An assessment of the serologic response to poliomyelitis vaccine in children, following passive immunization with gamma globulin, and in tuberculous children.

Poliomyelitis Vaccine Studies

By GORDON C. BROWN, Sc.D., ALAN S. RABSON, M.D., and DONALD E. CRAIG, Ph.D.

THE RECENT demonstration of the effectiveness of the Salk poliomyelitis vaccine in actual field trials in children (*1*) has concentrated interest on active immunization in controlling poliomyelitis. Nevertheless, the use of passive immunization in the form of gamma globulin (*2, 3*) may be indicated under circumstances requiring rapid protection. Since the action of the immune blood serum is almost immediate, but of short duration, it should be possible to combine this effect with the slower but longer lasting active immunity obtained with the vaccine. Before such passive-active immunization procedures are used, however, information must be obtained that the administration of gamma globulin does not interfere with artificially acquired active immunity.

Previous reports from the virus laboratory of the University of Michigan (*4*) have shown that gamma globulin does not prevent naturally acquired subclinical infection in human beings or the subsequent development of type-specific antibodies. Mixtures of antipoliomyelitis serum and virus have previously been reported to have very little antigenic effect in animals (*5–8*), but this was probably due to the fact that the virus was actually neutralized before inoculation.

Early work with virus and immune blood serum administered separately indicated that monkeys could be immunized in this manner although the techniques utilized were hardly adequate for the quantitative measurements attempted by the authors (*9–11*). More recently, Bodian (*8*) has described experiments in monkeys receiving gamma globulin in one leg and live virus in the other leg, and concluded that there was no interference with the antigenicity of the vaccine. Howe (*12*) found that a formalin-inactivated brain tissue vaccine was antigenic in a small number of humans when gamma globulin was inoculated at another site, but none of his subjects received the vaccine without the blood derivative.

The purpose of this paper is to report the

Dr. Brown is professor of epidemiology and Dr. Craig is a research associate at the virus laboratory, department of epidemiology, University of Michigan School of Public Health. Dr. Rabson is a former epidemic intelligence officer, Communicable Disease Center, Public Health Service, Atlanta, Ga. *Their studies were aided by a grant from the National Foundation for Infantile Paralysis and by the assistance and cooperation of Dr. Edna M. Jones, associate physician, Maybury Sanatorium, and of the staff of the Wayne County Training School.*

serologic results in children given gamma globulin 3 days prior to the administration of poliomyelitis vaccine, and, in addition, to describe the results of control administrations of the same vaccine to children hospitalized with tuberculosis.

Materials and Methods

During the summer of 1954 a small quantity of poliomyelitis vaccine was made available for research purposes through the courtesy of Dr. Jonas E. Salk. This particular lot of material (lot 309) had in fact been used in some areas for the nationwide field trial in 1954, but only as a third dose in conjunction with other lots. It was also used later in the summer and fall of that year in a separate study of infants and preschool children (*13*) during which it was discovered that, after this additional time of exposure to the merthiolate preservative, an unfortunate loss of antigenicity had occurred.

For most of the children in the present studies, the poliomyelitis vaccine was administered according to the same schedule used in the 1954 field trial; namely, 3 injections of 1.0 ml. each were given intramuscularly in the left deltoid muscle. The second injection was given 1 week after the first. The third injection was given 5 weeks after the first. A small group of children, however, received only 2 inoculations. The second inoculation was given at an interval of 8 to 10 weeks after the first.

When gamma globulin was used, it was administered intramuscularly in the gluteus maximus in quantities of 0.28 ml. per pound of body weight. The globulin was from the same lot (lot 212) used in a previous study (*4*) in which maximal levels of circulating antibodies were observed in 3 days and persisted for no longer than 3 weeks following this dosage.

Passive-Active Immunization

In June 1954, 27 boys, ranging in age from 8 to 10 years, at the Wayne County Training School, Northville, Mich., volunteered for the study. First, blood specimens were taken, then the boys were weighed and inoculated with gamma globulin as described. Three days later they received the first of 3 injections of vaccine. Blood specimens were taken 2 weeks after the last inoculation, or 7 weeks after the first injection. The serums were separated and stored at 4° C. until tested. Neutralization tests were performed by mixing equal volumes of original serum dilutions of 1:4, 1:8, 1:16, 1:64, 1:256, and 1:1024 with the 3 types—type 1 (Mahoney), type 2 (MEF-1), type 3 (Saukett)—of poliomyelitis virus calibrated to yield 100 tissue culture doses per inoculum as calculated by the 50 percent endpoint method (TCD_{50}). After the virus-serum mixtures had been incubated for 1 hour at room temperature, they were placed in tubes containing cultures of HeLa cells and incubated at 37° C. Appropriate tissue, virus titration, and immune serum controls accompanied each test. Microscopic

Figure 1. Serum antibody titer changes in 27 paired serums taken from Wayne County Training School boys before and after inoculation with gamma globulin and poliomyelitis vaccine.

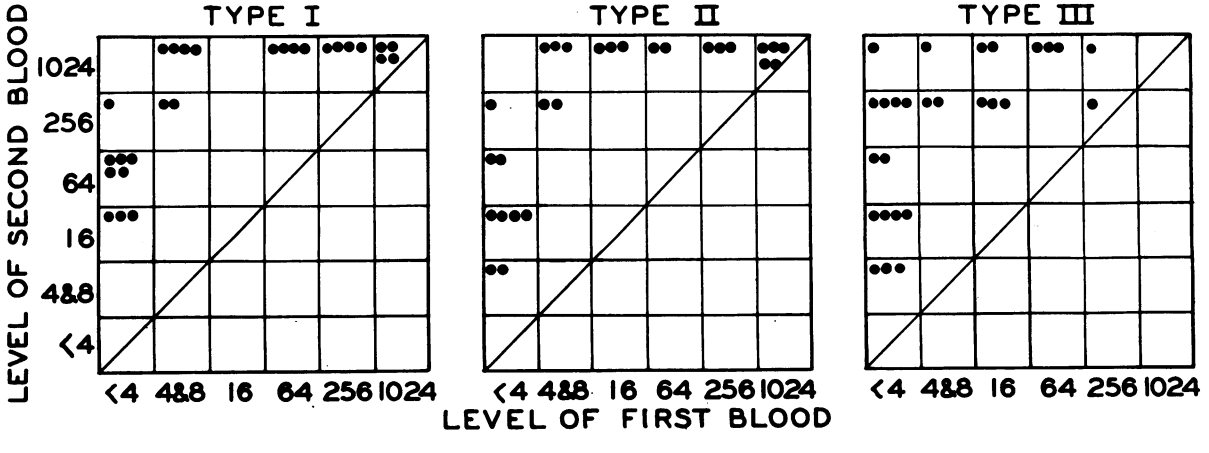
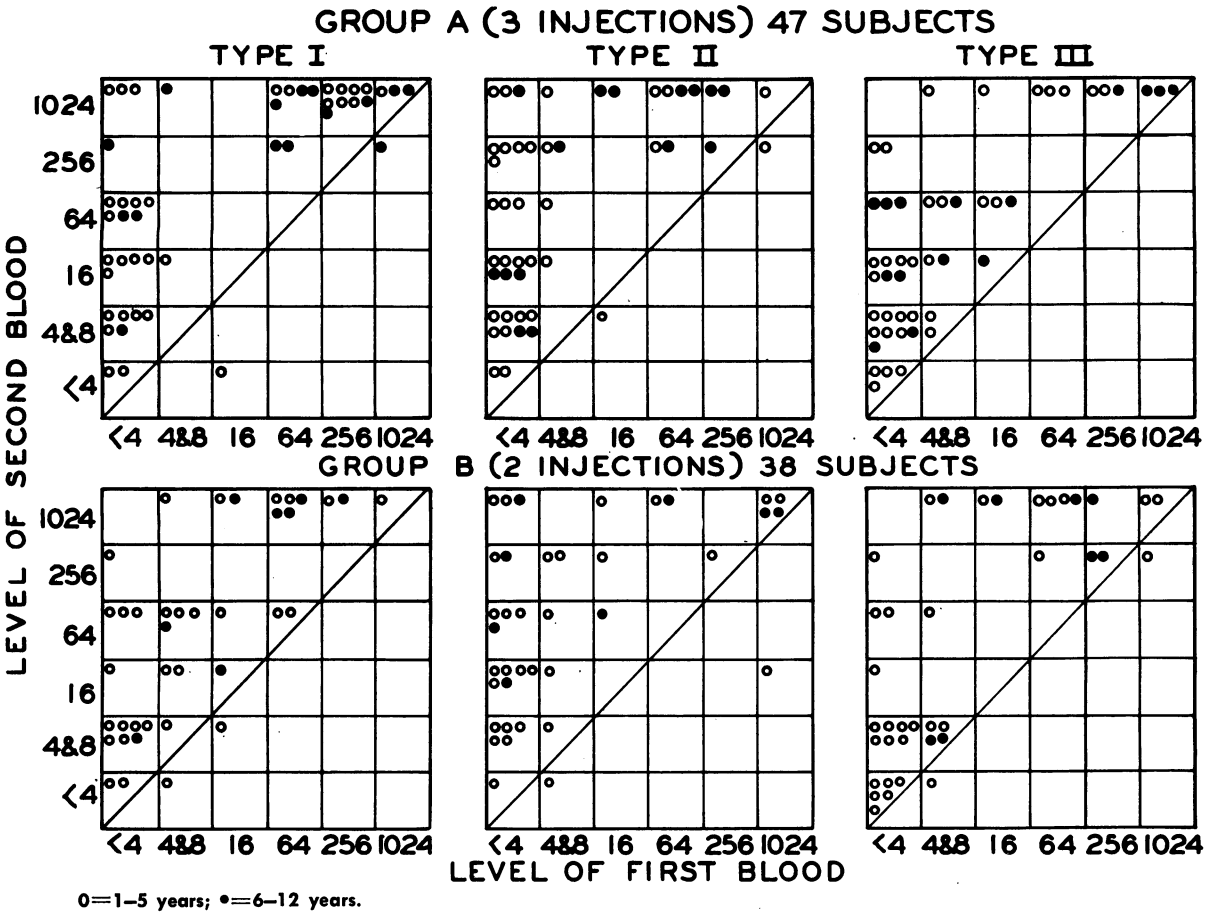


Figure 2. Serum antibody titer changes before and after poliomyelitis vaccination of 47 tuberculous children in Maybury Sanatorium.



evidence of cytopathogenicity was accepted as lack of neutralization.

The serologic response of the children who had been inoculated with gamma globulin 3 days before the administration of the first dose of poliomyelitis vaccine is shown in figure 1. In this figure the numerals at the bottom represent serum antibody levels before vaccination, and the numerals at the left indicate the titers after vaccination. Thus, any circle below the diagonal line would represent a drop in titer; any circle in the diagonal line of squares would indicate no change in titer, and any circle above the diagonal shows that the antibody titer has increased to the titer indicated on the coordinates. In spite of the small number of children studied, it is readily apparent that there was a marked increase in the serum antibody titer in most subjects as shown by the predominance of circles above the diagonal line. The median response in the children with undetectable antibodies prior to immunization was between 16-fold and 64-fold, with the more marked increase being observed against types 1 and 2 virus.

Most of the postvaccine titers of the children with demonstrable serum antibodies before vaccination reached the limit of the dilutions employed, namely 1 : 1024, and some of the titers would undoubtedly have been higher had the dilutions been extended. The controls for this experiment are represented in the results that follow.

Active Immunization

In June 1954, 85 children hospitalized for tuberculosis in the Maybury Sanatorium of the Detroit City Board of Health at Northville, Mich., were selected for study. These patients

ranged in age from 1 to 12 years with a predominance of children of 1 through 6 years. Most of the children had been nonambulatory for 6 months to a year. Blood specimens were first obtained from all; then vaccine was administered according to two schedules of inoculation.

Forty-seven children received 3 injections of 1 ml. intramuscularly at 0, 1, and 5 weeks, as given to the training school group after gamma globulin injections. Thirty-eight children, however, received only 2 inoculations at an interval of 8 to 10 weeks. Two weeks after the last injection, blood specimens were obtained from all the subjects again, and the serums were filed at 4° C. until tested.

For purposes of clarification, the children receiving 3 inoculations will be classified as belonging to group A and those with only 2 inoculations as group B. Five months after the first inoculation, blood specimens were taken from 21 group A and 25 group B subjects. Ten months after the start of the experiment, specimens were obtained again from 19 of the children (11 of group A and 8 of group B) who were still in the sanatorium, following which a booster inoculation of vaccine (lot E5721) was given, and blood specimens were taken 3 weeks later. These latter individuals, then, were children who had been studied over a period of approximately 1 year during which time 5 blood specimens had been obtained, before and after primary and secondary inoculations. All serums were tested for neutralizing antibodies, as described previously.

Figure 2 presents the serum antibody changes in the tuberculosis patients in Maybury Sanatorium after they received the primary inoculation of the vaccine. The upper part of the figure portrays the changes in the children who received 3 injections (group A), and the lower half shows the results for the group who received 2 injections. It will be seen that regardless of the serum antibody titer prior to vaccination, most of the subjects responded well. Very few persons failed to respond, and

Figure 3. Group A—Composite results of antibody response to standard virus types after 3 injections of Salk vaccine, lot 309.

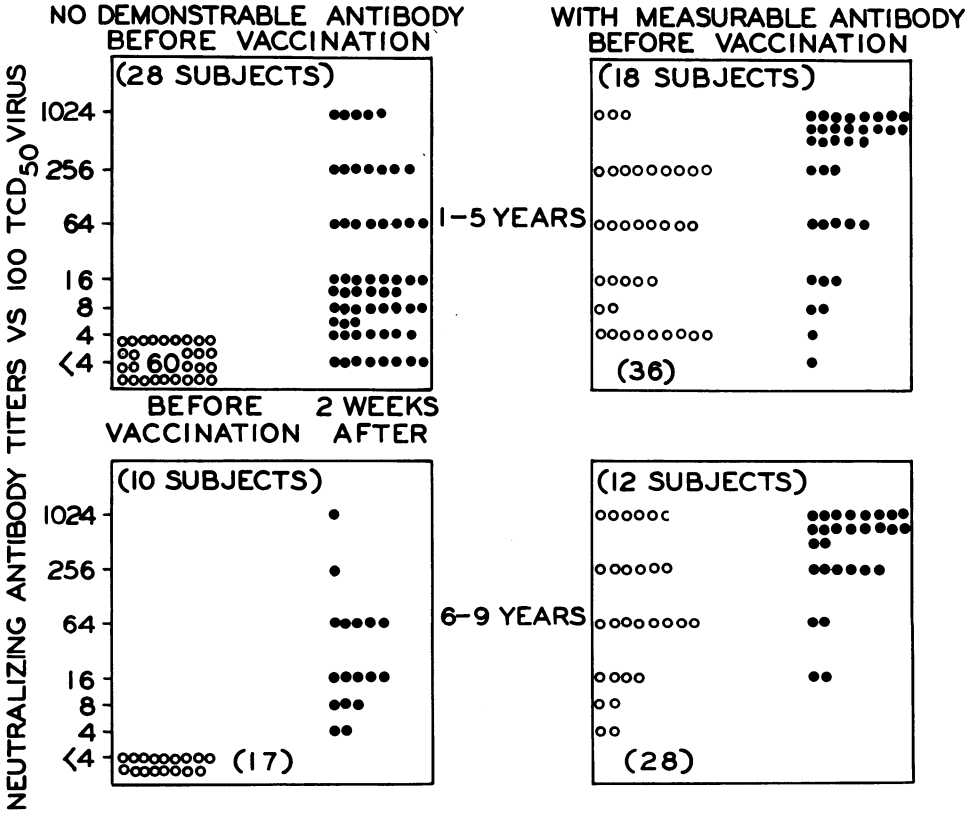
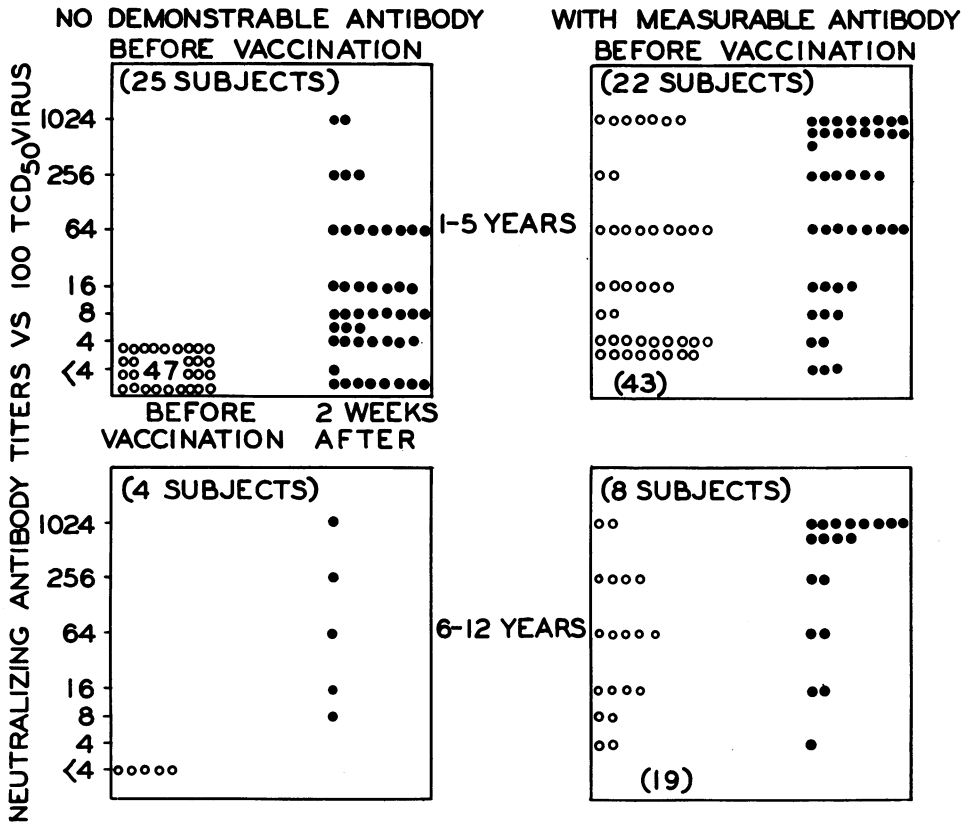


Figure 4. Group B—Composite results of antibody response to standard virus types after 2 injections of Salk vaccine, lot 309.



most of the failures are related to the type 3 component of the vaccine.

The data on the 47 children receiving 3 inoculations are reorganized as composite charts in figure 3, illustrating the development of demonstrable antibodies where none could be detected prior to vaccination and also portraying the results in the children who had antibodies before vaccination. In each of the four charts the antibody levels before vaccination are shown on the left, and the levels 2 weeks after vaccination are shown in the right-hand column. Thirty-two of the children were from 1 to 5 years of age, and, with the exception of one 12-year-old, the other 15 were from 6 to 9 years old. Thirteen children in the 1- to 5-year group had no demonstrable antibodies to any type of virus; 6 had antibodies to only 1 type; 9 had antibodies to 2 types, and only 4 had antibodies to all 3 types. Twenty-eight subjects were lacking in demonstrable antibodies to either one, two, or to all three types of virus, and the 60 such instances with the titers obtained after vaccination are shown in the upper left portion of the composite chart (fig. 3). A median antibody titer of 16 was observed. This duplicates exactly the median antibody response to vaccine in the 10 subjects of the 6- to 9-year age groups having 17 instances of no antibodies before vaccination although the younger age group appeared to have fewer antibodies initially. In those individuals with measurable antibodies to a given type of virus, the increase in the 1- to 5-year age group from a median of 64 before vaccination to a median of 1,024 after vaccination is again duplicated exactly in the older group.

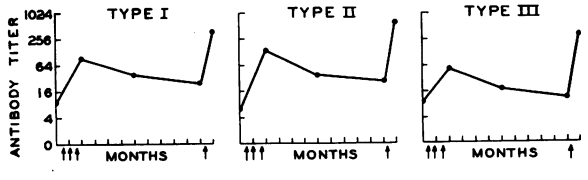
Figure 4 represents the composite results of antibody changes in children receiving only 2 injections of vaccine. Thirty of the subjects were from 1 to 5 years of age, and only 8 were from 6 to 12 years old. Eight children in the 1- to 5- year group had no demonstrable antibodies to any type of virus, 6 had antibodies to only

1 type, 11 had antibodies to 2 types, and only 5 to all 3 types. The composite chart shows that, when antibodies were not present before vaccination, the median rise in homologous titers was eightfold. In the 22 children 1 to 5 years old with 43 instances of demonstrable antibodies to virus (upper right of the chart), the median homologous titers changed from 16 before vaccination to 256 after vaccination, a 16-fold increase. The number of children in the 6- to 12-year age group was too small for accurate analysis, but the antibody increases in these few are obvious and of the same order.

As mentioned previously, blood specimens were obtained from 46 subjects in the study 5 months after the first inoculation of vaccine. Neutralization tests with these serums showed a median fall in antibody titer against all 3 types of virus regardless of whether the children had received two or three inoculations. The decrease was most marked in the type 1 antibodies and least in the type 3. The primary response to the latter had been less, however. This progressive decrease in measurable antibody is further emphasized in figure 5, which shows the geometric mean of the antibody levels in a series of 5 serums taken over a period of almost a year in those subjects who received 3 inoculations of vaccine at the times indicated by the first 3 arrows and a subsequent inoculation 10 months after the start of primary immunization, as indicated by the single arrow.

The tests revealed a good primary response to vaccination, followed by the gradual decline over the intervening 10 months to a point only slightly greater than that seen before vaccination. The effect of the secondary inoculation in these children, however, was quite pronounced and might have been numerically greater had the test dilutions been extended

Figure 5. Geometric mean of antibody level in 19 tuberculous children (groups A and B, Maybury Sanatorium) bled 5 times during interval from before vaccination to after receiving poliomyelitis booster injection.



since many serums neutralized 100 TCD_{50} of virus at the highest dilution employed (1:1024). The titers following the secondary vaccination in all children against all 3 types of virus were much higher than before vaccination, and in many persons the increase ranged from less than 1:4 to greater than 1:1024.

Discussion

The extent of the serologic response in children inoculated with gamma globulin 3 days prior to the first injection of poliomyelitis vaccine proves definitely that this dosage, which is twice that usually administered to humans, does not interfere with the development of active, artificially stimulated antibodies. The titers induced in this study were judged to be more than adequate when compared with the titers obtained following vaccination in the field trial of 1954 and when compared more specifically with titers in tuberculosis patients receiving injections from the identical lot of vaccine, on the same schedule and at the same time. These antibody titers reflect the response to the vaccine itself and not the residual passively acquired antibodies of the gamma globulin since previous studies with identical quantities of the same lot of gamma globulin injected in persons without previous antibody showed that only very low titers of antibody not exceeding 1:4 could be detected and then for a period not more than 3 weeks after injection. Thus, the conclusion is inescapable that poliomyelitis vaccine is capable of inducing the formation of antibodies quite uninfluenced by the presence of circulating artificially acquired antibodies. This observation has importance in view of the possible epidemiological circumstances which might indicate the advisability of a course of passive-active immunization in human beings.

The results of vaccinating patients hospitalized for tuberculosis not only serve to control the above results inasmuch as the lot of vaccine and the schedule of inoculation were the same but also illustrate the immunological response to a virus vaccine in nonambulatory persons infected with a debilitating bacterial disease. The 16-fold response to primary vaccination regardless of the existence of demonstrable an-

tibodies before inoculation shows that children hospitalized for tuberculosis are equally as good subjects for immunization as are normal children. In fact, the importance of immunizing institutionalized persons is emphasized by the frequency with which epidemics have occurred under such conditions. In this very hospital, 12 cases of poliomyelitis occurred among 80 children in 1952, 2 years prior to this study.

As expected, there appeared to be no correlation whatsoever between the individual antibody responses and the character or extent of the tuberculosis. Furthermore, the response of the young children between 1 and 5 years of age was judged as good as that of the older children regardless of whether they received 2 or 3 inoculations of vaccine. This important observation of the efficacy of vaccine in the younger age group is a forerunner of more extensive data representing studies in infants and preschool children (*13*) to be published soon from this laboratory.

The gradual decrease in antibody titer during the period following primary vaccination has been described elsewhere (*14–16*), but the sharp booster effect of subsequent inoculations in the individuals in this study not only demonstrates the beneficial effect of the booster but suggests strongly that, regardless of the level of demonstrable antibodies at that time, significant immunization did persist after the primary stimulation.

Summary

The serologic response of children inoculated with gamma globulin 3 days prior to active immunization with poliomyelitis vaccine was measured by the virus laboratory, University of Michigan School of Public Health. The consistent rise in antibody titer in the children in the study demonstrates that passive immunization of this extent has no suppressive effect on the individual's response to the vaccine.

The vaccination of children hospitalized for tuberculosis resulted in antibody levels consistent with the response of normal children and indicated that immunization of tuberculous individuals should be practiced. The effect of 2 inoculations was equally as good as that of 3 injections, and no significant difference was observed in the response of 1- to 5-year-old children as compared with that of older children, 6 to 12 years of age.

The booster effect of secondary vaccination almost 1 year later is demonstrated and discussed.

REFERENCES

(*1*) Francis, T., Jr., and others: Evaluation of 1954 field trials of poliomyelitis vaccine. Summary Report. Am. J. Pub. Health 45: 1–63, May (Part 2) 1955.

(*2*) Hammon, W. McD., and others: Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis. 1. Plan of controlled field tests and results of 1951 pilot study in Utah; 2. Conduct and early follow-up of 1952 Texas and Iowa-Nebraska studies; 3. Preliminary report of results based on clinical diagnosis; 4. Final report of results based on clinical diagnosis. J. A. M. A. 150; 739–749, 750–756, 757–760, Oct. 25, 1952; and 151: 1272–1285, Apr. 11, 1953.

(*3*) Hammon, W. McD., and others: Effect of passive immunity on infection with the poliomyelitis viruses. *In* Poliomyelitis papers and discussions presented at the Third International Poliomyelitis Conference. Philadelphia, J. B. Lippincott Co., 1955, pp. 159–166.

(*4*) Brown, G. C., Rabson, A. S., and Schieble, J. H.: Effect of gamma globulin on subclinical infection in familial associates of poliomyelitis cases. 1. Quantitative estimation of fecal virus; 2. Serological studies and virus isolations from pharyngeal secretions. J. Immunol. 73: 54 (1954) and 74: 71 (1955).

(*5*) Rhoads, C. P.: Immunity following the injection of monkeys with mixtures of poliomyelitis and convalescent human serum. J. Exper. Med. 53: 115 (1931).

(*6*) Kramer, S. D., and Schaeffer, M.: Experimental poliomyelitis; Active immunization with neutralized mixtures of virus and serum. Proc. Soc. Exper. Biol. & Med. 31: 409 (1933).

(*7*) Kramer, S. D.: Active immunization against polio; A comparative study. III. Active immunization of monkeys with exactly neutralized mixtures of virus and serum. J. Immunol. 31: 191 (1936).

(*8*) Bodian, D.: Experimental studies on passive immunization against poliomyelitis. I. Protection with human gamma globulin against intramuscular inoculation, and combined passive and active immunization. Am. J. Hyg. 54: 132 (1951).

(*9*) Goldbloom, A., Brodie, M., and Moffatt, W.: Active immunization against poliomyelitis in monkeys. Am. J. Dis. Child. 40: 923 (1930).

(*10*) Brodie, M., and Goldbloom, A. J.: Active immunization against poliomyelitis in monkeys. J. Exper. Med. 53: 885 (1931).

(*11*) Brodie, M.: Active immunization against poliomyelitis. J. Exper. Med. 56: 493 (1932).

(*12*) Howe, H. A.: Antibody response of chimpanzees and human beings to formalin-inactivated trivalent poliomyelitis vaccines. Am. J. Hyg. 56: 265 (1952).

(*13*) Brown, G. C., and Smith, D. C.: Immunization of infants and preschool children with poliomyelitis vaccine. To be published in Journal of the American Medical Association.

(*14*) Salk, J. E., Bazeley, P. L., Bennette, B. L., Krech, V., Lewis, L. J., Ward, E. N., and Youngner, J. S.: Studies in human subjects on active immunization against poliomyelitis. II. A practical means for inducing and maintaining antibody formation. Am. J. Pub. Health 44: 994, August 1954.

(*15*) Salk, J. E.: Considerations in the preparation and use of poliomyelitis virus vaccine. J. A. M. A. 158: 1239, Aug. 6, 1955.

(*16*) Brown, G. C.: The effect of booster inoculations on the serological status of children vaccinated with poliomyelitis vaccine. Am. J. Pub. Health 45: 1401, November 1955.

Increase in Nuclear Reactors

The number and heat capacity of nuclear reactors in the United States, used to generate electric power, will increase predictably, according to the Division of Sanitary Engineering Services, Public Health Service. There will be a parallel increase in the quantity of radioactive byproducts, it is said, with a corresponding need for protective practices.

The first reactor began operation in 1942. By the end of 1955, there were 22 reactors in operation, with a heat capacity of 100 megawatts. As of February 1956, approximately 40 nuclear reactors, about one-half of which are for the production of nuclear power, were in some stage of design or construction. Seven new reactors, with a heat capacity of 90 megawatts, are expected to begin operation during 1956.

Radioactive wastes from reactors must be segregated or diluted to tolerable concentrations. At present, underground storage and ocean burial are among the methods used or proposed for segregation. Both the economy and the adequacy of these methods are under continuing investigation in anticipation of the probable increase in volume of radioactive wastes.

It is estimated that nuclear power plants completed during 1964 alone will have a power level exceeding 2.25×10^6 kilowatts, producing wastes each year of 3.3×10^{10} gallons containing 10 millicuries per gallon. An indication of the magnitude of this activity is that the entire flow of the Mississippi River would not be sufficient to dilute to permissible concentrations the fission products from these plants. Such a method of dilution, of course, is obviously least likely to be used.