

POLIOVIRUS VACCINES IN MINERAL OIL ADJUVANT

II. Clinical Reaction and Antibody Response

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THE problem of antigenic deficiency and lot to lot variability in antigenic potency of Salk poliomyelitis vaccine¹ has been resolved by the development of a highly purified and concentrated vaccine containing standard amounts of viral antigen.²⁻⁴ The disadvantage of such vaccine is in the need for many-fold concentration of antigen with resultant high cost for preparation. Studies were undertaken to prepare purified killed vaccine in Freund's incomplete mineral oil adjuvant⁵ with a view toward obtaining a satisfactory neutralizing antibody response with the use of a reduced amount of viral antigen. A second objective was to measure the capacity of the adjuvant vaccine to overcome the effect of poliovirus antibody of maternal origin in suppressing active antibody response to the vaccine^{2, 6-9} when administered to very young infants. The present report describes the clinical and serologic findings among infants given aqueous or adjuvant vaccines. A previous study¹⁰ in this series describes the preparation of the adjuvant vaccine and summarizes the animal toxicity and immunization data.

MATERIALS AND METHODS

Poliovirus Vaccines.—Preparation of the vaccines was described previously.¹⁰ The diphtheria, pertussis, tetanus (DPT) combined and DPT-Salk poliovirus vaccine, combined (Tetravax), were lots of commercial material prepared by Merck Sharp and Dohme Laboratories. The aqueous purified vaccine contained 4.6 γ of poliovirus antigen (Type I, 3.0 γ ; II, 0.6 γ ; III, 1.0 γ) in the 0.5 ml. dose and the purified adjuvant vaccine contained only one tenth this amount, i.e., 0.46 γ of antigen (Type I, 0.3 γ ; II, 0.06 γ ; III, 0.1 γ).

Patients.—All were babies who were admitted to the Well Baby Clinic of the Bellevue Hospital Center, New York, New York. Most infants were one to 2 months of age at time of initial vaccination. The age range was 3 weeks to 4 months. Nearly all mothers of the infants had received a booster dose of Salk vaccine sometime during their pregnancy. Most infants were from a low socioeconomic group.

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Received for publication April 29, 1963.

Vaccine Regimen.—Infants were selected serially to receive purified aqueous (Lot 10A), purified adjuvant (Lot 10B), or DPT-Salk poliovirus vaccine combined. The aqueous purified poliovirus vaccine dose was 0.5 ml. and the purified adjuvant vaccine dose was 0.1 ml. The dose of DPT-Salk poliovaccine combined was 1.0 ml. Aqueous purified and DPT-Salk combined vaccine were administered in three doses given one month apart whereas the purified adjuvant vaccine regimen consisted of only two doses given one month apart. Infants who received purified aqueous or adjuvant vaccine were given DPT in the opposite arm. All infants received a booster dose of the same vaccine 9 months following the initial vaccine dose. The infants were bled prior to vaccination and again 4 weeks following the third and the booster doses of vaccine. All sera were tested for content of neutralizing antibody by the usual metabolic inhibition test procedure. All serum samples from the primary immunization series were examined in the same test and the pre- and post-booster samples were titrated together in another test. In total, 31 children completed the purified aqueous vaccine series, 34 purified adjuvant, and 31 Salk-DPT.

FINDINGS

Reactions.—No local reaction was recorded for any child who received the adjuvant or any other vaccine. There was neither redness, swelling, nor nodule formation. Systemic reactions consisting of fever and irritability occurred among a few of the children in all of the groups and this was interpreted to be due to the reaction to DPT or to an intercurrent infection.

Neutralizing Antibody Response.—Table I summarizes the seroconversion data among the infants who received the vaccines. It is difficult to appraise primary antibody responses to poliovaccine in children with maternal antibody since such antibody may be so high as to obscure the active immune response.

TABLE I. SEROCONVERSION RATES IN INFANTS TO AQUEOUS, ADJUVANT, OR SALK-DPT POLIOVIRUS VACCINES

BLEEDING TIME	AQUEOUS (4.6 γ) (31 INFANTS)				ADJUVANT (0.46 γ) (34 INFANTS)				DPT-SALK (31 INFANTS)			
	SEROLOGIC STATUS			% POS.	SEROLOGIC STATUS			% POS.	SEROLOGIC STATUS			% POS.
	RISE	STAT.*	TOTAL		RISE	STAT.	TOTAL		RISE	STAT.	TOTAL	
Type I												
Post-dose 3†	29%	26%	55%		15%	9%	24%		3%	13%	16%	
Pre-booster				83				52				37
Post-booster				100				90				80
Type II												
Post-dose 3	10%	6%	16%		9%	15%	24%		7%	7%	14%	
Pre-booster				55				42				52
Post-booster				96				80				92
Type III												
Post-dose 3	45%	10%	55%		24%	9%	33%		23%	13%	36%	
Pre-booster				84				48				60
Post-booster				100				87				100

*Stationary (no change).

†Two doses only of adjuvant vaccine were given in the primary series.

Maternal antibody, however, has a half-life of about 3 weeks^{6, 11} and it is reasonably safe to assume that all instances in which there is either an increase in antibody titer or no drop in titer after the third vaccine dose represent a primary reaction. Based on this criterion, 55 per cent of recipients of aqueous vaccine responded to type I virus, 16 per cent to type II, and 55 per cent to type III following the initial series of three doses of purified aqueous vaccine. Similarly, 24 per cent, 24 per cent, and 33 per cent of recipients of two doses of adjuvant vaccine responded to the three types, respectively, and 16 per cent, 14 per cent, and 36 per cent of infants who received Salk-DPT vaccine developed active immunity.

Such criterion does not, however, detect poor responders whose level of vaccine-induced antibody is well below that of antibody of maternal origin. Hence, a better measure of primary response to vaccine is in observation of the serologic status at the time prior to the booster dose. In the present group, this was at 10 to 13 months of age at a time when all antibody of maternal origin would have disappeared. Based on this criterion, it is seen that 83 per cent, 55 per cent, and 84 per cent (Types I, II and III, respectively) of infants responded to the initial three doses of the highly potent purified vaccine, in spite of the initial very high level of maternal antibody (see Fig. 1). Similarly, 52 per cent, 42 per cent, and 48 per cent of infants given adjuvant vaccine responded to the primary vaccination series even though only a very small amount of antigen was used (0.46 γ) and only two doses of vaccine were given. The primary responses to the three 1 ml. doses of DPT-Salk poliovaccine were roughly of the same order of magnitude as to the adjuvant material.

Even though some of the children failed to respond detectably to the primary series, nearly all were sensitized so as to respond to the booster dose of vaccine. It is seen that 96 to 100 per cent of infants responded to aqueous vaccine, 80 to 90 per cent to adjuvant vaccine, and 80 to 100 per cent to DPT-Salk vaccine.

Another criterion for measuring antibody response is in an examination of the mean antibody titers measured at various time periods, as shown in Fig. 1. By far the best antibody responses were achieved using three doses of 4.6 γ of aqueous purified vaccine. This was especially evident after the booster dose of vaccine had been given. The DPT-Salk vaccine given in three doses performed least well with only mediocre height of responses, even though nearly all infants had developed detectable antibody. The adjuvant vaccine given was more effective than the DPT-Salk vaccine in spite of the small amount of antigen and the fact that only two doses were given, but was less effective than three doses of the highly potent aqueous purified material.

DISCUSSION

The adjuvant vaccine given in 0.1 ml. amount to very young infants in the present study was remarkably innocuous. Cutler and co-workers¹² have recorded reactions following intramuscular administration of 23,917 half ml. doses of adjuvant, Salk, or purified poliovaccine¹⁰ in 15,420 persons. Of the latter, 8,497 received the second dose. Of the 8,497 persons, 0.1 per cent developed tissue

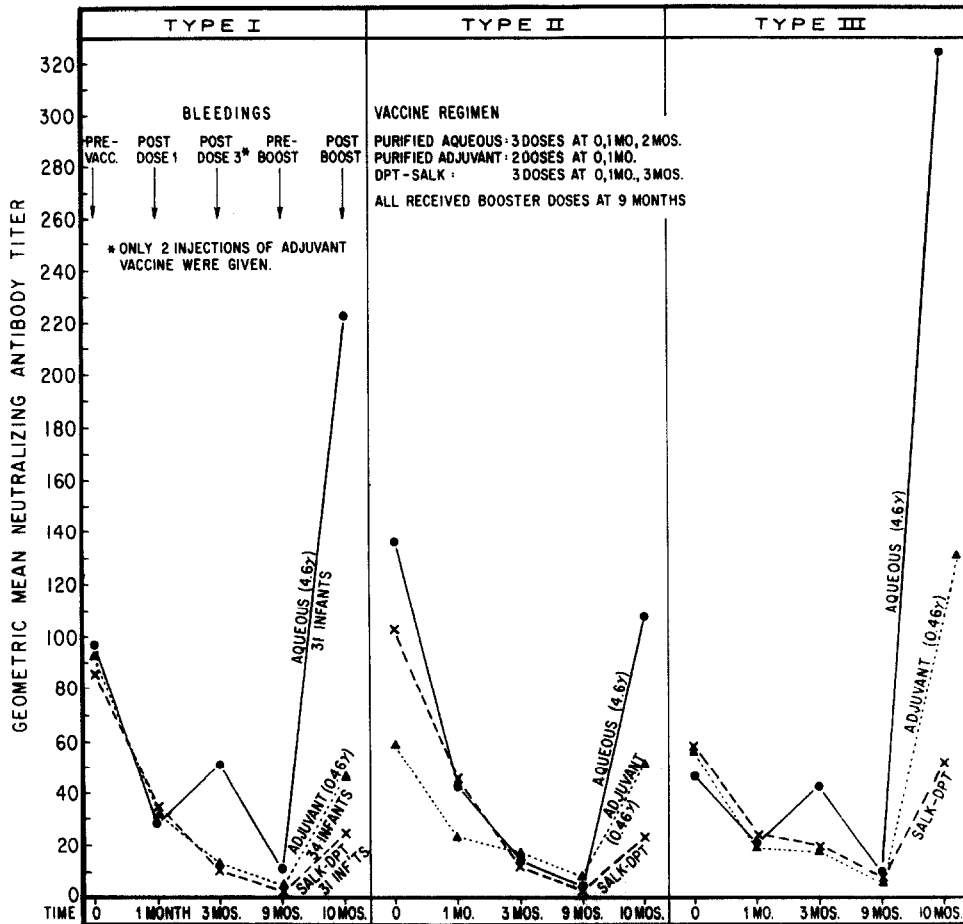


Fig. 1.—Summary of geometric mean neutralizing antibody titers among infants according to vaccine received and time.

induration and 0.1 per cent showed nodule formation following the first dose of vaccine. Two of the patients with nodules required biopsy. Pain, tenderness, and transient swelling were noted with greater frequency among the group. Only one of 69 persons under one year of age who received the vaccine developed signs or symptoms post-vaccination. Reactions were noted with greater than twice the frequency among recipients of adjuvant vaccine prepared by Dr. Salk than among those who received vaccine prepared in these laboratories. No adequate explanation could be given for the differences which were observed. Cutler and his associates concluded that the adjuvant poliovaccine used "is accompanied by an order of magnitude of reaction, subjective or objective, which is so low as to pose no barrier to widespread use of the preparation for all age groups." The absence of detectable reaction in the present study is consistent with this general conclusion.

The purified poliovaccine adjuvant preparation employed in the present

study performed remarkably well even though (a) only two doses were given, (b) it was given to infants with an overwhelming amount of maternal antibody, and (c) the vaccine contained only 0.46 γ of viral antigen. This was only one tenth the amount of antigen contained in the aqueous vaccine which was given in three doses and was only one sixth the amount of viral antigen required (total 2.6 γ ; 2.0 γ type I, 0.4 γ type II, and 0.4 γ type III) to effect sero-conversion following two doses in greater than 90 per cent of persons who are initially seronegative.⁴ Three doses of adjuvant vaccine containing possibly a slightly greater amount of type II antigen might reasonably have effected sero-conversion in near 100 per cent of persons on primary immunization or, at least, following the booster dose. The need for three rather than two doses of vaccine in the primary series in infants with maternal antibody was clearly shown by Perkins and associates⁷ and was the procedure recommended by the Committee on Control of Infectious Disease of the American Academy of Pediatrics.¹³

The mechanism for such high level of efficacy of the adjuvant vaccine cannot be stated. It is possible, however, that the enhanced stimulation of the antibody forming mechanism alone was adequate to overcome maternal antibody. Alternatively, or possibly in conjunction, the oil emulsion may have permitted penetration of the antigen into the antibody-forming cells without significant exposure to maternal antibody.

SUMMARY

Purified aqueous poliovirus vaccine containing a total of 4.6 γ of viral substance per dose was highly effective in immunizing very young infants against poliovirus. Eighty-three, 55, and 84 per cent of infants responded serologically with neutralizing antibody when given three doses of vaccine in the primary series. Essentially all responded with high titer when the booster dose of vaccine was given 7 months later. Emulsified mineral oil adjuvant vaccine, containing only 0.46 γ of viral antigen per dose and given in only two doses, effected sero-conversion of 52 per cent, 42 per cent, and 48 per cent of infants to poliovirus types I, II, and III, following the primary immunization series and 90 per cent, 80 per cent, and 87 per cent, respectively, were converted to the three types following the booster dose. Even better results would likely have been obtained had three doses been given initially. A generally poorer antibody response was obtained with the use of Salk vaccine than with the aqueous and adjuvant purified vaccines. None of the infants developed significant local or systemic reaction to the vaccine. Roughly, 30 children were included in each of the three vaccine groups in the study.

The authors are indebted to W. P. M. Fisher for technical assistance in performing the neutralizing antibody titrations.

REFERENCES

1. Kokko, U. P., and Murray, R.: Human and Animal Antibody Responses to Commercial Poliomyelitis Vaccine, *Bull. W. H. O.* **22**: 263, 1960.
2. Hilleman, M. R., Charney, J., Tytell, A. A., Weihl, C., Cornfeld, D., Ichter, J. T., Riley, H. D., Jr., and Huang, N.: Investigation Into the Development and Clinical Testing of a Poliomyelitis Vaccine Containing Standardized Amounts of Purified Poliomyelitis Virus Antigens, *Acad. Med. New Jersey, Spec. Bull.* **6**: 106, 1960.

3. Charney, J., Tytell, A. A., Machlowitz, R. A., and Hilleman, M. R.: Development of a Purified Poliomyelitis Virus Vaccine, *J. A. M. A.* **177**: 591, 1961.
4. Wehl, C., Cornfeld, D., Riley, H. D., Jr., Huang, N., and Cramblett, H.: Purified Poliomyelitis Vaccine—Clinical Appraisal, *J. A. M. A.* **176**: 409, 1961.
5. Freund, J.: The Mode of Action of Immunologic Adjuvants, *Adv. Tuberc. Res.* **7**: 130, 1956.
6. Perkins, F. T., Yetts, R., and Gaisford, W.: A Comparison of the Responses of 100 Infants to Primary Poliomyelitis Immunization With Two and With Three Doses of Vaccine, *Brit. M. J.* **1**: 1083, 1959.
7. Perkins, F. T., Yetts, R., and Gaisford, W.: Responses of 6- and 9-Months-Old Infants to Two and Three Doses of Poliomyelitis Vaccine, *Brit. M. J.* **2**: 530, 1959.
8. Rannon, L., Goldblum, N., Skalska, P., and Gotlieb, A.: Antibody Response of Newborns to Formalinized Poliomyelitis Vaccine (Salk Type), *Am. J. Hyg.* **72**: 244, 1960.
9. Brown, G. C., and Kendrick, P. L.: Serologic Response of Infants to a Multiple Vaccine for Simultaneous Immunization Against Diphtheria, Pertussis, Tetanus, and Poliomyelitis, in Relation to the Presence of Specific Maternal Antibody, *Am. J. Pub. Health* **50**: 1529, 1960.
10. Hilleman, M. R., Hampil, B., and Sweet, B. H.: Poliovirus Vaccines in Mineral Oil Adjuvant, I. Animal Toxicity and Serologic Response Data, *J. ALLERGY* **34**: 317, 1963.
11. Plotkin, S. A., Koprowski, H., and Stokes, J., Jr.: Clinical Trials in Infants of Orally Administered Attenuated Poliomyelitis Viruses, *Pediatrics* **23**: 1041, 1959.
12. Cutler, J. C., Lesesne, L., and Vaughn, I.: Use of Poliomyelitis Virus Vaccine in Light Mineral Oil Adjuvant in a Community Immunization Program and Report of Reactions Encountered, *J. ALLERGY* **33**: 193, 1962.
13. Committee on Control of Infectious Diseases, American Academy of Pediatrics: Report. Immunization Against Poliomyelitis, *Pediatrics* **26**: 331, 1960.