissue Culture Fluids Containing Each of the Three Types of Poliomyelities Virus Used in the Preparation of Vaccine for Field Tests in the Summer of 1954

	Type 1 Mahoney		Type 2 MEF-1		Type 3 Saukett	
Vaccine Lots	Pre-Filt.	Post-Filt.	Pre-Filt.	Post-Filt.	Pre-Filt.	Post-Filt.
A-1 - A-9	6.83	6.92	6.21	6.10	7.16	7.06
B-1 - B-9	6.72	6.39	6.42	6.31	6.46	6.32
Average	6.77	6.71	6.32	6.21	6.81	6.69

Titers are expressed as the negative log of the 50 per cent infectious end point for tissue cultures using 0.5 ml. inoculum in roller tubes prepared with trypsinized monkey kidney cell suspension.

Figure 4—Rates of Destruction of Infectivity of Type 1 Component (Mahoney Strain) for Each of 18 Different Lots of Poliomyelitis Virus Vaccine Used in Field Studies in the Summer of 1954

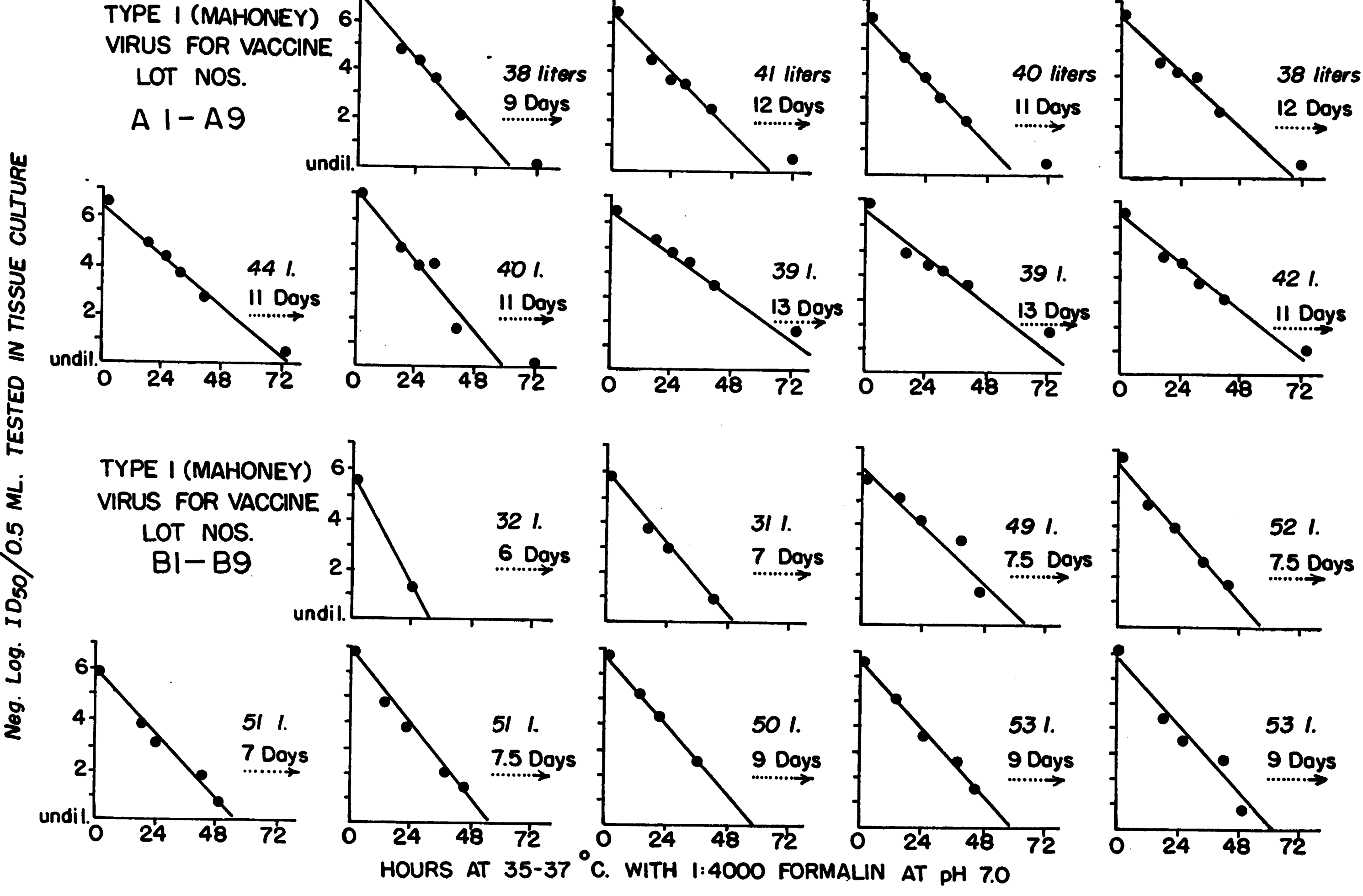
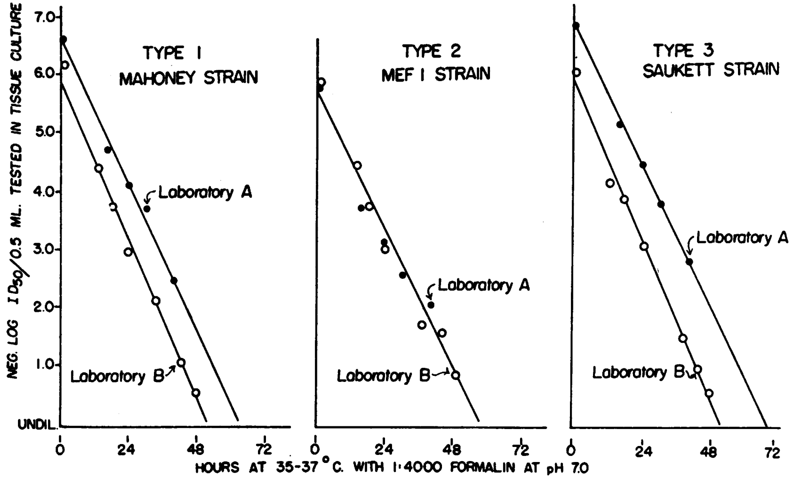


Figure 5

Composite of Data on Rates of Destruction of Infectivity of Types 1, 2, and 3 Virus for 13 Lots of Vaccine Prepared by One Manufacturer and 20 Lots Prepared by Another

Points Indicate Mean of Virus Titers at Intervals After Addition of Formalin to Warmed Fluids



formation, in turn, will then be correlated with the experience to be derived, in the course of this first season of exposure to the natural disease, to furnish the basis upon which may be established the minimal requirements for inducing a level of antibody that might be associated with protection against paralysis.

The degree of variation or, conversely, the degree of consistency, in the initial infectious titers of the fluids containing Types 1, 2, and 3 virus that were converted into the 18 lots of vaccine are shown in Figure 2. Each virus type is considered separately and the different batches of the three respective strains are arranged in the order of their infectious titer. As is shown in Figure 3, in which the three strain components of each lot of vaccine are placed in relation to one another, any one of the 18 lots of vaccine may have been composed of strain pools that differed widely in their rank on the scale shown in Figure 2.

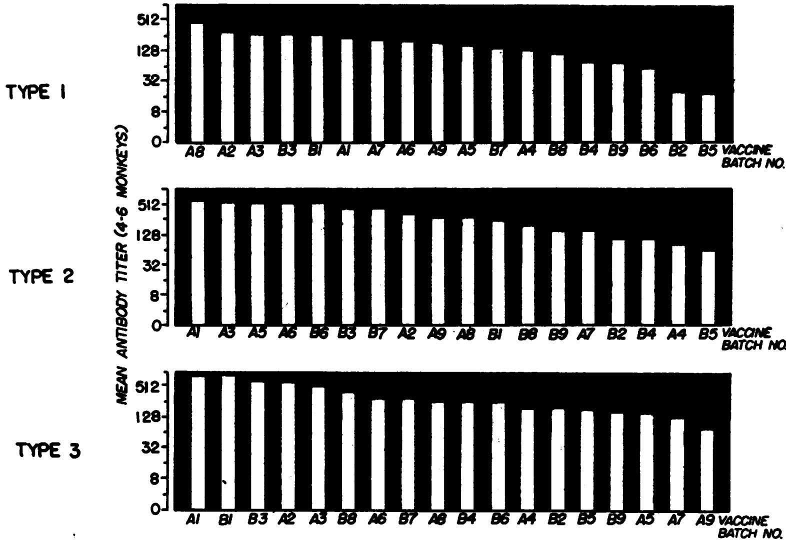
These virus-containing fluids, prior to treatment with formaldehyde, were filtered to remove not only bacteria or other organisms, but all tissue particles or precipitate that might have been present. Table 1 shows the mean titer of the 18 strain pools of each type before and after filtration. These data indicate that, by and large, little or no virus activity was lost by the methods used for filtration, although in some of the earliest batches filtration losses were encountered.

Figure 4 contains a summary of data on inactivation of the Mahoney strain representing the Type 1 component of each of the 18 lots of vaccine. In addition to showing the data upon which the rate of inactivation was ascertained and the point at which virus activity could no longer be measured, there is also indicated the extent to which treatment was continued before the effect of the formaldehyde was interrupted, either by reducing the temperature to 1–4° C., or by neutralizing its effect by treatment

Figure 6

Antigenic Capacity for Monkeys of Each of Three Components in 18 Different Batches of Poliomyelitis Vaccine Used in the Field Studies in the Summer of 1954

Three Doses—One ml. Each, I.M.—One Week Apart, Bled One Week After Last Dose



with sodium bisulphite. The duration of treatment was determined by the data indicating the rate of destruction of infectivity and was prolonged more than necessary beyond the minimal time required for destruction of infectivity or for optimal retention of antigenicity. Similar information is available for the Types 2 and 3 component of each lot of vaccine. A composite showing rates of destruction of infectivity of the three different virus types is shown in Figure 5.

When the vaccines so prepared were inoculated into monkeys to determine whether or not they possessed an arbitrarily established minimum antigenic potency, differences between batches were observed again. Figure 6 shows the geometric mean level of antibody produced in groups of from four to six monkeys after the last of three weekly injections of 1 ml. each given intramuscularly. The heights of the columns indicate the mean titer of antibody observed. The data, arranged in descending order for each type separately, represent the results of the experience in preparing 54 different batches of monovalent vaccine for the three separate immunologic entities and indicate that some of the vaccines were more potent, some were less potent, and that the relationships varied for the different types in the different batches of vaccine.

An examination of similar data (Figure 7) on antibody response one week after the second, rather than the third dose of vaccine, in the same group of monkeys reveals, especially at the extremes, a somewhat greater degree of variation among the batches. This is to be expected, since the third dose raises the response which may have been poor after the second dose, and in this way tends to reduce the difference between vaccines. Although there are changes in relative position among the vaccines

Figure 7

Antigenic Capacity for Monkeys of Each of Three Components in 18 Different Batches of Poliomyelitis Vaccine Used in the Field Studies in the Summer of 1954

Two Doses—One ml. Each, I.M.—One Week Apart, Bled One Week After Second Dose

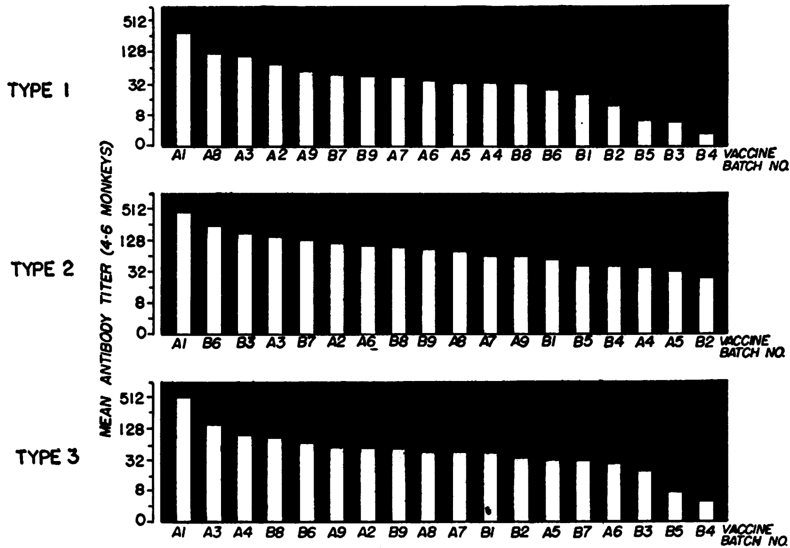
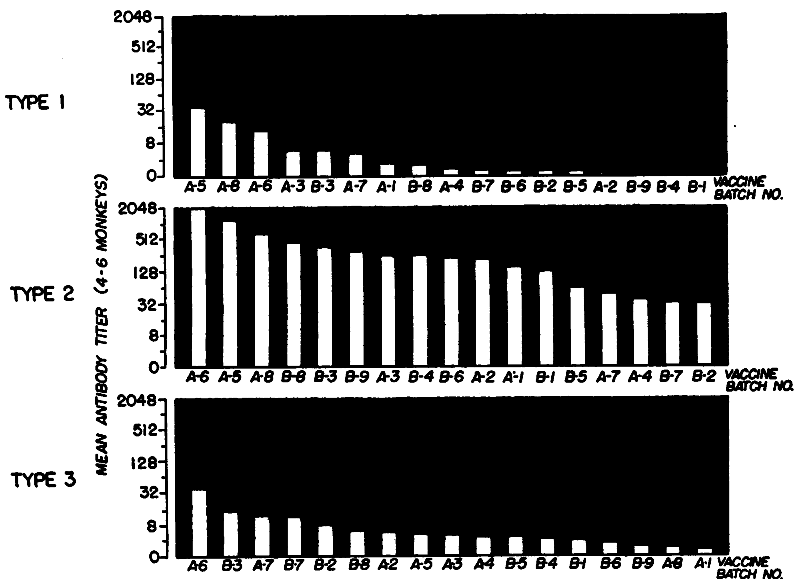


Figure 8

Mean Titer of Antibody in Cynomolgus Monkeys Four Weeks After a Single Administration of Ten ml. of 18 Different Batches of Poliomyelitis Vaccine Divided Among Four Intramuscular Sites



listed in both charts, the vaccines that are low on the scale tend to remain low in both, and here as well there are exceptions. The significance of these observations in relation to establishing requirements of vaccine potency for use in man is being determined.

An interesting observation has been made in other groups of monkeys inoculated on a single occasion with 10 ml. of vaccine divided between four sites (Figure 8). Blood drawn four weeks later revealed a rather poor response, or none at all, to the Types 1 and 3 components of the vaccine, but a good response to the Type 2 component. This suggests that vaccines made of each of the strains selected to represent the three types are not of equal antigenic capacity, even though the infective titers of the fluids prior to their inactivation were very similar. It is too early to conclude whether the differences here observed represent a strain-difference, or a type-difference, or merely a difference among lines of the same strain. Nevertheless, it does provide an interesting point for further study and is reminiscent of differences in antigenic capacity among the influenza viruses.

It is of interest to note from the foregoing that 3 ml. of vaccine, and even 2 ml., given in divided doses of 1 ml. each, a week apart, was more effective in inducing antibody response to Types 1 and 3 virus than was 10 ml. given on one occasion. For the Type 2 component, 3 ml. given in divided doses of 1 ml. each was measurably better than was 10 ml. given all at once. This reaffirms, once again, the importance of divided doses for providing greater effects with less vaccine.

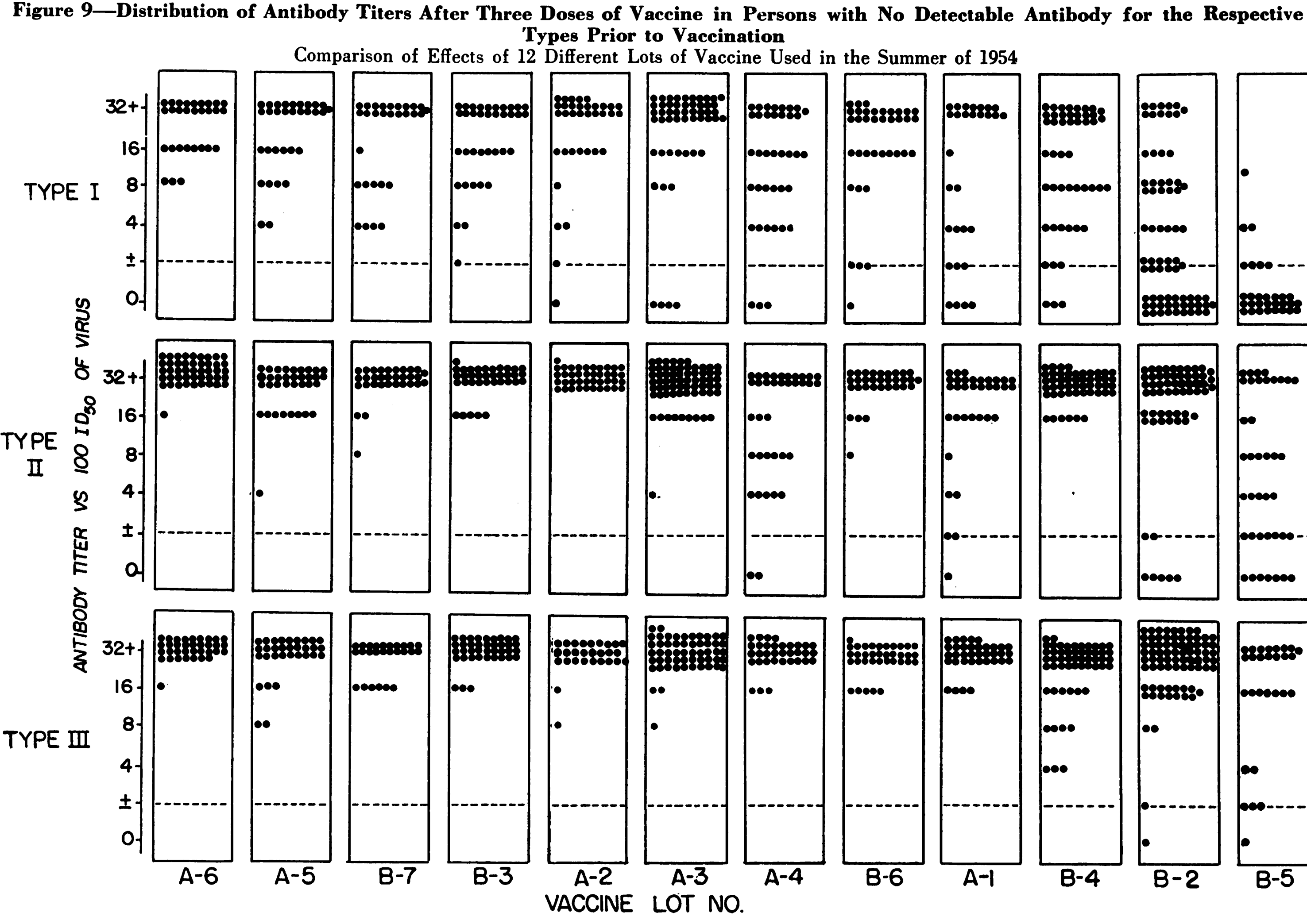
Antigenic Activity in Man of the Different Lots of Vaccine—The vaccines that have been described in the foregoing were inoculated into groups of human subjects of various ages, but for the most part in children between five and nine years of age. The purpose was to determine the degree to which materials, which had previously been tested in monkeys, were antigenic in man by determining the proportion of individuals in whom measurable antibody was engendered by three inoculations spaced at approximately 10 days to two weeks between the first and second, and approximately five to six weeks between the first and third. It had been known from other studies in human subjects that better responses could be obtained with the same quantities of vaccine if longer intervals were allowed between inoculations. Since studies on the optimal interval were not yet far enough along to indicate the best interval, the schedule indicated was adopted.

Since all of the serologic tests have not been completed, nor have all the available data on antibody response after each of the three inoculations been analyzed, there is presented in Figure 9 a summary of the effect of the vaccine in terms of the number of instances in which antibody appeared where, prior to vaccination, there had been none. Antibody measurements were made using one hundred 50 per cent doses of virus of each type in a tissue culture system in which the color change of phenol red was used as the indicator of virus activity.

The data in Figure 9 indicate the proportion of instances in which conversion occurred from no detectable antibody to a measurable level following inoculation with each of 12 of the 18 different lots of vaccine used in the field studies; data on the other six lots are still to be compiled. It is clearly evident that all lots for which information is now available induced antibody response rather uniformly for the Type 3 virus; as for the Type 2 component, the response was equally good to all but two lots (B-2 and B-5) in which a moderately good effect was observed. The principal defections were observed in the Type 1 component

Figure 9—Distribution of Antibody Titers After Three Doses of Vaccine in Persons with No Detectable Antibody for the Respective Types Prior to Vaccination

Comparison of Effects of 12 Different Lots of Vaccine Used in the Summer of 1954



of lots B-2 and B-5. The reason for the poor response is evident in part from data on infectivity titer of the fluids used for conversion into vaccine and was due in the case of B-2 to losses in the course of filtration. The tissue culture fluids used for preparing vaccine lot B-5 were not from the same source as was the fluid used for preparing the rest of the 18 lots and was of relatively lower titer. It had also been found that these same batches of vaccine, when tested in monkeys, were the least antigenic. Other evidence, indicating the deleterious effect of 1:10,000 dilution of merthiolate on the stability of the antigen, suggests that this also played a role in affecting the performance of vaccines of borderline concentrations. The effect of merthiolate is most marked on Type 1. This will be elaborated upon elsewhere and is discussed below.

Discussion

In this paper is presented a portion of the more extensive report on noninfectious vaccines for poliomyelitis that was made before the Third International Poliomyelitis Conference in Rome in September, 1954. The data assembled for this meeting were intended, in part, to provide answers that could be given at that time to some of the many questions concerning the first experiences with the first lots of vaccine prepared on a large scale.

The observations to which it is desired to draw attention at this time are in relation to the degree of antibody response induced in human subjects by the different batches of vaccine. It is clearly evident that all but two (lots B-2 and B-5) of the group of 12, for which data are presented here, induced the formation of measurable amounts of antibody for each virus type in all, or almost all, of the subjects tested. Data are now available on six additional lots of vaccine (Numbers A-7, A-8, A-9, B-1, B-8, and B-9), and one of these (B-1) performed in a manner similar to B-2 and B-5. It is of interest that even among the three lots (B-1, B-2, and B-5) it appears that the deficiencies were more marked for Type 1 than for Types 2 or 3.

These early data will be supplemented shortly by a much fuller account showing the actual antibody levels induced following each of the three inoculations, as well as the differences in responses observed among those with no antibody to any of the three types prior to vaccination and those who possessed some antibody to one or more types. Suffice it to point out here that, although these groups contained a substantial proportion of children with no antibody to any of the three types prior to vaccination, antibody was formed in many instances to levels of 32 or higher. Observations at higher dilutions will be contained in a report to follow.

The poor response to the three lots of vaccine that did not produce the expected effect was not due entirely to poor virus content of fluids converted into vaccines, nor to filtration losses—human response correlates with antigenicity tests in monkeys. The poor response was due in part, at least, to the deleterious effect upon antigenicity of the 1:10,000 dilution of Thimerosal (merthiolate) which was added merely as a preservative, or as a suppressant of microorganisms that might accidentally have been introduced in the course of manufacture or in the course of use of a multiple dose vial. Although the effect of Thimerosal on vaccine stability will be fully documented in another report, it now appears that a concentration of 1:10,000 of this chemical is not a satisfactory preservative for this vaccine. Since vaccines without preservatives are prepared routinely in other countries and some are similarly prepared even in this country, it is clear that one means of avoiding the

untoward influence of merthiolate would be to modify the handling procedure to make its use unnecessary, at least until a satisfactory concentration of this chemical or an adequate substitute can be recommended.

Correlation of the data on human antibody response with other information, such as infectivity data before and after filtration and data on antigenicity derived from monkeys inoculated with vaccine handled at different temperatures, has provided what appears to be a full explanation for the variations among batches when tested by measurements of antigenicity in human subjects. These and other data have provided the basis upon which recommendations have been made for further large-scale preparation and standardization of vaccines for additional studies, or for use, if the results of the field tests so indicate.

Summary

The principles involved in the preparation of vaccines for the field studies of vaccination against poliomyelitis, carried out in the spring and summer of 1954, are discussed. A summary is presented of some of the early data derived from studies of antibody response in man, and in laboratory animals, of the antigenic effects of the different lots of vaccine used. It appears that of 18 batches of vaccine employed, there were three that were less active antigenically than were the rest and that this effect was not equal for all, but was most marked for one of the components of these particular lots. Reasons for the variations are fully understood and have provided the basis for recommendations for further large-scale preparation and for standardization of vaccine for future studies.

ACKNOWLEDGMENTS—The virus fluids used for conversion into the vaccines referred to were prepared at the Connaught Medical Research Laboratories, University of Toronto, Canada; one exception is cited. The raw fluids were converted into vaccines in the laboratories of Eli Lilly Company, Indianapolis, Ind., and Parke, Davis and Company, Detroit, Mich. The data on infectivity measurements before and after filtration and on rates of destruction of infectivity were derived from the protocols submitted with each batch of vaccine. The remainder were derived from tests in this laboratory.

In addition to our appreciation for contributions from all those whose effort at the laboratories mentioned facilitated this undertaking, we are most appreciative of the contributions made by the subjects who participated, and particularly to the many members of the laboratory staff, without whose devoted assistance none of this would have been possible.

We wish to acknowledge, also, the generous assistance of the National Foundation for Infantile Paralysis, not only for their financial support, but for their active participation in many phases of these studies.

A New Poisoning Control Center

Duke University Hospital, Durham, N. C., recently established a new emergency Poison Control Center to meet the rising incidence of accidental poisoning. The center is serving as an information bureau, as well as a treatment center for cases of poisoning. It is establishing contact with the nation's drug houses and the Federal Food and Drug Administration, so that it will at all times know the ingredients of every poisonous product, the newest form of treatment, and the correct dosage. The center is under the direction of Jay M. Arena, M.D., associate professor of pediatrics, and Haywood M. Taylor, professor of toxicology and associate professor of biochemistry.