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DIPHTHERIA AND TETANUS TOXOIDS COMBINED WITH PERTUSSIS AND POLIOMYELITIS VACCINES*
CLINICAL TRIAL OF A QUADRUPLE ANTIGEN

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SHORTLY after the introduction of poliomyelitis vaccine for general use in children in 1955, consideration was given to the addition of this new antigen to the already widely accepted multiple antigen Diphtheria Toxoid, Pertussis Vaccine and Tetanus Toxoid (combined), or DPT. The first point for investigation was the stability of poliomyelitis vaccine in the presence of the other components of the mixture and the selection of a suitable preservative for the preparation as a whole. The second point for investigation was the response of infants under one year, and in particular under six months, to the poliomyelitis vaccine component, since the first dose of DPT is usually administered between three and six months of age in order to induce an early active immunity to whooping cough.

TABLE I.—COMPARISON OF POTENCIES OF POLIOMYELITIS VACCINES AND DPT POLIO VACCINES CONTAINING CORRESPONDING LOT, AVERAGE OF 10 LOTS

	*Geometric mean antibody levels in monkeys**			
	DPT polio vaccine		*Poliomyelitis vaccine*	
Type	*Level*	*Log_2 level*	*Level*	*Log_2 level*
1	≧ 67**	≧6.06	≧ 34	≧5.10
2	≧≧140	≧≧7.13	≧≧116	≧≧6.86
3	≧105	≧6.72	≧ 63	≧5.98

*10-16 animals used in test of each vaccine lot.
**Reciprocal of serum dilution.

as DPT Polio Vaccine, were prepared for further stability studies, and in such a manner that any lot showing suitable characteristics might ultimately be used for clinical trials. The formalin-inactivated poliomyelitis vaccine component, containing benzethonium chloride 1:40,000 as a preservative, was from a lot previously released by the Laboratory of Hygiene, Ottawa, for general distribution. To this were added ultrafiltered, concentrated formol diphtheria and tetanus toxoids specially prepared with benzethonium chloride 1:20,000 as a preservative, and concentrated *H. pertussis* vaccine prepared in a manner similar to that used for general distribution but with benzethonium chloride as a preservative. The final product contained, in each ml., 40 Lf diphtheria toxoid, 8 Lf tetanus toxoid, 15,000 million *H. pertussis* in phase 1, and 0.92 ml. trivalent, formalin-inactivated poliomyelitis vaccine.

In Table I are summarized data on comparisons of the potencies, in monkeys, of 10 lots of poliomyelitis vaccine, and the poliomyelitis vaccine components of the DPT Polio Vaccines prepared from corresponding lots. The serum levels shown are the reciprocals of the geometric means of the titres produced in monkeys. These are also expressed as logarithms to the base 2.

From the table it can be seen that DPT Polio Vaccine produced somewhat higher levels for each type than did poliomyelitis vaccine alone. However, the difference is significant at the 5% level ($t = 3.2$, $P < 0.02$) for type 1 only.

In Table II are shown the results of stability tests on the lot of DPT Polio Vaccine used in the clinical trial. These data show no loss of potency over a period of 14 months at 4° C.

CLINICAL TRIAL

The lot for which data are presented in Table II was selected for clinical trial on the basis of its stability at six months and its suitability for other reasons. It had been held for eight months at 4° C. by the time the first dose was administered in the trial and 10 months when the last dose was given.

TABLE II.—STABILITY OF POLIOMYELITIS VACCINE COMPONENT IN DPT POLIO VACCINE

Age of DPT polio vaccine (months)	*Number of animals*	*Log_2 of geometric mean antibody level in monkeys*		
		Type 1	*Type 2*	*Type 3*
0	6	6.33 ± 1.03	6.83 ± 1.03	≧ 7.17 ± 1.38
6	16	≧ 5.75 ± 0.45	≧ 7.25 ± 0.37	≧ 5.75 ± 0.80
11	16	4.17 ± 0.72	6.38 ± 0.62	4.63 ± 0.98
14	16	≧ 6.50 ± 0.35	≧ 8.16 ± 0.36	≧ 8.25 ± 0.33

Through the co-operation of Dr. Lloyd A. Clarke, Medical Officer of Health, and Dr. John S. Kitching, Deputy Medical Officer of Health of Hamilton, Ontario, the trial was carried out in one of their immunization clinics. Fifty-one infants received 3 doses of the DPT Polio Vaccine. Of these, 12

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TABLE III.—RESPONSE (PERCENT CONVERSION) TO POLIOMYELITIS VACCINE AND TO DPT POLIO VACCINE PRIMARY VACCINATION—Q SERIES (INFANTS 3-12 MONTHS)

Series	Total in Series	Negative to type 1 Ratio	%	2 Ratio	%	3 Ratio	%
DPT polio; polio vaccine (72)	51	23/24	96%	24/24	100%	24/24	100%
Control polio vaccine only (72)	8	5/5	100%	5/5	100%	6/6	100%
K_2 series polio vaccine only (49 or 58)	54	26/35	74%	33/33	100%	32/41	78%
				Tri-negative			
DPT polio; polio vaccine (72)	51	12/13	92%	13/13	100%	13/13	100%
Control polio vaccine only (72)	8	4/4	100%	4/4	100%	4/4	100%
K_2 series polio vaccine only (49 or 58)	54	13/19	68%	19/19	100%	16/19	84%

K_2 series—2 doses (lot 49 or 58) DPT polio and control (lot 72)—3 doses

were three months of age, 21 were four months, 6 were five months and the remaining 12 were evenly distributed over 6-12 months. Owing to restrictions imposed by the available infant population in that locality, only 8 controls received poliomyelitis vaccine alone from the master lot used in the preparation of the DPT Polio Vaccine.

Each infant received three doses of DPT Polio Vaccine, or poliomyelitis vaccine alone, at intervals of four weeks. Those under six months of age received 0.5 ml. for the first dose and two additional doses of 1.0 ml. Those over six months received three doses of 1.0 ml. No undue reactions were observed. Blood samples were obtained from each infant before immunization and 14 days after the third dose. These samples were assayed for antibodies to the three types of poliovirus, and diphtheria and tetanus antitoxin titres were determined. Studies of agglutination titres for *H. pertussis* were given lowest priority and have not yet been completed in those cases where serum samples remain after the other assays.

TABLE IV.—DPT POLIO VACCINE. DIPHTHERIA ANTITOXIN TITRES: Q SERIES (51 INFANTS 3-12 MONTHS)

Units/ml. serum	Pre-immunization number of samples	14 days Post-immunization number of samples
<0.01	29	5*†
= or > 0.01	22	46
= or > 0.1	2	36
= or > 1.0	1	14

*All had maternal antibody of > 0.01 < 1.0.
†Retitration of these 5 samples:
3/5 < 0.001
1/5 = or > 0.008
1/5 = or > 0.002

Fig. 1 shows the percentage distribution of poliomyelitis antibodies to the three types of poliovirus before and after initial vaccination. The vertical line over the histogram of each type indicates the median level for the 51 infants. Before vaccination, approximately 50% of infants possessed antibody which was undoubtedly of maternal origin, especially in those under 6 to 8 months of age. After vaccination there was a marked shift in antibody level. The median level rose from 4 to 64 in type 1 and from 4 to 128 in types 2 and 3.

Table III shows the percentage of infants originally without antibody (negative in a dilution of 1 in 4) either for a single type (negative for type) or for all three types (triple negatives) who developed antibody upon vaccination. Of those negative for type, only one infant failed to respond to type 1; all responded to types 2 and 3. An identical picture is shown in the triple negatives. This response is significantly better for types 1 and 3 than in the K_2 series[1] where the infants received only 2 doses of poliomyelitis vaccine alone for initial immunization. All infants responded to the three types after vaccination with the control lot of poliomyelitis vaccine from which the DPT Polio Vaccine was prepared, but the numbers are too small for comparisons.

Table IV presents the diphtheria antitoxin titres before and after immunization. A significant number (25) showed a maternal antibody level of 0.01 unit or more before vaccination. Five of the infants failed to achieve a level of 0.01 unit 14 days after the third dose.

All infants responded to tetanus toxoid (Table V). The protective level is generally accepted as being ± 0.1 unit per ml.; 90% of the infants exceeded this by 10-fold to 1000-fold or more. The one infant with maternal antibody responded well.

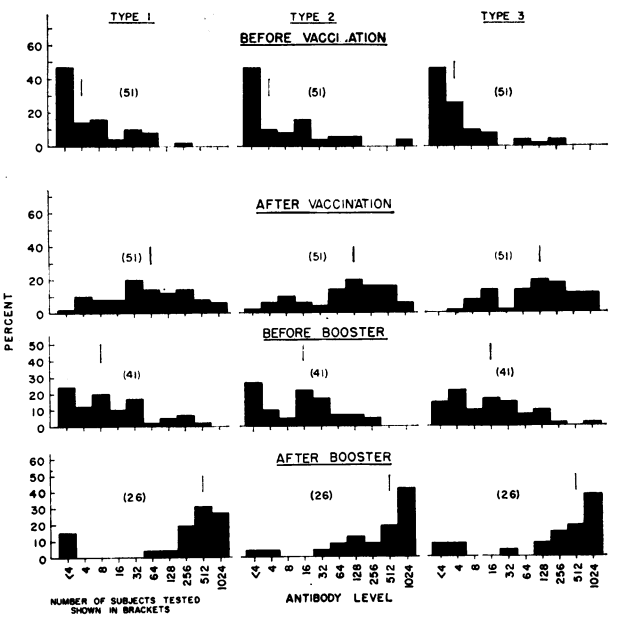

Fig. 1.—Percentage distribution of poliovirus antibody levels: infants 3 to 12 months of age given DPT Polio Vaccine (Q series).

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TABLE V.—DPT POLIO VACCINE. TETANUS ANTITOXIN TITRES: Q SERIES (51 INFANTS 3-12 MONTHS)

Units/ml. serum	*Pre-immunization number of samples*	*14 days Post-immunization number of samples*
<0.1	50	0
= or > 0.1	1*	51
= or > 1.0	0	46
= or > 10		23

*Rose to > 1 < 10

RESPONSE TO A RECALL DOSE

Twelve months after the initial course of three injections, a fourth dose, 1.0 ml., of DPT Polio Vaccine was administered. Of the original 51 infants, blood samples were obtained from 41 before the recall dose, and from 26 two to three weeks after the recall dose. From Fig. 1 it may be seen that 12 months after the initial three doses of DPT Polio Vaccine there were small declines in antibody levels. For type 1, 76% had a titre of > 4, for type 2, 73% and for type 3, 85%. The median titres dropped from 64 to 8 for type 1 and from 128 to 16 for types 2 and 3.

TABLE VI.—DPT POLIO VACCINE. Q SERIES (INFANTS 3-12 MONTHS): INFANTS FAILING TO RESPOND AFTER RECALL DOSE.

Subject No.	*Age at vaccination*	*Specimen**	*Antibody Titre Type 1*	*Type 2*	*Type 3*
QB 7.....	5 mo.	A	256	≷1024	64
		B	64	256	64
		C	<4	<4	<4
		D	<4	<4	<4
QB 10.....	3½ mo.	A	64	32	4
		B	16	8	16
		C	<4	<4	4
		D	<4	64	256
QB 27.....	12 mo.	A	<4	<4	<4
		B	<4	64	128
		C	<4	4	16
		D	<4	≷1024	256
QB 30.....	2½ mo.	A	64	16	64
		B	4	4	16
		C	<4	<4	<4
		D	<4	32	<4

*Specimen A—pre-vaccination
B—post-initial vaccination
C—pre-recall
D—post-recall

After the recall dose (Fig. 1) the median titre rose from 8 to 512 for type 1, and from 16 to 512 for types 2 and 3. Fifteen per cent of infants failed to respond to type 1, 4% to type 2 and 8% to type 3. In Table VI are shown those infants who failed to respond. In each instance maternal antibody was present before immunization in a titre of 1:64 or greater, except in No. QB 27, an infant of 12 months, who was initially triple negative and who failed to respond to type 1, either after initial immunization or after the recall dose.

In Table VII are shown the diphtheria antitoxin titres of 38 of the original 51 infants one year after initial immunization. Seven had less than 0.01 unit and 31 more than 0.01 unit. In only 23 of these infants was a post-recall sample obtained. The five infants with less than 0.01 unit

TABLE VII.—DPT POLIO VACCINE. DIPHTHERIA ANTITOXIN TITRES: Q SERIES (INFANTS 3-12 MONTHS). RESPONSE TO RECALL DOSE 12 MONTHS AFTER PRIMARY IMMUNIZATION

Units/ml. serum	*No. pre-recall specimens—38*	*No. post-recall specimens—23*
<0.01	7 (5)*	0
= or > 0.01	31 (18)	23
= or > 0.1	14 (7)	23
= or > 1.0	1 (1)	22
= or > 10.0	1 (1)	19

*Figures in parentheses represent the number of infants from whom both pre-recall and post-recall specimens were obtained.

responded to the recall dose; all had more than 0.1 unit, 22 out of 23 had 1.0 unit or more and 19 out of 23 had 10.0 units or more.

One year after initial immunization only one of 35 infants had less than 0.1 unit tetanus antitoxin (Table VIII). All responded well to the recall

TABLE VIII.—DPT POLIO VACCINE. TETANUS ANTITOXIN TITRES: Q SERIES (INFANTS 3-12 MONTHS). RESPONSE TO RECALL DOSE 12 MONTHS AFTER PRIMARY IMMUNIZATION

N.I.H. units /ml. serum	*No. pre-recall specimens—35*	*No. post-recall specimens—22*
<0.1	1 (1)*	0
= or > 0.1	34 (20)	22†
= or > 1.0	10 (6)	22
= or > 10.0	2 (2)	18
= or > 100.0	0 (0)	8

*Figures in parentheses represent the number of infants from whom both pre-recall and post-recall specimens were obtained.
†Post-recall specimen only from one infant.

dose and attained a titre of at least 1.0 unit. Of the 22 infants 18 had 10 units or more and 8 had 100 units or more.

EFFECT OF MATERNAL IMMUNITY

The relationship of maternal antibody at the time of initial vaccination to the results after a recall dose of DPT Polio or poliomyelitis vaccine (controls) is shown in Table IX. After six months of age maternal antibody had, for the most part, disappeared. There was a slightly higher percentage response in those infants with no maternal antibody. This is most evident in the age group 2 to 5 months.

TABLE IX.—RESPONSE TO VACCINATION IN INFANTS AT DIFFERENT MONTHS OF AGE WITH AND WITHOUT MATERNAL ANTIBODY. THREE DOSES DPT POLIO VACCINE OR POLIO VACCINE WITH RECALL DOSE ONE YEAR LATER

	Proportion positive (conversion) 14 to 21 days after recall dose					
	Initially negative to type			*Initially positive to type (maternal antibody)*		
Age in months	*Type 1*	*Type 2*	*Type 3*	*Type 1*	*Type 2*	*Type 3*
2-5	9/9	11/11	11/11	13/16	13/14	12/14
6-8	2/2	1/1	1/1	—	1/1	1/1
9-12	3/4	3/3	4/4	—	1/1	—
Total 2-12	14/15 =93%	15/15 =100%	16/16 =100%	13/16 =81%	15/16 =94%	13/15 =87%

DISCUSSION

The age at which immunization with DPT Polio Vaccine should be initiated is determined by the desire to achieve immunity to whooping cough as early as possible. At three months of age a considerable proportion of infants (in this series between 40 and 50%) possess maternal antibody to both poliomyelitis and diphtheria. While the antibody levels after primary immunization were highly satisfactory, it was not possible to determine with any degree of accuracy, in those possessing maternal antibody initially, what proportion of the antibody after vaccination was maternal or actively acquired. One year after the initial series it was evident that a small number of infants had very low levels of antibody against the three types of poliovirus and less than 0.01 unit of diphtheria antitoxin. Even the tetanus antitoxin levels had declined, one infant showing a titre of less than 0.1 unit; here maternal antibody was not a factor. After the recall dose, it was evident that although levels had declined, the infants were sensitized by the initial immunization to respond to the recall dose; 85% responded to type 1 poliovirus, and over 90% to types 2 and 3. The response to diphtheria and tetanus toxoids was 100%, the majority possessing high titres. Since agglutinin titres to *H. pertussis* are not a significant measure of immunity, these determinations were given lowest priority and in most instances insufficient volumes of serum remain to carry out this procedure.

While it is evident from these data that maternal immunity may interfere, in some measure, with the responses to poliomyelitis vaccine and to diphtheria toxoid, it is also evident in the case of diphtheria toxoid that this interference may be overcome by the use of a good antigen initially, since pertussis vaccine acts as an adjuvant to diphtheria toxoid, and by the administration of an additional dose spaced some months after the initial series. It is also evident that while this additional dose is in the nature of a recall or reinforcing dose, it must be considered as an essential part of the establishment of good immunity in infants.

This trial was carried out under rigorous conditions. The first dose in those under six months was reduced to 0.5 ml. and the fourth dose was administered a full 12 months after the initial series. While it has been suggested in the past, in the case of DPT, that the first dose *may* be reduced to 0.5 ml. for infants under six months if it is desired to reduce reactions, it may be well to use the full 1.0 ml. dose, in the case of DPT Polio Vaccine, in order to increase the effectiveness of the type 1 poliovirus antigen and to administer the recall dose less than one year after the initial series. In addition it is evident that every effort must be made to increase the amount of type 1 antigen in poliomyelitis vaccines. This investigation is already under way.

SUMMARY

A stable "quadruple" antigen, DPT Polio Vaccine, has been prepared. The response of infants, the majority under six months, to three doses, followed by a fourth dose 12 months after the primary series, was highly satisfactory. Eighty-five per cent responded to type 1 poliovirus, and over 90% to types 2 and 3; 100% responded well to diphtheria and tetanus toxoids.

While the presence of maternal immunity interfered in some measure with the response to poliomyelitis vaccine and to diphtheria toxoid, the fourth dose, administered 12 months after the initial three doses, elicited the recall type of response and established a sound immunity.

The fourth dose, or first recall dose, must be considered essential in establishing immunity in infants.

The first dose of DPT Polio Vaccine should not be reduced in infants under six months, in order not to diminish the volume of the type 1 poliomyelitis vaccine component.

REFERENCE

1. MACLEOD, D. R. E. *et al.*: *Canad. M. A. J.*, 81: 443, 1959.

RÉSUMÉ

Les laboratoires de recherches médicales Connaught ont réussi à mettre au point sous forme stable un antigène quadruple comprenant de l'anatoxine antidiphtérique et antitétanique combinée à du vaccin antipoliomyélitique et anticoquelucheux. L'immunité conférée par trois injections de ce quadrivalent chez des nourrissons, suivies d'une quatrième un an plus tard, fut très satisfaisante. On trouva des anticorps I dans 85% des sujets et des anticorps II et III dans 90%. La réponse à la diphtérie et au tétanos fut de 100%. Bien que la présence d'anticorps maternels s'interposa dans une certaine mesure à l'action du vaccin antipoliomyélitique et de l'anatoxine antidiphtérique, la quatrième injection administrée douze mois après les trois injections du début produisit un effet de rappel et établit une immunité solide. Cette quatrième injection est donc une partie essentielle du programme d'immunisation, chez les enfants. Chez les nourrissons de moins de six mois la dose entière d'antigène quadruple doit être administrée afin de ne pas diminuer la portion d'antigène type I de la poliomyélite.

MEDICAL SOCIETY INSTALS DRUG STORE READING RACKS

Doctors' reception rooms aren't the only good places for distributing medical public relations literature. The Medical Society of the State of Pennsylvania is working with the state pharmaceutical association to use drug stores as additional distribution centres. When a local pharmaceutical association expresses interest in pamphlet distribution, the state medical society dispatches a representative to explain the program to the group. If the druggists agree, each member is supplied with a pamphlet rack and a quantity of pamphlets to display in his drug store. The society replenishes the supply periodically with up-to-date materials.

The whole operation is carefully coordinated with the state pharmaceutical association. That organization notifies its county groups of the program through its own publications and relays their interest back to the medical society. The state pharmaceutical association also supplies mailing lists and advises the medical society when to restock the druggists' literature.

It's all part of Pennsylvania's "Safeguard Your Health" educational program. TV and radio stations, newspapers, doctors' offices and now drug stores are being utilized to build a health education system that reaches almost everyone in the state.—*The PR Doctor* (Communications Division, American Medical Association), May 1959.