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Diphtheria Immunization With Fluid Toxoid and Alum Precipitated Toxoid*

Preliminary Report

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THE studies were planned to investigate the height and duration of the antitoxic immunity following immunization with fluid and alum precipitated diphtheria toxoid. Conflicting reports [1, 2, 3, 4] on the comparative values of these two antigens made such a study seem important. We present a comparison of results from 1 dose of alum precipitated toxoid and 2 doses of fluid toxoid at 4 months, 1 year, and 2 years after injection; and a comparison of 2 doses of alum precipitated toxoid and 3 doses of fluid toxoid at 4 months after injection.

METHODS

The study is being carried out on free-living children primarily from rural schools in Saginaw County, a county of about 1,110 square miles. A total of 1,800 children are included in the report. The following procedures were used in immunization:

1. 1 injection of fluid toxoid
2. 2 injections of fluid toxoid (2 weeks apart)
3. 2 injections of fluid toxoid (3 weeks apart)
4. 3 injections of fluid toxoid (3 weeks apart)
5. 1 injection of alum precipitated toxoid
6. 2 injections of alum precipitated toxoid (3 weeks apart)

In addition a group of unselected children who received no injection were blood titrated at the end of 4 and 12 months. In a strictly controlled group alternate children in each school received the 2 immunizing agents. In this way 2 doses of fluid and 1 of alum precipitated toxoid were compared in some schools, and 3 doses of fluid compared with 2 doses of alum precipitated toxoid in other schools. The results from these controlled groups are analyzed separately as well as combined

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with the results obtained when alternate schools received the 2 immunizing agents. The schools included in the investigation number 110.

It was decided to follow the immunity response by blood titrations only. The Schick test was not used because of the well known fact that the Schick test itself has an antigenic effect, especially when given to a child already having antitoxin circulating in its blood. Therefore, before giving immunizing treatment, 4–5 ml. of blood were taken from the children. The children were bled again in 4 months and 12 months, and will be bled every 12 months thereafter for the duration of the study so that not only the height of the antitoxin level but the relative permanence of that level will be determined. A 1 ml. injection of the fluid or alum precipitated toxoid was given subcutaneously in the upper arm. Whenever a second or a third injection was given, a 3 weeks' interval usually elapsed between the injections. A small group was given injections with a 2 weeks' interval.

The toxoid used was obtained from a commercial firm by Dr. W. T. Harrison of the National Institute of Health. It was felt advisable to use such a product because it was widely 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 intrinsic antigenic efficacy for which we have no method of determination. The preparation used had a high average potency. The National Institute of Health tests showed 20 L_f/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 tests for alum precipitated toxoid. On the re-solution of the alum precipitated toxoid it was likewise found to have 20 L_f/ml.

RESULTS

Of the 1,800 children, 358, or 20 per cent, had titratable antitoxin (.001 unit or more per ml.) in their circulating blood at the time of the first injection

TABLE I

Age Distribution of Children in the Study

Age	*1 A. P.*	*2 A. P.*	*1 Fluid*	*2 Fluid*	*3 Fluid*	*Titration (No Toxoid)*	*Total*
8 Mos.	1	1	1	..	..	..	3
1 Yr.	2	5	..	1	..	..	8
1½ Yr.	2	2	..	2	..	..	6
2	1	2	2	2	1	1	9
3	8	3	2	5	3	2	23
4	37	13	2	10	9	1	72
5	97	60	15	38	41	2	253
Total	148	86	22	58	54	6	374—20.8%
6	156	98	15	94	82	..	445
7	146	59	7	60	49	3	324
8	101	35	8	18	40	4	206
9	73	32	4	22	29	..	160
10	41	25	5	22	22	2	117
Total	517	249	39	216	222	9	1,252—70. %
11	14	6	..	16	19	..	55
12	15	9	1	16	13	..	54
13	8	8	1	11	13	1	42
14	4	2	1	5	7	..	19
15	..	..	1	1	2	..	4
Total	41	25	4	49	54	1	174— 9.2%
Grand Total	706	360	65	323	330	10	1,800

of antigen. The majority showed a marked rise in antitoxin content following the injections and of course are not included in the results analyzed in this report. This leaves 1,442 children with less than .001 unit of antitoxin at the time of the first injection, and this paper is concerned with a comparison of the antitoxin levels in these children following the different methods of immunization described.

Table I shows the age distribution of the 1,800 children. Of these, 374 or about 28 per cent, are of preschool age; 1,252, or 70 per cent, are between the ages of 6 and 10; and 174, or 9.2 per cent are between the ages of 11 and 15. Included are 201 cases from Genesee County, studied in coöperation with Dr. L. V. Burkett, Genesee County Health Commissioner, Flint, Mich.

As stated, part of the children were in a strictly controlled group with each alternate child receiving different immunizing procedures. These results are entered in Table II under "controlled." For the rest of the children the two immunizing agents were given in alternate schools. This group is classified as "uncontrolled" in Table II. It is evident that the results in the two groups are strictly comparable. The number of children developing .001 unit or more of antitoxin is made the basis of analysis in this table. The two groups proved equally comparable when .01 or 0.1 unit was the basis of comparison. To conserve space, analyses at these levels are not included here. Since the "controlled" and "uncontrolled" groups are comparable, the results from the two groups are pooled in the remainder of the report to simplify presentation.

Table III shows the comparison of the antitoxin response of the children to different immunizing procedures. Of the children who received no immunizing injection 11 per cent developed antitoxin at the end of 4 or 12 months. This is rather high for a community with the low carrier rate (see later) we have found in Saginaw County. However, all of these children merely changed from less than .001 to .001 unit and may very well represent only the degree of unreliability of the titrations at this low level. The group that received one injection of fluid toxoid responded very poorly by the end of 10 days and the antitoxic response was still low at the end of 4 and 12 months.

The group that received one injection of alum precipitated toxoid also responded very poorly at the end of 10 days, having 27 per cent with .001 or more units of antitoxin.

One hundred and sixty children who received 2 injections of fluid toxoid at 3 week intervals showed an antitoxic

TABLE II

Comparison of Antitoxin Response to the Different Immunizing Procedure in Controlled and Uncontrolled Groups

(Antitoxin level at time of 1st injection <.001)

Immunizing Preparation and Procedure	Group	4 Months			12 Months		
			Children With .001 or More			Children With .001 or More	
		No. in Group	No.	%	No. in Group	No.	%
2 injections fluid	Controlled	129	82	63.5	88	55	62.5
3 wk. interval	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 wk. apart	Uncontrolled	49	46	93.8	49	47	95.9
2 injections A. P.	Controlled	138	138	100.0	..	..	....
3 wk. apart	Uncontrolled	10	10	100.0	..	..	....

TABLE III

Comparison of Antitoxin Response to the Different Immunizing Procedures (All cases <.001 antitoxin level at primary titration)

Immunizing Procedure	10 Days			4 Months			12 Months			18 Months			24 Months		
	No. in Group	Children With .001 or More		No. in Group	Children With .001 or More		No. in Group	Children With .001 or More		No. in Group	Children With .001 or More		No. in Group	Children With .001 or More	
		No.	%		No.	%		No.	%		No.	%		No.	%
No injection	..	..	....	61	6	11.5	19	2	10.5	..	..	....	..	..	....
1 injection Fluid	86	18	20.9	12	2	16.6	10	2	20.0	..	..	....	..	..	....
2 injections Fluid 2 wk. apart	..	..	....	14	12	85.6	15	9	60.0	..	..	....	13	6	46.1
2 injections Fluid 3 wk. apart	..	..	....	160	103	64.4	111	67	60.4	9	4	44.4	..	..	....
1 injection A. P.	80	22	27.5	352	327	93.0	327	285	87.3	19	15	79.0	152	118	77.6
3 injections Fluid 3 wk. apart	..	..	....	162	159	98.1	49	47	96.0	..	..	....	..	..	....
2 injections A. P. 3 wk. apart	..	..	....	148	148	100.0	..	..	....	..	..	....	..	..	....

response in 64 per cent at the end of 4 months. This percentage was reduced to 60 per cent at the end of 12 months, and 45 per cent at the end of 18 months.

For comparison the antitoxic response may be noted when the interval was reduced to 2 weeks. The figures indicate that 85 per cent showed a response at the end of 4 months, 60 per cent at the end of 12 months, and even 46 per cent at the end of 24 months. The difference between these and the 3 week interval results is not statistically significant with the numbers concerned.

Better response was observed in the group of 352 children who received one injection of alum precipitated toxoid. In this group 93 per cent showed an increase in antibody at the end of 4 months. As time went on some loss of antitoxin was noticed, although 75.7 per cent still maintained the increased antitoxin level at the end of 2 years. The difference between these and the 2 fluid toxoid injection results is statistically significant and can be considered as proved under the conditions of this study.

Of 162 children who received 3 injections of fluid toxoid at 3 week intervals, 98 per cent showed an increase in circulating antitoxin at the end of 4 months, and 96 per cent at the end of 12 months.

Of 148 children who received 2 injec-

TABLE IV

Comparison of Antitoxin Levels 10 Days After a Single Injection of Fluid or A. P. Toxoid in Children Having <.001 at Time of Injection

	Units of Antitoxin per 1 ml. of Serum												
	<.001		.001<.004		.004<.01		.01<.04		.04<.1		.1 plus		Total Number
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
10 days after one injection of fluid	68	79.0	6	7.0	..	....	1	1.0	3	4	8	9.0	86
10 days after one injection of A. P.	58	72.5	6	7.5	..	....	1	1.3	..	....	15	18.7	80

TABLE V

Comparison of Antitoxin Levels 10 Days After One Injection of Fluid or A. P. Toxoid in Children Having <.001 Unit or More

Kind of Toxoid	No. of Cases	Units of Antitoxin per ml. of Serum		Number at Time of 1st Injection	Number 10 Days Later
Fluid	41	<.001			3
		.001	<.004	9	0
		.004	<.01	3	0
		.01	<.04	7	0
		.04	<.1	6	0
		.1 plus		16	38
Alum precipitated	30	<.001			1
		.001	<.004	6	0
		.004	<.01	3	0
		.01	<.04	8	0
		.04	<.1	6	0
		.1 plus		7	29

tions of alum precipitated toxoid and who were followed for a period of 4 months only, 100 per cent showed an antitoxin response within 4 months. Twelve months have not elapsed since the last injection.

Table IV shows the antitoxin response at the end of 10 days after a single injection of either fluid or alum precipitated toxoid. Those receiving fluid toxoid who did not have titratable antitoxin to start with showed an increase in 21 per cent only, although it can be seen from the table that some show a surprisingly high rise in antitoxin level. Groups which received alum precipitated toxoid injections showed an increase in antitoxin in 28 per cent of the children at the end of 10 days.

A group of children who had more than .001 unit of antitoxin demonstrated prompt response to antigenic stimulation. This can be seen in Table V. These results are interesting because of the frequent questions as to the advisability of giving immunizing injections after exposure. In cases of exposure to diphtheria it might be a very desirable procedure to give some kind of antigen to children. Of the 4 chil-

TABLE VI

Distribution of Antitoxin Levels After Various Immunizing Procedures and Following Different Intervals

(All children having <.001 unit before injection)

No. of Cases	Time of Titration	<.001 No.	<.001 %	.001<.004 No.	.001<.004 %	.004<.01 No.	.004<.01 %	.01<.04 No.	.01<.04 %	.04<.1 No.	.04<.1 %	.1 plus No.	.1 plus %
		Antitoxin Response from Two Doses of Fluid Toxoid, 3 Weeks Apart											
		Units of Antitoxin per ml. of Serum											
160	4 months	57	35.6	26	16.2	24	15.0	23	14.4	12	7.5	18	11.3
111	12 months	44	39.7	25	22.5	11	9.9	20	18.0	2	1.8	9	8.1
9	18 months	5	55.6	1	11.1	0	0.0	2	22.2	0	0.0	1	11.1
		Antitoxin Response to One Dose Alum Precipitated Toxoid											
352	4 months	25	7.1	68	19.3	58	16.5	96	27.2	46	13.1	59	16.8
327	12 months	42	12.8	69	21.1	61	18.7	107	32.7	18	5.5	30	9.2
19	18 months	4	21.0	4	21.0	4	21.0	6	31.8	1	5.2	0	0.0
152	24 months	34	22.4	33	21.7	28	18.4	35	24.0	9	5 9	13	8.6
		Antitoxin Response to Three Doses of Fluid Toxoid 3 Weeks Interval											
162	4 months	3	1.9	13	8.0	24	14.8	52	32.1	29	17.9	41	25.3
49	12 months	2	4.1	4	8.2	5	10.2	16	32.6	12	24.5	10	20.4
		Antitoxin Response to Two Doses of A. P. Toxoid 3 Weeks Interval											
148	4 months	0	0.0	2	1.4	4	2.7	21	14.2	41	27.7	80	54.0

dren who failed to show an increase in antitoxin, 3 had a titer of less than .002 unit and 1 a titer of .004 unit per ml. before injection. Whether these represent a negative phase or the unreliability of titrations at this low level of antitoxin cannot be said. This phase of the problem is receiving further detailed study.

Table VI shows the actual antitoxin levels achieved at different intervals following the several immunizing procedures. It is evident that a procedure which causes more children to develop an appreciable antitoxin titer also produces more with antitoxin in higher concentrations.

In our series of 360 children receiving 2 injections of alum precipitated toxoid, we observed no reactions indicative of sensitivity following the second injection. It is indeed suggestive that 2 injections of alum precipitated toxoid produce the highest antitoxin level of all procedures tried. As we started this group in the spring, we are not in a position to present observations for longer than 4 months.

Table VII shows a comparison of response in preschool and school children receiving one injection of alum precipitated toxoid and fluid toxoid. There may be a tendency for the preschool children to show a better response to the immunizing treatment than the school children but the figures are not large enough for the differences to be significant. Further work is being done on this point.

In addition to following the immunity response, a continual diphtheria carrier survey is being carried on in the schools. Throat cultures are taken from all the school children irrespective of whether they have received toxoid or not. This is felt to be essential for the intelligent evaluation of the immunizing results obtained 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

TABLE VII

Comparison of Antitoxin Response in Preschool and School Age Groups Having <.001 at Time of Injection

		4 Months			12 Months			24 Months		
			Children With .001 or More			Children With .001 or More			Children With .001 or More	
Immunizing Procedure	*Age Group*	*No. in Group*	*No.*	*%*	*No. in Group*	No.	*%*	*No. in Group*	No.	*%*
1 injection	5 yrs. and under	63	61	96.8	59	53	89.9	33	28	84.8
A. P. toxoid	6 yrs. and over	316	292	92.4	268	232	86.6	..	..	....
2 injections	5 yrs. and under	29	19	65.5	12	9	75.0	..	..	....
Fluid toxoid	6 yrs. and over	131	84	64.2	99	58	58.6	..	..	....

TABLE VIII

Saginaw County Diphtheria Carrier Survey

			No. of Positive KL					
			Pathog.		Non Pathog.		No Virulence Test Made	
Year	*No. of Cultures Taken*	*Per cent Pos. KL*	*No.*	*%*	*No.*	*%*	*No.*	*%*
1936	3,522	.4	8	.23	3	.09	3	.09
1937	6,636	.5	14	.21	14	.21	6	.09
1938	9,188	.37	9	.1	21	.23	5	.05
Total	19,346	.42	31	.16	38	.2	14	.07

exposure to virulent diphtheria organisms is a rarity.[5] An attempt is being made to take throat cultures at regular intervals during the months of highest morbidity rate. Every positive culture is now being examined for virulence and the child is tested repeatedly as long as it remains a carrier. Of the 19,346 throat cultures taken 83 were found KL positive, of whom 31 were found virulent (see Table VIII). The antitoxin level of all diphtheria carriers is determined at frequent intervals in order to evaluate influences of harbored diphtheria bacilli on the immunity response of the child.

The diphtheria carrier survey covered 119 schools, and 7,347 children were cultured.

DISCUSSION

This paper is 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, and 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 striking a balance 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.

The fact that no abscesses or severe reactions have followed the 2 injections of alum precipitated toxoid augurs well for the future of this method of immunization.

In most studies of a diphtheria immunizing procedure the Schick test is used. On the other hand, in much of the routine immunization a pre-Schick test is not used and probably will be used less and less in the future as preschool immunization increases. Thus, since the Schick test may possibly have an antigenic effect, it becomes important from the practical point of view to evaluate diphtheria immunization in the absence of the Schick test, as done in this study.

FitzGerald, et al.[6] report a better antitoxin response to 3 doses of fluid toxoid than to 2 injections of alum precipitated toxoid, just the opposite of our results. There is no significant difference between their results and ours with 2 doses of alum precipitated toxoid. There is a significant difference between their results and ours with 3 injections of fluid toxoid, our results being definitely lower than theirs. Perhaps this is due to a difference in the toxoids used, but it may be unsound to compare their fluid toxoid results with ours or with their own alum precipitated toxoid results since their 3 dose fluid toxoid results are based on those children who were originally Schick positive and not on the basis of those who had originally no titratable antitoxin. In other words, in contrast to their other groups (and to ours) their 3 dose fluid toxoid group had the benefit of the stimulus from a Schick test, and also might perhaps have contained some children who gave positive Schick tests but had titratable antitoxin below the .01 unit level and so would give marked response to any immunizing procedure. on the other hand, Fraser and Halpern [8] in a study of 32 children observed a similar high response to 3 injections of

fluid toxoid. Here again some of the children received a pre-Schick test but all were titrated for antitoxin content before injection making the conditions more comparable to ours. Perhaps these latter results do point to some undetermined difference in the fluid toxoids used. It is conceivable that different technics or environments influence the results but one might expect these factors also to effect a difference in the alum precipitated toxoid results.

SUMMARY

The antitoxin response of children to several diphtheria immunization procedures has been determined. In the increasing order of the response they induce, they are, under the conditions of our study: 1 dose fluid toxoid, 2 doses of fluid toxoid at 3 week interval, 1 dose of alum precipitated toxoid, 3 doses of fluid toxoid at 3 week interval, and 2 doses of alum precipitated toxoid at 3 week interval.

REFERENCES

1. Wells, D. M., Graham, A. H., and Havens, L. C. *A.J.P.H.*, 22:648–650 (June), 1932.
2. Graham, A. H., Murphree, L. R., and Gill, D. G. *J.A.M.A.*, 100:1096–7 (Apr.), 1933.
3. Fraser, D. T., and Halpern, K. C. *Canad. Pub. Health J.*, 26:469 (Oct.), 1935.
4. Straus, H. W. *J. Lab. & Clin. Med.*, 22:893 (June), 1937.
5. Jensen, Claus. *Proc. Roy. Soc. Med.*, 30:71 102 (July), 1937.
6. FitzGerald, J. G., Fraser, D. T., McKinnon, N. E., and Ross, M. A. *Bull. New York Acad. Med.*, 14:566 (Sept.), 1938.

Tribute to a Public-Spirited Physician

Legend on a Memorial Statue, St. Paul's Cathedral, London, England

William Babington, M.D., F.R.S., Fellow of the Royal College of Physicians, born May 21, 1756, died April 29, 1833.

"Eminently distinguished for science, beloved for the simplicity of his manners and the benevolence of his heart, respected for his inflexible integrity and his pure and unaffected piety. In all the relations of his professional life he was sagacious, candid, diligent and humane; firm in his purpose, gentle in execution; justly confident in his own judgment, yet generously open to the opinion of others. Liberal and indulgent to his brethren but ever mindful of his duty to the public.

"To record their admiration of so rare a union of intellectual excellence and moral worth and to extend to future generations the salutary influence which his living example can no longer diffuse, this monument has been erected by the public subscription of his contemporaries. A.D. 1837."