Diphtheria Immunization with Fluid Toxoid and Alum-Precipitated Toxoid*

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THE purpose of this study was to investigate the height and duration of the antitoxin response following injection with fluid or alum-precipitated toxoid. When the study began in 1936, immunization against diphtheria was attempted largely by one injection of alum-precipitated toxoid, or two or three injections of fluid toxoid. It was felt to be highly desirable to determine which of these was the method of choice. The most conclusive study would have been a comparison of the resistance to actual exposure to diphtheria following the different immunizing procedures. Since the low incidence of diphtheria made this impossible, it was decided that the next most valuable study would be a comparison of the antitoxin response and the durability of that response.

METHODS

The study has been carried out on 2,487 free-living children from rural schools and communities in Saginaw County, Mich., a county of about 1,110 square miles.

The immunity response has been followed by blood serum titrations alone. No Schick tests were used because the Schick test itself has an antigenic effect, especially on a child with circulating antitoxin in its blood. Eliminating the Schick test made it possible:

1. To observe the comparative antitoxin response to the various immunizing procedures alone.
2. To follow the duration of the response without giving additional secondary stimulations.
3. To determine the responses to be expected in routine immunization when the Schick test is not used. The decreasing use of the Schick test made this important.

Therefore, before giving any antigenic stimulation, the children were bled. Four or five ml. of blood were taken in vacuum tubes (Keidel or Kimball). Each child was bled again 4 months after the first injection and at 12 months and every 12 months thereafter for the duration of the study.

The blood was titrated essentially by the method of Fraser.[1] The clotted blood was centrifuged at approximately 1,200 r.p.m. for 1 hour, the serum drawn off aseptically, and stored at 2–10° C. The serum of children not previously immunized was first tested to determine if it contained more or less than 0.001 unit of antitoxin per ml. by comparing the reactions in the skin of a rabbit on the intradermal injections of the following mixtures:

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Serum Control	0.2 ml. serum + 0.2 ml. saline
Test of Serum for 0.001 Unit	0.2 ml. serum + 0.2 ml. toxin dilution
Standard Control	1.0 ml. N.I.H. antitoxin + 1.0 ml. toxin dilution

The antitoxin in the standard control was the standard diphtheria antitoxin of the National Institute of Health. This was diluted 1:10 with 66 per cent neutral glycerine, in which dilution it keeps indefinitely in the cold. It was then made up to 1:6,000 with saline just before use. At this dilution 1 ml. contained 0.001 unit. The same toxin (No. 358) was used throughout the study and was diluted 1:6,700–1:7,200, varying a little with each new bottle. The optimum toxin dilution was determined by mixing several dilutions of the toxin with each of four dilutions of the National Institute of Health standard antitoxin, namely, 0.001 unit per ml., 0.002 unit per ml., 0.004 unit per ml., and 0.01 unit per ml. That dilution of toxin was chosen which showed the greatest difference in size between the reactions —usually that dilution which gave a + reaction on injection of the 0.001 unit mixture, a ± reaction to the 0.002 unit, and a negative reaction to the 0.004 and 0.01 unit mixtures. The toxin dilution was always added to the serum, the tubes were shaken, and allowed to stand ½ hour at room temperature. The syringes were filled while waiting, and the intradermal injections of 0.1 ml. of the mixtures made within a second ½ hour period. Clipped white rabbits were used, weighing from 5 to 8½ pounds. Forty-eight injections were placed on each rabbit, including six control injections, three at the front and three at the back. Readings were made on the 3rd and 4th day after injection by comparing the size of the reactions with those of the control injections. The reactions on the front half of the rabbits were compared with the controls on the front half, and those on the rear half with the rear controls.

If the serum proved to contain more than 0.001 unit it was retitrated, using the following dilutions:

0.01 Unit	0.1 ml. serum	+ 0.9 ml. saline A
0.1 Unit	0.2 ml. A	+ 1.8 ml. saline B
1.0 Unit	0.2 ml. B	+ 1.8 ml. saline

After this titration determined the range in which the titer fell, the serum was again tested. The units tested for in the study were:

0.002	0.02	2.0	16.0
0.004	0.04	4.0	32.0
0.01	0.1	8.0	

The toxin was diluted for all mixtures in Fraser's buffer diluent which has the following formula:

Sorensen's borate—boric acid buffer:
- 12.404 gm. H_3BO_3
- 100 ml. N NaOH
- Water to 1 liter

To one liter add 818.1 ml. 0.1 N HCl to pH 7.9

- 1,070 ml. buffer pH 7.9
- 36.4 gm. NaCl
- 2,210 ml. H_2O

Autoclave 1 hour at 120° C.

0.85 gm. gelatin — Autoclave 1 hour at 120° C.

Add gelatin and sterile H_2O to make 4,280 ml. after autoclaving.

The following procedures were used in immunization of children:

1. 1 injection of fluid toxoid
2. 2 injections of fluid toxoid (3 weeks apart)
3. 3 injections of fluid toxoid (3 weeks apart)
4. 1 injection of alum-precipitated toxoid
5. 2 injections of alum-precipitated toxoid (3 weeks apart)

The toxoid used was obtained from a commercial firm by Dr. W. T. Harrison of the National Institute of Health so that it would be representative of toxoid generally available. The alum-precipitated toxoid was prepared from the fluid toxoid used in the study. This was felt to be important because of the possibility that two different preparations of toxoid might vary in some in-

trinsic antigenic efficacy for which we have no method of determination. The National Institute of Health tests showed the fluid toxoid to contain 20 L_f per ml. and the alum-precipitated toxoid to stimulate production of an average of 2–4 units of antitoxin in guinea pigs in the National Institute of Health control test for alum-precipitated toxoid. On the re-solution of the alum-precipitated toxoid it was likewise found to contain 20 L_f per ml. These tests were repeated and verified by the Michigan Department of Health.

A diphtheria carrier survey was carried out in the schools during the study. Throat cultures were taken at intervals from all the school children, irrespective of whether they had received immunizing injections. The throat cultures were examined by the Bureau of Laboratories of the Michigan Department of Health according to the following procedure:

Inoculate a tellurite plate and a Loeffler's plant with the specimen submitted. Incubate at 37° C. for 18–24 hours. Prepare smears from the Loeffler's slant and stain with Loeffler's methylene blue. If diphtheria-like organisms are found, re-incubate the tellurite plate for another 24 hours. Subculture typical colonies to Loeffler's medium and incubate 24 hours at 37° C. Wash off the growth with 2.0 ml. of tryptose broth. Inoculate 1 ml. subcutaneously into the abdominal wall of a guinea pig weighing 250–300 gm., and 1 ml. into a control pig of equal weight which has been previously injected intraperitoneally with 500 units of diphtheria antitoxin.

Autopsy pigs at death or at the end of 72 hours. Consider any cultures as toxigenic if the unprotected guinea pig exhibits edema, necrosis at site of inoculation, and hemorrhagic suprarenals, and the protected control guinea pig is normal at the end of the test period. Consider any culture as non-toxigenic if both pigs are normal at autopsy.

The carrier survey was felt to be essential to the evaluation of the antitoxic response obtained by the various immunizing procedures because one might expect a much higher antitoxin response in a locality with a high carrier rate of virulent diphtheria organisms and a lower response in a locality where exposure to virulent diphtheria organisms is a rarity.[2]

RESULTS

There are several reasons why the results of this study may be considered indicative of a safe minimum response to be expected from each of the immunizing procedures studied. They are:

1. The children were free-living children from 150 rural schools and their vicinities. A higher antigenic response might be expected from urban children or institutional children.

2. The children were living in a low diphtheria environment as shown by the results of the carrier survey (Table 1) and the incidence of diphtheria (Table 2). A higher response would be expected in the presence of a higher carrier or diphtheria rate.

3. The children were living in a northern state. Children in a southern state might be expected to give a greater antigenic response.[3]

TABLE 1

Saginaw County Diphtheria Survey
(Total Number of Cultures Taken: 31,363)

Time	*Number of Children*	*Number of Children with Positive KL Culture*		*Number of Children with Pathogenic KL Culture*		*Number of Children with Non-pathogenic KL Culture*		*Number of Children with Positive KL Culture (No Virulence Test Made)*	
		Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
1936–1940	13,998	121	0.86	35	0.25	72	0.51	14	0.10

TABLE 2

Incidence of Diphtheria

Year	*1936*	*1937*	*1938*	*1939*	*1940*	*1941*
Cases of Diphtheria	4	3	0	0	0	0

TABLE 3

Age Distribution of Children in the Study

Age	1 A.P.	2 A.P.	1 Fluid	2 Fluid	3 Fluid	1 Fraser	2 Fraser	3 Fraser	Titr. No. Toxoid	Total	Per cent
8 mo.	1	1	1	..	..	..	..	..	..	3	....
1 yr.	5	4	..	2	..	..	..	..	..	11	....
1½ yr.	2	2	..	2	..	..	..	..	..	6	....
2 yr.	7	2	3	3	6	1	..	1	1	24	....
3 yr.	10	5	2	8	6	..	..	1	2	34	....
4 yr.	38	12	2	19	18	1	..	2	1	93	....
5 yr.	94	75	13	51	70	9	6	66	2	386	....
Total	157	101	21	85	100	11	6	70	6	557	22.3
6 yr.	153	123	22	110	158	6	3	65	..	640	....
7 yr.	131	77	8	70	84	1	1	24	4	400	....
8 yr.	89	51	10	31	59	1	..	10	4	255	....
9 yr.	57	50	6	34	62	..	..	4	..	213	....
10 yr.	31	25	5	31	36	..	..	..	2	130	....
Total	461	326	51	276	399	8	4	103	10	1,638	65.9
11 yr.	20	9	2	14	31	..	..	2	..	78	....
12 yr.	23	13	3	20	27	..	..	..	..	86	....
13 yr.	14	4	3	15	19	..	..	..	1	56	....
14 yr.	13	2	7	8	14	..	..	..	..	44	....
15 yr.	6	..	2	2	5	..	..	..	2	17	....
16 yr.	6	..	1	..	..	..	..	..	..	7	....
17 yr.	4	..	..	..	..	..	..	..	..	4	....
Total	86	28	18	59	96	..	..	2	3	292	11.8
Grand Total	704	455	90	420	595	19	10	175	19	2,487	100.0

The fact that 65.9 per cent of the children were 6 to 10 years of age, and 22.3 per cent were in the preschool age group (Table 3) makes the results of the study directly applicable to routine immunization against diphtheria, since these are the age groups usually concerned.

For part of the study, alternate children in a group were given two different immunizing procedures in order to have a strictly controlled comparison. These were called " Controlled Groups." For the rest of the study, different immunizing procedures were used in alternate schools or communities. These are listed as " Uncontrolled Groups " (Table 4).

It is evident that the results are comparable between the " Controlled " and the " Uncontrolled " groups when the responses of those children having less that 0.001 unit of antitoxin at the time

TABLE 4

Comparison of Antitoxin Response to the Different Immunizing Procedures in Controlled and Uncontrolled Groups
(Antitoxin level at time of 1st injection <0.001)

Immunizing Preparation and Procedure	Group	Four Months: No. in Group	Four Months: Children with 0.001 or More: No.	Four Months: Children with 0.001 or More: Per cent	Twelve Months: No. in Group	Twelve Months: Children with 0.001 or More: No.	Twelve Months: Children with 0.001 or More: Per cent
2 Injections fluid	Controlled	129	82	63.5	33	55	62.5
3 Weeks apart	Uncontrolled	31	20	64.5	23	12	52.1
1 Injection A.P.	Controlled	116	105	90.5	101	86	85.1
	Uncontrolled	236	222	94.0	226	199	88.0
3 Injections fluid	Controlled	113	113	100.0	..	..	....
3 Weeks apart	Uncontrolled	49	46	93.8	49	47	95.9
2 Injections A.P.	Controlled	138	138	100.0	..	..	....
3 Weeks apart	Uncontrolled	10	10	100.0	..	..	....

TABLE 5

Comparison of Antitoxin Response to the Different Immunizing Procedures
(All Cases having <0.001 Unit at Time of Injection)

Immunizing Procedure		*4 Days*				*10 Days*				*4 Months*				*12 Months*			
		Number of Cases	*Cases with 0.001 Unit or More*	*Cases with 0.01 Unit or More*	*Cases with 0.1 Unit or More*	*Number of Cases*	*Cases with 0.001 Unit or More*	*Cases with 0.01 Unit or More*	*Cases with 0.1 Unit or More*	*Number of Cases*	*Cases with 0.001 Unit or More*	*Cases with 0.01 Unit or More*	*Cases with 0.1 Unit or More*	*Number of Cases*	*Cases with 0.001 Unit or More*	*Cases with 0.01 Unit or More*	*Cases with 0.1 Unit or More*
1 Injection 1 ml. Fluid	No.	34	1	1	0	130	17	10	5	39	8	3	1	39	4	2	0
	%	..	3	3	0	..	13	8	4	..	21	8	3	..	10	5	0
2 Injections 1 ml. Fluid	No.	..	..	..	..	..	..	..	..	243	157	71	18	235	126	53	15
	%	..	..	..	..	..	..	..	..	..	65	29	7	..	54	23	6
3 Injections 1 ml. Fluid	No.	..	..	..	..	..	..	..	..	566	528	380	114	544	490	335	115
	%	..	..	..	..	..	..	..	..	..	93	67	20	..	90	62	21
1 Injection 1 ml. A.P.	No.	19	0	0	0	87	22	16	15	374	345	210	54	367	316	170	27
	%	..	0	0	0	..	25	18	17	..	92	56	14	..	86	54	7
2 Injections 1 ml. A.P.	No.	..	..	..	..	..	..	..	..	310	310	296	149	300	298	268	110
	%	..	..	..	..	..	..	..	..	..	100	96	48	..	99	89	37

Immunizing Procedure		*24 Months*				*36 Months*				*48 Months*				*60 Months*			
		Number of Cases	*Cases with 0.001 Unit or More*	*Cases with 0.01 Unit or More*	*Cases with 0.1 Unit or More*	*Number of Cases*	*Cases with 0.001 Unit or More*	*Cases with 0.01 Unit or More*	*Cases with 0.1 Unit or More*	*Number of Cases*	*Cases with 0.001 Unit or More*	*Cases with 0.01 Unit or More*	*Cases with 0.1 Unit or More*	*Number of Cases*	*Cases with 0.001 Unit or More*	*Cases with 0.01 Unit or More*	*Cases with 0.1 Unit or More*
1 Injection 1 ml. Fluid	No.	35	4	1	0	25	3	1	1	8	1	0	0	..	..	..	..
	%	..	11	3	0	..	12	4	4	..	13	0	0	..	..	..	..
2 Injections 1 ml. Fluid	No.	226	113	48	13	168	77	32	5	87	42	17	2	..	..	..	..
	%	..	50	21	6	..	46	19	3	..	48	20	2	..	..	..	..
3 Injections 1 ml. Fluid	No.	388	335	218	62	175	163	98	26	42	40	24	6	..	..	..	..
	%	..	86	56	16	..	93	56	15	..	95	57	14	..	..	..	..
1 Injection 1 ml. A.P.	No.	394	317	139	24	359	256	92	18	297	199	66	15	114	76	25	6
	%	..	81	35	6	..	71	26	5	..	67	22	5	..	67	22	5
2 Injections 1 ml. A.P.	No.	299	295	262	94	167	164	143	52	..	..	..	..	..	..	..	..
	%	..	99	88	31	..	98	86	31	..	..	..	..	..	..	..	..

TABLE 6

Comparison of Antitoxin Levels in a Group of Children Having Three Injections of Fraser's Fluid Toxoid (0.5 ml.–0.5 ml.–1 ml.) in Children Having < 0.001 Units of Antitoxin at the Time of Injection

	Kind of Toxoid	No. of Children	Units of Antitoxin per One ml. of Serum: < 0.001 No.	< 0.001 Per cent	0.001 < 0.004 No.	0.001 < 0.004 Per cent	0.004 < 0.01 No.	0.004 < 0.01 Per cent	0.01 < 0.04 No.	0.01 < 0.04 Per cent	0.04 < 0.1 No.	0.04 < 0.1 Per cent	0.1 Plus No.	0.1 Plus Per cent
Four Months	Fraser	139	18	13	12	9	26	19	39	28	24	17	20	14
	U.S. P. H. S. Toxoid	428	21	5	53	12	57	13	132	31	71	17	94	22
	Total	567	39	7	65	11	83	15	171	30	95	17	114	20
Twelve Months	Fraser	127	13	10	8	6	21	17	37	29	19	15	29	23
	U.S. P. H. S. Toxoid	417	41	10	62	15	63	15	111	27	54	13	86	21
	Total	544	54	10	70	13	84	15	148	27	73	13	115	21

of injection are compared. This suggests that, in studies on free-living children with no history of previous diphtheria immunization, it may not be necessary to have strictly controlled groups using alternate children if the children having less than 0.001 unit of antitoxin are used and if the diphtheria environments are similar. Since the results with the two groups are similar, they are combined in the rest of the paper to simplify presentation.

The antitoxic response of all children having less than 0.001 unit of antitoxin per ml. of circulating serum at the time of the first immunizing injection is given in Table 5. The response is analyzed at three different antitoxin levels; that is, in terms of the number and percentage of children developing 0.001 unit or more, 0.01 unit or more, or 0.1 unit or more of antitoxin per ml. of circulating serum.

The results in Table 5 indicate that:

1. Under the conditions of this study, two injections of alum-precipitated toxoid or three injections of fluid toxoid at 3 week intervals cause the highest and consequently the most lasting antitoxic response.

2. One injection of alum-precipitated toxoid is superior to 2 injections of fluid toxoid.

3. The immunizing procedure which raises the most children from no detectable antitoxin to a detectable level also raises the most children to higher antitoxin levels.

4. Some antitoxin response may be under way within 10 days of an injection of either alum-precipitated or fluid toxoid. This is in accord with the observations of Jensen.[4]

Table 5 reëmphasizes the increased antigenic efficiency conferred by the alum-precipitation of toxoid, since the average antitoxin response to a single injection of alum-precipitated toxoid is so much greater than that to either one or two injections of fluid toxoid.

Fitzgerald, et al.,[5] and Fraser and Halpern[6] observed an increase in antitoxin following injection of alum-precipitated toxoid comparable to those we are reporting. They observed a much greater response to 3 injections of fluid toxoid than we found. In an attempt to solve this discrepancy, a group of children were injected at 3 week intervals with 0.5, 0.5, and 1.0 ml. of fluid toxoid.* The resulting antitoxin response of children having less than 0.001 unit of antitoxin at the time of injection is shown in Table 6. It is evident that the results with the Fraser toxoid and dosage was comparable to

* Obtained from the Connaught Laboratories through the coöperation of Dr. Fraser.

TABLE 7

Comparison of Antitoxin Response to the Different Immunizing Procedures
(All Cases Having 0.001 Unit or More at the Time of Injection)

Immunizing Procedure		4 Months				12 Months				24 Months				36 Months				48 Months				60 Months			
		Number of Cases	Cases with 0.001 Unit or More	Cases with 0.01 Unit or More	Cases with 0.1 Unit or More	Number of Cases	Cases with 0.001 Unit or More	Cases with 0.01 Unit or More	Cases with 0.1 Unit or More	Number of Cases	Cases with 0.001 Unit or More	Cases with 0.01 Unit or More	Cases with 0.1 Unit or More	Number of Cases	Cases with 0.001 Unit or More	Cases with 0.01 Unit or More	Cases with 0.1 Unit or More	Number of Cases	Cases with 0.001 Unit or More	Cases with 0.01 Unit or More	Cases with 0.1 Unit or More	Number of Cases	Cases with 0.001 Unit or More	Cases with 0.01 Unit or More	Cases with 0.1 Unit or More
1 Injection	No.	12	12	12	12	12	11	11	11	11	9	9	9	3	2	2	2	1	1	1	1	..	..	..	..
1 ml. Fluid	%	..	100	100	100	..	92	92	92	..	82	82	82	..	..	..	..	..	..	..	..	..	..	..	..
2 Injections	No.	65	65	63	59	52	49	46	46	39	35	33	30	28	27	25	24	13	12	12	10	..	..	..	..
1 ml. Fluid	%	..	100	97	91	..	94	88	88	..	90	85	77	..	96	89	86	..	92	92	77	..	..	..	..
3 Injections	No.	104	104	104	100	99	99	98	95	74	74	74	71	27	27	27	26	6	6	6	6	..	..	..	..
1 ml. Fluid	%	..	100	100	96	..	100	99	96	..	100	100	96	..	100	100	96	..	..	..	..	..	..	..	..
1 Injection	No.	126	124	116	97	119	114	99	80	115	109	96	67	79	69	60	35	62	52	41	28	29	27	20	14
1 ml. A. P.	%	..	98	92	77	..	98	83	70	..	95	84	58	..	87	76	44	..	84	66	45	..	93	69	48
2 Injections	No.	71	70	70	65	66	65	64	57	45	45	44	37	30	30	29	25	..	..	..	..	..	..	..	..
1 ml. A. P.	%	..	99	99	92	..	99	97	86	..	100	98	82	..	100	97	83	..	..	..	..	..	..	..	..

that obtained in the rest of the study with fluid toxoid in three 1 ml. doses at 3 week intervals. We again did not obtain the high antitoxic response observed by the Canadian workers. We are unable to explain the discrepancy.

The response to the different immunizing procedures of all children having 0.001 unit or more at the time of injection is analyzed in Table 7.

As was to be expected, the response of these children to all immunizing procedures was excellent. There is a suggestion that fluid toxoid is more effective than alum-precipitated toxoid in boosting the antitoxin level where detectable circulating antitoxin already exists. This is only of academic interest, since the immunizing of the non-immune is the important problem. The actuality of this difference could not be gauged unless the results were analyzed on the basis of the amount of antitoxin present at the time of the first injection. Table 8 gives a comparison of the response of those children having 0.001 to 0.004 units of antitoxin at the time of injection.

Although the numbers are of necessity small, Table 8 does carry the same suggestion as Table 7 that fluid toxoid causes a better response than alum-precipitated toxoid when antitoxin is already present in a detectable amount. It is also interesting to note that even when 0.001 to 0.004 unit of antitoxin is present, three doses of fluid toxoid cause a better response than two, and two doses of alum-precipitated toxoid cause a better response than one.

It can be seen that there is little difference in the response of the different age groups to the immunization procedure used. It is important to know that the response of the preschool group is as good as any other, since this is the most important group to immunize from the standpoint of lowering the diphtheria mortality rate. Blum [7] likewise found the response of children in the age group 2–4 to be excellent following tetanus antitoxin immunization, but reported that those in the age group 1–2 gave a lower response.

There were no reactions of an allergic nature. From a total of 1,614 injections of alum-precipitated toxoid, two definite abscesses (both sterile) and two reactions suspicious for abscesses were observed. Three of these were in children having less than 0.001 unit of antitoxin and in one having 0.1 unit of antitoxin at the time of the first alum-precipitated injection. We attribute these to technic.

TABLE 8

Response of Children Having 0.001 to <0.004 Units at Time of Injection

Immunizing Procedure		*4 Months*		*1 Year*		*2 Years*		*3 Years*		*4 Years*	
		Number of Cases	*Cases with 0.1 Unit or More*	*Number of Cases*	*Cases with 0.1 Unit or More*	*Number of Cases*	*Cases with 0.1 Unit or More*	*Number of Cases*	*Cases with 0.1 Unit or More*	*Number of Cases*	*Cases with 0.1 Unit or More*
2 Injections	No.	13	10	12	7	11	5	9	5	4	2
1 ml. Fluid	%	..	77	..	58	..	46	..	56	..	50
3 Injections	No.	22	19	23	20	18	16	10	9	..	..
1 ml. Fluid	%	..	86	..	88	..	89	..	90	..	..
1 Injection	No.	27	13	30	10	32	6	24	2	20	1
1 ml. A. P.	%	..	48	..	33	..	19	..	8	..	5
2 Injections	No.	16	14	14	10	15	10	10	7	..	..
1 ml. A. P.	%	..	88	..	82	..	67	..	70	..	..

TABLE 9

Response of Children in Different Age Groups to the Various Immunizing Procedures (All Children Having <0.001 Unit at Time of Injection)

Age Group	Immunizing Procedure		4 Months			1 Year			2 Years			3 Years		
			Number of Cases	Cases with 0.001 Unit or More	Cases with 0.01 Unit or More	Number of Cases	Cases with 0.001 Unit or More	Cases with 0.01 Unit or More	Number of Cases	Cases with 0.001 Unit or More	Cases with 0.01 Unit or More	Number of Cases	Cases with 0.001 Unit or More	Cases with 0.01 Unit or More
Under 2 Years	3 Injections + 1 ml. Fluid	No.	3	3	3	2	2	2	2	2	2	..	..	..
		%	..	..	..	..	..	..	..	..	..	..	..	..
	1 Injection + 1 ml. A. P.	No.	1	1	1	1	1	1	..	..	..	..	..	..
		%	..	..	..	..	..	..	..	..	..	..	..	..
	2 Injections + 1 ml. A. P.	No.	3	3	3	4	4	4	4	4	4	..	..	..
		%	..	..	..	..	..	..	..	..	..	..	..	..
2 to 5 Years	3 Injections + 1 ml. Fluid	No.	118	114	82	121	116	89	67	62	46	28	27	20
		%	..	97	69	..	96	74	..	93	69	..	96	71
	1 Injection + 1 ml. A. P.	No.	54	51	38	57	53	36	69	66	35	53	44	10
		%	..	94	70	..	93	63	..	96	51	..	83	19
	2 Injections + 1 ml. A. P.	No.	68	67	67	63	63	58	67	67	62	..	..	..
		%	..	100	99	..	100	92	..	100	93	..	..	..
6 to 10 Years	3 Injections + 1 ml. Fluid	No.	328	314	236	324	305	206	247	221	143	122	117	67
		%	..	96	72	..	94	64	..	89	58	..	96	55
	1 Injection + 1 ml. A. P.	No.	206	199	123	205	192	109	214	191	84	209	179	68
		%	..	97	60	..	94	53	..	89	39	..	86	32
	2 Injections + 1 ml. A. P.	No.	230	230	221	218	218	204	200	199	170	..	..	..
		%	..	100	96	..	100	94	..	99	85	..	..	..
11 Years and Over	3 Injections + 1 ml. Fluid	No.	55	51	43	53	49	37	43	38	27	14	12	10
		%	..	93	78	..	92	70	..	88	63	..	86	71
	1 Injection + 1 ml. A. P.	No.	16	14	6	10	8	4	15	10	7	9	5	3
		%	..	87	37	..	80	40	..	67	47	..	56	33
	2 Injections + 1 ml. A. P.	No.	10	10	10	12	12	12	11	11	9	..	..	..
		%	..	100	100	..	100	100	..	100	82	..	..	..

DISCUSSION

This study was concerned solely with the antitoxin response to different immunizing procedures. No attempt is made to prove that any one procedure is the procedure of choice for routine immunization against diphtheria, for two reasons:

1. The ultimate basis for the evaluation of any immunization method is whether or not it protects against diphtheria, and not necessarily whether or not it confers Schick negativity or raises the antitoxin level to any definite point.

2. It is entirely possible that a procedure which results in a lower level of antitoxin than some other may still be the method of choice from the public health standpoint; that is, it might be less expensive, be easier to administer, and confer a high enough percentage of immunity to be the most efficient use of the public health dollar in prevention of diphtheria. It may be a question of choosing between the conferring of the highest possible immunity to the individual on the one hand, and the reduction of diphtheria by conferring a lower

but sufficient level of immunity to the community as a whole on the other.

This study emphasizes the comparatively poor antitoxin response to two injections of fluid toxoid with a 3 week interval and justifies the discontinuance of this procedure for immunization against diphtheria.

This study suggests that the Schick test may be omitted in routine immunization. Certainly there is nothing to be gained by the pre-Schick test in the preschool group—and it is doubtful whether there is any reason to Schick test following two injections of alum-precipitated toxoid or three injections of fluid toxoid until the child enters school—and then, as indicated in the subsequent paper,[8] a single injection of fluid or alum-precipitated toxoid would be more logical.

SUMMARY

The antitoxin response of children to several diphtheria immunization procedures has been determined. In the decreasing order of the response they induce in children having less than 0.001 unit of antitoxin per ml. of serum at the time of injection, they are, under the conditions of our study: two doses of alum-precipitated toxoid at 3 week interval, three doses of fluid toxoid at 3 week interval, one dose of alum-precipitated toxoid, two doses of fluid toxoid at 3 week interval, and one dose fluid toxoid.

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