

POLIOVIRUS VACCINES IN MINERAL OIL ADJUVANT

I. Animal Toxicity and Serologic Response Data

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SALK poliomyelitis vaccine, while highly efficacious in preventing poliomyelitis, exhibits marked variation in antigenic potency from lot to lot¹ and does not protect all persons, even after the fourth dose.² This deficiency in immunizing capacity is further accentuated in attempts to immunize infants who have circulating polio antibody of maternal origin.³⁻⁷ Such maternal antibody may effect considerable suppression of immune response, especially for vaccines of low potency. An effective means for increasing immunogenicity of killed polio-vaccine was achieved by the development of a highly purified poliovirus vaccine containing a precisely measured amount of each viral antigen.⁷⁻⁹ This vaccine effected serologic response in more than 90 per cent of initially seronegative persons following but two doses of vaccine but was disadvantageous in the need for many-fold concentration of poliovirus with resultant high cost for preparation. An effort was made to improve potency and to cut cost by using a reduced amount of viral antigen emulsified in Freund's incomplete mineral oil adjuvant.¹⁰ Early attempts along these lines were recorded by Salk and his co-workers.^{11, 12}

The present report describes the preparation of both purified and ordinary Salk poliovirus vaccines in mineral oil adjuvant and presents the findings in studies to measure toxicity and antibody responses in animals. A second report¹³ in this series describes the findings in infants given the purified vaccine in adjuvant. Studies to measure clinical reactions to and public acceptance of the emulsified poliovirus vaccines were recorded earlier by Cutler and associates.¹⁴

MATERIALS AND METHODS

Poliovirus Vaccines.—The purified and Salk poliovirus vaccines were prepared by the Merck Sharp and Dohme Biological Laboratories and released by the Division of Biologies Standards, National Institutes of Health, for commercial distribution. The method for preparing purified poliovirus vaccine has been described elsewhere.^{7, 8} Lot 8 of aqueous purified poliovaccine contained 1.5 μ g of Type I (Parker), 0.2 μ g of Type II (MEF-1), and 0.4 μ g of Type III

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(Saukett) poliovirus antigen per usual 0.5 ml. human dose. Lot 10 contained 3.0, 0.6, and 1.0 μ g of each component, respectively. Salk vaccines were prepared by usual production procedures and 1.0 ml. is the usual human dose.

Emulsification.—Incomplete Freund adjuvant without added Mycobacteria was used.¹⁰ By this method, the aqueous vaccine was emulsified with an equal volume of a sterile filtered mixture containing 90 per cent light mineral oil (Drakeol 6VR, Pennsylvania Refining Co., Butler, Pa.) and 10 per cent emulsifier (Arlacel A, mannide mono-oleate, Atlas Powder Co., Wilmington, Del.). Emulsification was carried out aseptically in a New Brunswick fermenter. The lot of Arlacel A employed was certified by Dr. B. S. Berlin, University of Michigan, and by ourselves in accordance with the Tentative Draft, Minimum Requirements: Influenza Virus Vaccine, Emulsified in Mineral Oil, U. S. Department of Health, Education and Welfare, National Institutes of Health, dated Jan. 30, 1956. The Drakeol was tested and certified by our laboratories in accordance with the above Tentative Minimum Requirements. The emulsified vaccines proved stable on the centrifuge test as described in the Minimum Requirements and there was no evidence of breaking of the emulsion when the vaccines were stored at 56° C. for 7 days.

Sterility and Safety Tests.—Filled container materials proved sterile when tested in Sabouraud's and thioglycollate medium by ordinary procedures after the emulsion was broken using sterile sesame oil. The filled container vaccine caused no untoward effect in mice or in guinea pigs when examined according to U. S. government safety regulations for biologics.

Monkey Antibody Response.—Groups of 12 initially seronegative rhesus monkeys were given 0.1 ml., 0.25 ml., or 0.5 ml. of mineral oil adjuvant vaccine by the intramuscular route according to the regimens indicated in the text. Aqueous control vaccines were administered in identical manner but were diluted 1:2, using medium 199, to provide antigens of viral content equal to those of the corresponding adjuvant preparations. The animals were bled immediately preceding the first vaccine dose and again at a later time period as stated in the text. The pre- and post-vaccination serum samples were assayed at the same time for their titers of poliovirus neutralizing antibody by the usual metabolic inhibition assay procedure. Control reference antisera were always included in the tests for purpose of computing monkey potency ratios* according to the regulations.¹⁵ The Salk vaccine contained type I strain Mahoney and the purified vaccine contained type I Parker. Both strains were used in the antibody assays.

FINDINGS

Tests for Toxicity.—The mineral oil adjuvant vaccine, as specified in the Tentative Draft of Minimum Requirements referred to above, is not ordinarily tested for toxicity. Instead, the individual components are assayed for degree of inflammatory response in intradermal tests in guinea pigs and in intra-

*This is a calculated value based on a simultaneous comparison of the antibody levels produced in monkeys by the vaccine under test with the antibody levels of reference serums distributed by the National Institutes of Health.

peritoneal tests in mice. The Arlacel A and mineral oil used in the present vaccines were so assayed and proved satisfactory. Additionally, the emulsified vaccines themselves were assayed by the same methods.

Table I shows the average skin-fold thickness of three sites, of 6 guinea pigs per test, given 0.1 ml. amounts of adjuvant vaccine intradermally. There was an expected inflammatory response evidenced by swelling and induration which reached a maximum on the seventh to ninth day after inoculation and which gradually subsided thereafter. In no instance was there hemorrhage, necrosis, or sloughing of the skin.

TABLE I. FINDINGS IN GUINEA PIG INTRADERMAL TOXICITY TESTS FOR SALK AND PURIFIED POLIOVIRUS VACCINES EMULSIFIED IN MINERAL OIL ADJUVANT

VACCINE		AVERAGE* SKIN-FOLD THICKNESS ($\frac{1}{64}$ INCH) AT INDICATED DAY					
LOT	KIND	1	3	7	9	12	14
A	Salk	7.5	8.5	16.0	16.6	14.2	10.3
B	Salk	7.3	8.6	17.3	18.6	15.0	10.8
C (Lot 8)	Purified	6.6	9.0	17.8	17.0	14.8	11.5
D	Salk	14.0	15.8	18.4	18.2	16.4	14.6
E	Salk	9.0	11.1	15.8	15.7	14.9	12.5
F	Salk	8.5	10.3	14.8	15.0	14.7	12.5
G	Salk	8.0	9.5	17.3	17.0	15.5	14.0
10B	Purified	8.1	13.0	15.5	15.7	12.8	12.4
Physiological saline solution (example)		6.0	7.1	7.8	7.5	7.0	7.4

*Six guinea pigs, three sites each.

Groups of 10 mice were inoculated intraperitoneally with 0.25 ml. amounts of each lot of adjuvant vaccine and were weighed at periodic intervals. Uninoculated mice were handled similarly for purpose of comparison (Table II). The rates of weight gain among the mice given adjuvant vaccine were essentially the same as for the uninoculated control mice. A single control example is shown in Table II. Autopsy of the test mice on the fourteenth day post-inoculation revealed no apparent ascites or adhesions.

Potency Ratios, Purified Vaccine.—(See Table III.) The antibody responses, as expressed in terms of potency ratios, were very markedly enhanced

TABLE II. FINDINGS IN MOUSE INTRAPERITONEAL TOXICITY TESTS FOR SALK AND PURIFIED POLIOVIRUS VACCINES EMULSIFIED IN MINERAL OIL ADJUVANT

VACCINE		AVERAGE* WEIGHT (GRAMS) AT INDICATED DAY			
LOT	KIND	0	3	7	14
A	Salk	11.5	12.9	16.8	22.8
B	Salk	11.7	12.9	17.3	24.5
C (Lot 8)	Purified	11.3	13.2	17.7	24.8
D	Salk	11.1	13.4	17.2	22.8
E	Salk	11.1	12.8	16.6	23.6
F	Salk	12.0	14.1	18.1	24.9
G	Salk	11.1	12.3	16.5	23.7
10B	Purified	12.6	15.4	18.8	24.9
Physiological saline solution (example)		12.0	15.5	18.8	23.9

*Ten mice.

TABLE III. COMPARISON, MONKEY POTENCY TEST RATIOS, AQUEOUS AND ADJUVANT PURIFIED POLIOVIRUS VACCINES (LOT 10B)

VACCINE REGIMEN			MONKEY POTENCY RATIO VS. TYPE			
TOTAL VOLUME AND VIRAL CON- TENT PER DOSE	NO. DOSES	VACCINE USED	I		II	III
			PARKER	MAHONEY	MEF-1	SAUKETT
0.10 ml.	1*	Aqueous†	0.02	0.03	0.10	0.00
(I—0.3γ		Adjuvant	0.70	0.23	0.32	0.08
II—0.06γ	2*	Aqueous†	0.12	0.05	0.26	0.04
III—0.1γ)	(28 days apart)	Adjuvant	10.00	4.00	4.40	1.67
0.25 ml.	1*	Aqueous†	0.00	0.00	0.03	0.00
(I—0.75γ		Adjuvant	0.87	0.76	0.73	0.22
II—0.15γ	2*	Aqueous†	0.25	0.08	0.30	0.06
III—0.25γ)	(28 days apart)	Adjuvant	18.39	13.00	8.80	5.41
0.5 ml.	3†	Aqueous	9.20	6.97	3.83	4.11
(I—3.0γ	(Standard NIH	(undiluted)				
II—0.6γ	test)	Ordinary				
III—1.0γ)						
NIH minimal passing ratio				0.29	0.25	0.16

*Animals were bled 56 days after first dose (28 days after second dose, if given).

†Three doses were given one week apart and the animals were bled 7 days following the third dose.

‡Aqueous vaccine diluted 1:2 to provide dose volume equal to that of adjuvant vaccine.

by incorporating the vaccine in mineral oil adjuvant. Two doses of adjuvant vaccine which contained only one tenth the amounts (0.1 ml. dose of half antigen, half adjuvant) of poliovirus antigens as the ordinary aqueous vaccine (0.5 ml. dose, undiluted) gave potency ratios which roughly approximated those obtained following three doses of the ordinary vaccine as measured in the standard N.I.H. assay. When two doses of adjuvant vaccine containing only one fourth (0.25 ml. dose) the ordinary aqueous dose (0.5 ml.) were used, the values obtained usually were greatly in excess of those obtained following three doses of the ordinary aqueous vaccine. Nearly all values obtained with adjuvant vaccine, even when only a single dose was employed, were greatly in excess of the minimal passing ratios required by the regulations for vaccine release.

TABLE IV. COMPARISON, MONKEY POTENCY TEST SEROCONVERSION RATES AND GEOMETRIC MEAN

VACCINE REGIMEN*			I	
TOTAL VOLUME PER DOSE	NO. DOSES	VACCINE USED	PARKER	
			RATE (%)	G.M.†
0.1 ml.	1	Aqueous	1/11 (9)	—
		Adjuvant	11/11 (100)	60
	2	Aqueous	8/10 (80)	8
		Adjuvant	11/11 (100)	1024
0.25 ml.	1	Aqueous	0/12 (0)	—
		Adjuvant	11/12 (92)	30
	2	Aqueous	8/12 (75)	5
		Adjuvant	12/12 (100)	723
0.5 ml.	3	Aqueous	12/12 (100)	440
Standard NIH test				

*See legend, Table I, for details.

†G.M. = Geometric mean titer.

Seroconversion Rates and Mean Antibody Titers, Purified Vaccine.—The findings presented in Table IV show the same marked increase in antibody response attending use of the vaccine emulsified in mineral oil adjuvant. Two doses of adjuvant vaccine, which contained only one tenth as much antigen as the ordinary aqueous vaccine, sufficed to convert all recipient animals to a seropositive status and the geometric mean antibody titers approximated those obtained following three doses of the ordinary aqueous vaccine.

Adjuvant Antibody Response, Salk and Purified Poliovirus Vaccines.—Table V shows the findings obtained in mineral oil adjuvant experiments with Salk poliovirus vaccines and with purified poliovirus vaccine lot 8. Lots A and B of Salk adjuvant vaccine were prepared from a single lot of starting material as were lots D, E and F. Many-fold increase in antibody response was obtained both for Salk vaccine and for purified poliovaccine through use of the mineral oil adjuvant. These lots of vaccine shown in Table V were the same as employed by Cutler and co-workers¹⁴ in large-scale studies in man. It should be noted, however, that the lot designations were changed so as to include a lot A of emulsified vaccine prepared by Dr. Jonas Salk.

Antigenic Stability.—Purified or Salk vaccine in adjuvant assayed 6 or 8 months after preparation gave values which were in the same range of activity as obtained for the material when tested fresh. The water-in-oil emulsions were stable for at least 2 years when stored in the refrigerator at 4° C.

DISCUSSION

A principal purpose for employment of any immunologic adjuvant is to achieve a more durable immunity of a higher level employing a smaller antigenic mass in a fewer number of doses. Freund's incomplete mineral oil adjuvant, such as employed in the present studies, consists of a water (containing antigen)-in-oil (paraffin) emulsion which is stabilized by addition of Arlacel A, an ester of mannitol and oleic acid (mannide mono-oleate). Antigenic potentiation appears to reside in (a) the persistence of antigen at the site of injection (slow release), (b) in the systemic dissemination of the antigen throughout the animal

ANTIBODY TITERS, AQUEOUS AND ADJUVANT PURIFIED POLIOVIRUS VACCINES (LOT 10B)

MONKEY NEUTRALIZING ANTIBODY RESPONSE VS. TYPE					
I		II		III	
MAHONEY		MEF-1		SAUKETT	
RATE (%)	G.M.	RATE (%)	G.M.	RATE (%)	G.M.
1/11 (9)	—	7/11 (64)	8	0/11 (0)	—
9/11 (82)	18	11/11 (100)	32	7/11 (64)	5
5/10 (50)	7	10/10 (100)	18	1/10 (10)	—
11/11 (100)	315	11/11 (100)	440	11/11 (100)	104
0/12 (0)	—	1/12 (8)	—	0/12 (0)	—
11/12 (92)	30	11/12 (92)	50	9/12 (75)	7
5/12 (42)	3	11/12 (92)	13	2/12 (17)	—
12/12 (100)	549	12/12 (100)	630	12/12 (100)	157
12/12 (100)	330	12/12 (100)	294	12/12 (100)	157

TABLE V. COMPARISON, MONKEY POTENCY TEST RATIOS AND GEOMETRIC MEAN ANTIBODY TITERS, AQUEOUS AND ADJUVANT, SALK AND PURIFIED POLIOVIRUS VACCINES

VACCINE*		MONKEY SEROLOGIC RESPONSE VS. TYPE							
		I				II		III	
		PARKER		MAHONEY		MEF-1		SAUKETT	
		RATIO	G.M.	RATIO	G.M.	RATIO	G.M.	RATIO	G.M.
Salk									
Adjuvant	A	—	—	1.87	90	5.81	478	7.15	274
Adjuvant	B	—	—	2.64	128	10.11	831	17.60	674
Aqueous	Control	0.38	17	0.25	11	0.73	59	0.30	8
Adjuvant	D	9.19	512	4.01	362	8.80	955	5.80	775
Adjuvant	E	4.00	212	2.46	137	7.16	512	11.61	446
Adjuvant	F	8.57	274	4.93	239	12.44	723	20.21	588
Aqueous	Control	0.57	22	0.62	21	2.05	119	0.78	27
Adjuvant	G	8.58	337	8.58	294	13.33	775	18.99	675
Aqueous	Control	1.62	78	0.76	59	2.71	167	1.67	68
Purified									
Adjuvant†	C (Lot 8)	24.27	1100	14.94	667	10.11	830	18.85	549
Aqueous†	Control	8.0	274	4.60	223	2.90	157	4.72	147
NIH minimal passing ratio				0.29		0.25		0.16	

*Aqueous vaccines were diluted 1:2 so as to contain the same amount of antigen as the corresponding adjuvant preparation. Vaccination schedule was according to the regulations, i.e., 3 doses, 0.5 ml., 1 week apart with bleeding 1 week after the third dose.

†Content: I, 0.75γ; II, 0.1γ; III, 0.2γ per 0.5 ml. dose.

in emulsified form to other sites of antibody formation, and (c) in the stimulation of formation and/or accumulation of mononuclear cells, in the local lesions, which may be responsible for antibody formation.

Consistent with the findings for other antigens, e.g., influenza,¹⁶ Freund's incomplete adjuvant was highly effective in potentiating a neutralizing antibody response to killed poliovirus antigen either in ordinary Salk vaccine or in the highly purified poliovaccine preparation. Far greater antibody response was achieved for the adjuvant vaccine than when equivalent aqueous antigen was given. Additionally, considerably higher antibody titers were generally attained than when greater numbers of injections of aqueous vaccine containing larger amounts of antigen were employed.

Gross pathologic reactions to the adjuvant vaccine were minimal. None of the monkeys showed any apparent local or systemic reaction to the vaccine. Induration and swelling on intradermal injection into guinea pigs were within the range of expectation compared with other adjuvant vaccines of this type. These reactions were limited and minor as evidenced by absence of hemorrhage, necrosis, or sloughing at the injection sites. Mice injected by the peritoneal route failed to show significant adverse clinical effect and there was no depression of expected weight gain. Clinical experiences with certain of these same lots of vaccine have been recorded earlier by Cutler and others¹⁴ and in a second report in this series.¹³

SUMMARY

Intramuscular administration in monkeys of Salk or purified poliovaccine in Freund's incomplete mineral oil adjuvant effected very marked potentiation

of neutralizing antibody response. Far higher levels of antibody were generally achieved with the use of fewer doses and lesser amounts of antigenic substance than when the corresponding aqueous material was used. There was no significant adverse clinical reaction to the adjuvant vaccines when given to monkeys by the intramuscular route and the reactions which occurred following intradermal injection into guinea pigs or intraperitoneal administration into mice were within the range of acceptability for such adjuvant material.

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