Diphtheria Toxin-Antitoxin and Toxoid*

A COMPARISON

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THESE two preparations, toxin-antitoxin and toxoid, are now being widely used for immunization of infants and young children against diphtheria. Toxin-antitoxin has been longest in use. Behring was the first to employ it experimentally as an immunizing injection in man. His preparation was the undiluted toxic broth with its toxin nearly neutralized by antitoxin. He never gave a clear explanation of how he prepared and standardized it. The breaking out of the war delayed the practical utilization of toxin-antitoxin in Europe.

Park and Zingher were the first to realize that by using the Schick test to determine the susceptibility of the children, and a retest to note the changes in reaction, we could study the immunizing effect of toxin-antitoxin injections in human beings. We demonstrated that immunity developed in about 85 per cent of those receiving 3 injections of our 3 L + preparation at intervals of 1 week, and by 1917 we realized that it lasted in the great majority of cases for at least several years. We also tried giving it at intervals of 2 weeks, but the results were only moderately better. Schroder followed the Schick reaction in the immunized children for a longer time and in 1925 found that the period of immunity extended to 10 years for at least 80 per cent of them. These were New York City children. As a rule the same children were not retested in order to avoid the possibility of the Schick test adding its immunizing effect. The long duration of immunization might have been due in some to the added immunizing effect of repeated infection from carriers.

In 1918 we began the serious attempt to immunize the whole child population of New York City. This earlier work was concerned mostly with children of school age, as most parents were not yet willing to have the injections given to the babies and very young children. We noted that in a small percentage of the children the injection gave

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quite a severe local reaction. To lessen this, we tried diluting the toxin before adding the antitoxin, and found that when as little as 0.1 L + dose of toxin was given in an injection, the immunizing effect was as good as when a 3 to 6 L + dose was used. Smaller amounts of toxin were less immunizing. The most important point is that the toxin-antitoxin to have its maximum effect has to have such a toxicity that a human dose kills a 250 gm. guinea pig in about 4 weeks. With decreasing toxicity the immunizing effect is gradually diminished.

This new preparation gave much less local reaction in children of school age, since this reaction was due, not so much to the specific toxin as to the other elements, especially the dissolved bacillus substance. It was found that even when the toxin-antitoxin was stored in good glass and kept cool the product became gradually somewhat less toxic. The reverse never happened with the 0.1 L + preparation except when it was frozen and then the increase in toxicity was not sufficient to be in the least dangerous. This characteristic of becoming less toxic is a real drawback, because many commercial preparations suffer this during transportation and the period of storage, and when this happens, 3 injections may cause only 50 to 75 per cent of the children to change from a Schick positive to a Schick negative state, instead of the expected average of about 80 per cent.

When toxin-antitoxin is accurately standardized, a full dose, injected just under the skin of the anterior surface of the arm, acts both as an immunizing injection and as a substitute for the Schick test. The result in the older children is not quite so accurate an index of immunity as that from the Schick test, but the error is on the safe side. This slight difference is due to the fact that a dose of suitable toxin-antitoxin is a little more toxic than the Schick test dose, and also to occasional nonspecific protein reactions. We have found that this use of toxin-antitoxin is a very valuable help among the school children of New York City, since without using the Schick test we are enabled to save fully 50 per cent of them from the second and third injections, as they are shown by their negative reactions to be immune. The readings are not made until the 5th or 6th day, to allow, in most cases, for the disappearance of the pseudo-reactions, which are more pronounced than with the Schick test. The 6th day is also a suitable time for the second injection in those who require it.

The possible objection, when horse antitoxin was used, that the approximately 0.001 c.c. of horse antitoxin in the immunizing dose sensitizes the injected children to later injections of therapeutic doses of an antiserum from the horse, has been removed by substituting antitoxin from the goat. We are convinced that the fear of sensitization

was greatly exaggerated, but it was wise to substitute goat antitoxin to remove any objection. This preparation of toxin-antitoxin is used generally in the United States and has given satisfaction. In New York City alone, during the past 15 years, more than 500,000 school children have been given toxin-antitoxin, and during the past 2½ years, owing to an intensive drive to stamp out diphtheria, inaugurated by the Commissioner of Health, Dr. Wynne, more than 250,000 infants and preschool children have received it. Deaths from diphtheria in New York City during 1930 were only 198 against 416 for 1929, and 800 for 1920. The figures for the first 8 months of 1931 are even better than those of 1930. During this time, the population has considerably increased. All this immunization work was performed without any accident.

Only 4 serious accidents have occurred from toxin-antitoxin injections in other cities of the world, and these would have happened just as readily if toxoid had been used. The first took place in the United States and resulted from the sending by mistake of a proved overtoxic preparation. The second and third happened in Austria and Russia and were caused by the accidental distribution and injection of a diluted diphtheria toxin for toxin-antitoxin; while the fourth occurred in Australia, due to the use of a toxin-antitoxin preparation which had no antiseptic in it and which was stored in large bottles. The contents of one of the bottles after having been opened several times became contaminated by virulent staphylococci.

Within recent years, efforts have been made by bacteriologists to obtain a better preparation than toxin-antitoxin. Park, Zingher and Schroder stated in 1924 that a toxin which had been changed to toxoid by long standing gave remarkably good immunizing results, though the marked pseudo-reactions occasionally caused by it made it less desirable, in their opinion, for school children who made the majority of those being immunized, than the new preparation of toxin-antitoxin. Glenny and his associates, by the use of formalin, reduced toxin to a slightly toxic toxoid, and found this, when its remaining toxicity was partially neutralized by antitoxin, an excellent immunizing agent. Like toxin-antitoxin, the toxoid was diluted and gave less reaction than undiluted toxoid.

The best immunizing material now in use is, however, the *non-toxic* diphtheria toxoid which Ramon developed. He adopted the suggestions of Glenny as to the use of formalin, and of Loewenstein of Vienna as to the value of non-toxic toxoid in tetanus. He was able also to prepare a stronger toxin, and therefore toxoid, than had previously been prepared. Starting with a highly potent toxin, he reduces

its toxicity by the addition of formalin and its storage at 37° C. until it is practically non-toxic. The greater the number of L + doses in the original toxin, the better the immunizing effect of the toxoid. With toxoid sent us by Ramon, or made by Povitzky in our laboratory, we have obtained no greater pseudo-reaction from an intramuscular injection of 0.5 c.c. in little children than from the equivalent dose of 1 c.c. of toxin-antitoxin, and but little more from 1 c.c. injections, but in school children the toxoid preparations produce on the average somewhat more marked reactions than do 1 c.c. injections of toxin-antitoxin.

The immunizing effects of our best toxoid have been better than from the best properly standardized toxin-antitoxin. We have therefore recently adopted the toxoid (Ramon) for the children of preschool age in New York City, but continue to use toxin-antitoxin for the school children and for such adults, as for instance, nurses, who require it. We may soon employ the toxoid entirely, especially, if we are able to reduce the substances in it which cause pseudo-reactions.

We have also recently tried out the inunction of toxoid mixed with lanolin (Loewenstein) and have obtained a change from a positive to a negative Schick reaction in about 70 per cent of the children on whom it was used. The 4 or 5 rubbings were made at weekly intervals. This method has certain advantages in cases where the mothers object to the use of the needle and in institutions where a nurse can apply it to the children as they enter. The ointment should be thoroughly rubbed into the skin. For general use it is more time consuming and less effective than the subcutaneous or intramuscular injection of toxin-antitoxin or toxoid.

INCREASE OF THE POTENCY OF DIPHTHERIA TOXOID THROUGH THE ADDITION OF ALUM

Two years ago, Glenny, working under the direction of O'Brien, found that the addition of sufficient alum to the toxoid to make a 0.2 to 0.5 per cent solution increased appreciably its power to develop antitoxin when injected in horses. We tried his methods with good results. It occurred to us that toxoid containing alum might be advantageously used in infants and children. Schroder injected about 100 children last spring with very favorable results. A report of this was made by one of us at the international meeting of the Microbiological Society at Paris last June. O'Brien told me that they had also tried it in a few children with favorable results. During the past 10 months, Schroder and Blum have injected additional children, and we have made a comparison of the results following the use of toxin-

TABLE I

A COMPARISON OF THE IMMUNIZING EFFECT OF DIPHTHERIA TOXOID, TOXOID PLUS ALUM AND TOXIN-ANTITOXIN IN GUINEA PIGS

Fraction of 1 c.c. Given and Number of Doses	Average Number of Minimal Fatal Doses Neutralized in Guinea Pigs After 1, 2 and 3 Doses of			
	Toxin-Antitoxin	Toxin-Antitoxin Alum	Toxoid	Toxoid and Alum
1/16	1.5	1.5	3	3
1/16, 1/16	3.0	5.0	6	15
1/16, 1/16, 1/16	5.0	15.0	40	60
1/8	1.5	1.5	5	10
1/8, 1/8	3.0	4.0	25	80
1/8, 1/8, 1/8	15.0	25.0	30 +	90 +
1/4	2.0	3.0	10	30
1/2	2.0	3.0	50	80
Total doses neutralized by 8 guinea pigs	33.0	58.0	169 +	368 +

Injections given at intervals of 1 week. Retests done at the end of 2 months. Note the much greater number of fatal doses neutralized by the guinea pigs receiving toxoid, especially by those receiving toxoid plus alum.

TABLE II

THE INCREASED EFFECT OF GIVING A DEFINITE QUANTITY OF TOXOID IN DIVIDED DOSES RATHER THAN IN A SINGLE ONE

	Number of m.l.d. overcome by guinea pig
A single dose of 1/2 c.c. of toxoid plus alum	80
A single dose of 1/4 c.c. of toxoid plus alum	30
Two doses of 1/8 c.c. with interval of 1 week	80
A single dose of 1/2 c.c. of toxoid	50
A single dose of 1/4 c.c. of toxoid	10
Two doses of 1/8 c.c. with interval of 1 week	20
Three doses of 1/16 c.c. with intervals of 1 week	40

antitoxin, toxoid, and toxoid with alum, in the children living in institutions. By dividing them into similar groups and giving children of similar age the injections, we felt we might obtain valuable information. Finally Welton, who has charge of our Otisville branch laboratory, injected some 500 guinea pigs with one or other of the 3 preparations. The results of these tests in children and in guinea pigs are shown in Tables I to VI.

DOSAGE OF TOXIN-ANTITOXIN

The almost universal custom is to give 3 injections of 1 c.c. each. A few have advised 4 or even 5 injections. More than 3 are hardly necessary, if properly prepared and preserved preparations are used. In 149 young children in which 1 c.c. of a well tested preparation was

TABLE III

THE COMPARATIVE VALUE OF INTERVALS OF 1 AND 2 WEEKS BETWEEN INJECTIONS. NUMBER OF INJECTIONS 2 AND 3. MATERIAL TOXOID WITH 0.2 PER CENT ALUM

No. of Pig	1st Dose	2nd Dose	2nd Dose	M.l.d. 8/12/31	Day of Death
	2 Injections				
	7/3/31 c.c.	7/10/31 c.c.	7/18/31 c.c.		
1	1/8	1/8		20	*
2	1/8	1/8		20	*
3	1/8	1/8		30	3rd
4	1/8	1/8		30	**
5	1/8	1/8		30	**
6	1/8	1/8		30	**
7	1/8	1/8		60	*
8	1/8	1/8		60	*
9	1/8	1/8		60	3rd
10	1/8	1/8		100	3rd
11	1/8	1/8		100	4th
12	1/8	1/8		100	2nd
13	1/8	1/8		150	4th
14	1/8		1/8	20	5th
15	1/8		1/8	20	2nd
16	1/8		1/8	30	*
17	1/8		1/8	30	*
18	1/8		1/8	30	2nd
19	1/8		1/8	30	*
20	1/8		1/8	60	**
21	1/8		1/8	60	*
22	1/8		1/8	60	2nd
23	1/8		1/8	100	3rd
24	1/8		1/8	100	*
25	1/8		1/8	100	3rd
26	1/8		1/8	150	*

* Destroyed 8/27/31 because of necrosis at site of toxin injection.
** Still living and healthy, discharged.
Pigs at beginning of immunization weighed from 260 to 300 gm.
Pigs at time of toxin injections weighed from 700 to 900 gm.
The difference shown in these two sets of guinea pigs is very slight.

recently injected by us we obtained 90 per cent of success. With over-neutralized preparations others have obtained at times as little as 50 per cent of immunization.

DOSAGE OF TOXOID

The potency of toxoid depends on the number of antigenic units contained in a specified amount. These can be approximately determined by the L+ doses of the original toxin or by the flocculation units of the toxoid when ready for use. A good preparation and one which should be produced by all biological laboratories contains at least 8 antigenic or flocculation units per c.c. A department of health

TABLE IV

THE COMPARATIVE VALUE OF INTERVALS OF 1 AND 2 WEEKS BETWEEN INJECTIONS. NUMBER OF INJECTIONS 2 AND 3. MATERIAL TOXOID WITH 0.2 PER CENT ALUM

No. of Pig	3 Injections				M.l.d. 8/12/31	Day of Death
	7/3/31 c.c.	7/10/31 c.c.	7/18/31 c.c.	7/31/31 c.c.		
27	1/8	1/8	1/8		30	8th
28	1/8	1/8	1/8		60	
29	1/8	1/8	1/8		60	5th
30	1/8	1/8	1/8		60	5th
31	1/8	1/8	1/8		60	3rd
32	1/8	1/8	1/8		100	*
33	1/8	1/8	1/8		100	*
34	1/8	1/8	1/8		100	**
35	1/8	1/8	1/8		100	**
36	1/8	1/8	1/8		150	**
37	1/8		1/8	1/8	30	*
38	1/8		1/8	1/8	60	**
39	1/8		1/8	1/8	60	*
40	1/8		1/8	1/8	60	**
41	1/8		1/8	1/8	60	**
42	1/8		1/8	1/8	100	**
43	1/8		1/8	1/8	100	**
44	1/8		1/8	1/8	100	**
45	1/8		1/8	1/8	100	**
46	1/8		1/8	1/8	100	**
47	1/8		1/8	1/8	150	**
48	1/8		1/8	1/8	150	**
49	1/8		1/8	1/8	150	**

* Destroyed 8/27/31 because of necrosis at site of toxin injection.
** Still living and healthy, discharged.
Pigs at beginning of immunization weighed from 260 to 300 gm.
Pigs at time of toxin injections weighed from 700 to 900 gm.
The guinea pigs having a 2-weeks interval certainly show somewhat more resistance to the toxin.

TABLE V

A COMPARISON OF THE IMMUNIZING POWER OF TOXOID WITH AND WITHOUT THE ADDITION OF ALUM IN INFANTS AND YOUNG CHILDREN LIVING IN HOME FOR HEBREW INFANTS
THREE INJECTIONS GIVEN AT INTERVALS OF 1 WEEK

	No. of Children 2 Mos. to 2 Yrs.	Number Immunized	Per cent Immunized	No. of Children 2 to 4 Yrs.	Number Immunized	Per cent Immunized
Toxoid 1/2 c.c.	49	44	89.8	33	31	94
Toxoid with alum 1/2 c.c.	35	34	97	21	21	100
Toxoid with alum 1/8 c.c.	43	41	95	13	13	100
Filtrate of Toxoid plus alum 1/2 c.c.	25	0	100	8	0	100

TABLE VI

A COMPARISON OF DIPHTHERIA TOXIN-ANTITOXIN, TOXOID AND TOXOID WITH THE ADDITION OF 0.2 PER CENT OF ALUM AS AN IMMUNIZING AGENT IN CHILDREN.* THREE INJECTIONS OF 1 C.C. OF TOXIN-ANTITOXIN AND 0.5 C.C. OF TOXOID WERE GIVEN. THE INJECTIONS WERE GIVEN AT INTERVALS OF 1 WEEK

Children from 5 to 14 years of age

Immunizing Substance	No. Children	No. Immunized	Per cent Immunized
Diphtheria Toxoid (not toxic)	243	228	93.7
Diphtheria Toxoid (plus alum)	112	110	98.2
Diphtheria Toxin-antitoxin	149	132	90.0

* The children the results of whose immunization are given in the above tables were cared for in 7 institutions. In order to attempt to make the comparison an accurate one, we selected an equal number of children from each institution for immunization by each of the substances. The children were also of similar age groups ranging from 2 to 14 years.

These were all excellent preparations. The toxoid plus alum gave the best results and the simple toxoid the next.

should know that a toxoid supplied by it is of at least this potency. If the toxoid is diluted it should contain at least 4 antigenic units in the dose advised. Such a toxoid is certainly more potent than toxin-antitoxin. Many believe that 2 injections of such toxoid are sufficient. With 3 injections of 0.5 c.c. each, Tables V and VI, we obtained about 94 per cent of successful results. Volk, of Pontiac, using our preparation, but giving only 2 injections of 0.5 c.c. each, obtained only 83.8 per cent in 246 school children. The retests were made at the end of 8 months. Of the children 23 received only 1 injection, and in these only 47 per cent of success was obtained at the end of 2 months and 63.5 per cent at the end of 8 months. It seems, therefore, that 3 doses are desirable if the dose is limited to 0.5 c.c. With 0.5 c.c. and 1 c.c., a somewhat higher percentage of immunization would be obtained, and even higher with 2 doses of 1 c.c. each; but with these there would be occasionally annoying but not serious reactions. Each person must weigh these facts and decide as to whether the possibility of the increased local reaction is a hindrance to the larger dose. It is important that health officials get the most potent preparations available.

MOST DESIRABLE AGE FOR IMMUNIZATION

All are agreed that we wish to treat the children at the earliest suitable age. We found some years ago that infants within a few days of birth did not respond well to injections of toxin-antitoxin. Approximately 2,000 infants were given the first injections on the 2d or 3d day after birth, and in addition 2 more at weekly intervals. There was practically no local or constitutional reaction to the injections, but when 100 of them were retested at the end of 1 year the immunization

was found to be quite disappointing. Recently Schroder has injected groups at ages of 1 month, 2 to 12 months, and over 1 year. With toxin-antitoxin her results were as follows:

No. of Infants	Age when Treated	Age when Retested	Results % Immunized
12	1 month	9 to 11 mos.	75
11	3–7 months	9 to 20 mos.	90
10	1 year	1.5 to 3 yrs.	90
4	1 year (toxoid)	17 months	100

These results taken together with those given me by Blum and shown in Table V, suggest that at 3 months or after, babies are suitable for immunization, so far as their immunity response is concerned. As to the most suitable age, other factors are of importance. In cities the new-born babies are usually immune through the transfer to them before birth of antitoxin from their mothers, while in the country the majority are susceptible. This passive antitoxic immunity lasts 6 to 12 months. For this reason the age chosen in cities as the desirable one is usually 6 to 9 months, while in the country it is usually 6 months. Another reason for waiting until the babies are at least 6 months of age is that very young infants are more apt to suffer from intestinal and other disturbances. Fortunately the earlier in life the toxin-antitoxin or toxoid is given, the less the annoyance—in fact in babies under 2 years an intramuscular injection of toxoid is not usually followed by any appreciable reaction. The toxoid gives on the average a local reaction 25 per cent more severe than does the toxin-antitoxin. Deep intramuscular injections give much less frequent and severe local reactions than subcutaneous ones.

We may sum up as follows: Non-toxic diphtheria toxoid or anatoxin is undoubtedly the best preparation for young children and probably for older ones because it is the most potent and stable immunizing agent. Suitable underneutralized toxin-antitoxin is however an efficient preparation and is only a little less immunizing than toxoid, and it has the advantage of causing less nonspecific reactions in older children. It is to be remembered that in most toxin-antitoxin preparations, the "toxin" is really mostly toxoid, since the toxin has been kept for a year or more. This allows it to become more stable and more efficient. It is difficult to decide which method should be preferred for older children. Each has its advantages. The use of toxoid mixed with lanolin, and rubbed into the skin has a distinct place, as for example, for children in institutions or in cases in which there are objections to the use of the "needle." Alum 0.1 or 0.2 per cent,

added to the toxoid adds to its immunizing power, as is also the case when it is added to toxin-antitoxin, only then to a much less extent.

It is of the greatest importance that the toxoid be made from highly potent toxin and that the antigenic units should be stated on the containers. If it is diluted, this should be stated on the label. Toxin-antitoxin should be properly standardized and have the desired toxicity. In large cities such as New York we find toxin-antitoxin has an advantage, in older children, for the injection acts not only as an immunizing dose, and gives on the average less local reaction, but because it is also a substitute for the Schick test. The exclusion of the negatively reacting children cuts down the number of later injections. For the second and third injections in these older children, either toxin-antitoxin or toxoid may be used.

A Schick retest 3 to 4 months after making the immunizing injections is always desirable but not essential, for only in this way can we be absolutely sure that the desired effect has been obtained. The tables here present some of the evidence upon which our opinions were formed.

NOTE: A part of the expense of this investigation was provided by the Committee on Administrative Practice of the American Public Health Association from an appropriation made to it by the Commonwealth Fund.

Premiums to Mothers, Liége, Belgium

TO overcome the indifference of women to prenatal work and to infant health work, the municipal council of Liége, on July 1, 1931, offered premiums of 200 francs each to expectant mothers who attended regularly a prenatal health center or were examined by their own physician at least once before the end of the 6th month of pregnancy and not less than 3 times during the entire pregnancy, and who have had their infants regularly examined either at a health center or by the family physician. The premium is paid 3 months after the birth of the child.—*Oeuvre Nationale de l'Enfance, Revue Mensuelle,* Brussels, July, 1931, p. 776.