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STUDIES ON DIPHTHERIA - TETANUS

Combined Immunization in Children in some elementary schools of Tehran

by

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The mortality rate from diphtheria in different areas of Iran is still high. Being the main governmental producer of the refined antitoxins and toxoids for the whole country we note that the need for diphtheria antitoxin since 1951 till the present remains stationary and on a high level. There is, therefore, since 1951 a growing tendency for immunization of children in Tehran and in large cities. Recently a program for mass vaccination was planned by the Ministry of Health with financial aids of the Seven Year Plan Organization.

During the spring of 1958 it appeared desirable to secure information concerning the immunity state of the children immunized with our prophylactic by the Health Department of the American Joint Distribution Committee in Iran.

The American Joint Distribution Committee (A.J.D.C.) is one of the important international welfare organizations having at the present, health, educational and welfre programs in 25 countries of the world.

For this study it was arranged by AJDC to explain the objects of

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the investigation to the parents of the children in order to obtain their consent for blood-tests.

The purpose of this paper is to describe the results obtained by Schick test and by in vivo control of sera in regard to diphtheria and tetanus immunity.

Material and Procedure

Children of two kindergartens as well as of seven elementary schools, were used for studies. Most of these children are vaccinated during infancy before entering kindergarten or school and have their corresponding files. So they made a particularly suitable group for the study of duration of immunity. As a rule children over 5 years are vaccinated with combined diphtheria and tetanus vaccine. The triple antigen, i.e. diphtheria and tetanus combined with pertussis vaccine is reserved solely for the young children under 5 years.

Choice of Prophylactic

From the well-known 9 prophylactics presented to the WHO experts meeting held at Dubrovnik, Yugoslavia, in 1952 (23) we manufacture and use the following preparations:

1 - F.T. (formol toxoid = anatoxine)

a) **Diphtheria toxoid**

This fluid product, largely used in Iran from 1940 to 1952 was prepared in collaboration with L.P. Deply under the auspices of G. Ramon (*) as described by Ramon (19,20) or by Loiseau and Philippe (14) in peptic or papainic digest of meat. It was for lack of pure reagents that a new simple broth was formulated during World War II by Delpy and Mirchamsy (4). The new preparation contains crude maltose prepared locally and brewer yeast.

This toxoid was delivered in 50 Lf/ml, two injections of 1 and 2 ml are made 3 to 4 weeks apart.

40 to 50 per cent. of children of 10 years and over show severe local and general reaction.

(*) We wish to thank Prof. G. Ramon, Hon. Director of Pasteur Institute, Paris, for his continuous help and advice throughout our works.

It seems very probable that the latter toxoid contains large quantities of natural adjuvant factors described by Lahiri (9). In fact it was possible for us to produce sera of high titer (1200 to 2000 U/ml) for many years by using this crude toxoid for hyperimmunization of horses.

Crude tetanus toxoid was first prepared in enzyme digest of meat according to the formule of Turpin et al (21); later it was replaced by Muller's medium (16, 17, 18). To increase the yield of toxin, the whole culture of Cl. tetani is, at present, submitted to freezing and thawing, as we have described elsewhere (15). Th toxoid is fractionated with ammonium sulfate by Levine's technique (10). The final product contains 1300 to 1600 Lf/mg P.N.

2-A.P.T.

With very good response, but causing untoward reactions, APT is prepared as described by Glenny and Barr (5), Linggood (2), Barr and her associates (1). This immunizing preparation contains:

Diphtheria toxoid	30 Lf)	
Tetanus toxoid	15 Lf)	1 ml
Alk (s04) 2, 12H20	30 mg	
Merthiolate (Ely Lilly & Co)	0.01%	

Purity: 200-300 Lf: mg P.N.

2 injections of 0.5 ml, 3-4 weeks apart are practised.

3-**P.T.A.P.**

Purified toxoid adsorbed on aluminium phosphate is one of the best known prophylactics which gives excellent immunity without causing much unpleasant reactions in young adults. We prepare the toxoid by Holt's technique (8).

The purification by frctionation with ammonium sulfate as well as the adsorption on aluminium phosphate nascent is done as described by Levine et al (11).

This prophylactic contains:

Diphtheria toxoid	30 Lf)	
Tetnus toxoid	15 Lf)	1 ml
AIPO4	7 mg)	

Purity: 1400 - 1600 Lf/mg N.P.

2 injections of 0.5 ml, 3 to4 weeks apart.

Titration of Antitoxin

1) Diphtheria Antitoxin

Tests to determine the antitoxin value of the sera were made in guinea pigs by intracutaneous injections of mixtures of toxin-sera, similar, in general, to the technique used in N.I.H., Utrecht-Holland.*

Our local breed of guinea pigs is suitable for this purpose. Varying dilutions of sera are mixed with equal volumes of the standard test toxin and after 30 minutes incubation in 22.C, 0.2 ml of each mixture is injected intradermally.

In general, each guinea pig receives 5 injections in each side, previously shaved and cleaned, The Lr to Lr/1000 of the standard toxin are carefully designed with Reference Danish antitoxin of State Seruminstitute, Cophenhagen. The reactions are recorded 48 hour after injection. We adopt, as standard reaction, the redness of 15 mm diameter with swelling, but without necrosis. The antitoxin value is estimated by comparison between dermal reaction of test serum and that of reference serum. The highest titer obtained is 27 A.U./ml and the lowest 0.001 A.U./ml.

2) Tetanus Antitoxin

Tetanus antitoxin is determined by Greenberg's method (6,7)** and our in breed Swiss mice are used throughout this investigation.

Schick - Test

Our Schick test toxin is a sterile dilution of a mature diphtheria toxin. The crude toxin is prepared in tryptic digest of meat, as described by Linggood et al (13). The sterile toxin matured 5 years under toluol in cold; it contains 45 Lf/ml and 200 LD50/ml. The Schick test dose, injected in 0.2 ml contains 1/50 LD50. The control raegent is the same diluted

(★) HMC wishes to thank Dr Tasman, Chief Toxoids and Antitoxins Laboratories of National Institute of Health, Utrecht, Holland, for his valuable help during his stary in Utrecht.

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toxin heated 30 minutes at 75oC. The Schick and control reagents are injected into the inner side of the right and left forearm respectively. The reations are recorded by two qualified persons after 2 and 4 days.

Results

One of the main objectives of active immunization is the sensitization of the subject to the stimulus of antigen. Bousfield in 1945 (3) was able to show that the degree of this sensitization in case of diphtheria is so high that even a small dose of toxin contained in a Schick test dose may stimulate production of antitoxin. In this investigation the bleeding is performed one week before Schick control.

The reults of Schick tests are summarized in table 1.

Table 1

Schick control in some elementary schools in Tehran June 1958

Name of School	Age year	Total No. children	Sex	Positive No.	Positive %	Pseudo positive	Pseudo negative	Negative	Negative %
Kuresh	7-12	502	mix.	17	3.4	15	2	468	96.6
Rouhi	5-10	103	,,	4	3.8	1	0	98	96.2
Ettehad	7-16	455	male	14	3.0	5	0	436	97.0
Ettehad	7-16	763	fem.	26	3.4	24	8	705	96.6
Cyrus	4-7	475	mix.	18	3.8	6	2	449	96.2
Bagh-Saba	5-8	41	,,	1	2.5	0	0	40	97.5
Fakhrabad	6-14	513	,,	8	1.5	15	1	489	98.5
Darasht	6-10	168	,,	—	0	7	1	160	100.0
Gorgan	6-10	189	,,	10	5.3	4	2	173	94.7
Total:		3209		98	3.0	77	16	3018	97.0

We may note from these figures that the adequate immunity is established in the group of children under our investigation. It is interesting to note that among 3209 Schick tested children 77 show pseudo-positive and 16 pseudo-negative reaction (2.8% pseudo-reactors).

The level of antitoxin value in total blood sera tested being only 2% under 0.01 U/ml. We believe, as it is mentioned by Barr and her associates in 1957 (2) that all these pseudo-reactors are immune and the small dose of Schick toxin may reinforce their immunity.

The responses to diphtheria toxoids are shown in table 2.

Among 125 samples of sera of children vaccinated 5 to 6 years ago with F.T., we have found 94 children (75%) with antitoxin level over 0.1 U/ml and even 47 children (37%) have 0.5 U/ml and more circulating antitoxin. We believe the natural boosting may play a role to reinforce the effect of antitoxin in a high position. In fact, amongst 48 children of both sexes aged 8 to 16 years and without any previous history of inoculation we have found only 13 (27%) under Schick-negative level. It is interesting to note that in Tehran the diphtheria morbidity rate is still high. According to the official figures of 1956 there were 604 notification s of diphtheria in Tehran with 106 deaths.

The population of Tehran being 1,512,082 (recent census) the morbidity rate is 40 per 100,000 inhabitants.

This figure, in comparison with those of the other countries where regular mass vaccination is carried out, is high.

The following table copied from the interesting article of Tasman and Lansberg (1957) (21) summarized the diphtheria morbidity in some of the Western countries.

TABLE 4

Cases of diphtheria, per 100,000 inhabitants; notified in various countries from 1941 to 1952

Country	1941	1944	1946	1948	1951	1952
Netherlands	60.65	656.49	286.41	44.01	26.86	26.98
England and Wales	121.68	54.65	28.07	8.22	1.59	0.96
Belgium	52.34	78.22	43.97	15.51	5.90	6.16
Denmark	23.90	83.87	24.05	3.75	0.72	0.30
U.S.A.	13.50	10.25	11.58	6.55	2.68	1.95

The second prophylactic used in this investigation is APT which, though very potent and useful for vaccination of children under 5 years, was not found satisfactory for mass vaccination of older children. In fact by using it a lot of local and general reactions occur.

From the 64 samples of sera of children vaccinated with 2 x 0.5 ml of APT 4 years ago, 48 (75%) had anti-toxin level over 0.1 U/ml and 2 (3%) were under Schick-negative level.

On the other hand PTAP used in Iran since 1954 seems to be a good prophylactic for vaccination of young older children. The reaction to the first injection in both sexes is generally mild. It is rare to see general or local severe reaction. However the number of local reactions to the second injection of PTAP seems to be greater; there are some cases with general reaction too.

The response to this prophylactic is good. From 389 children boosted 1 to 3 years before this test was done with PTAP, 324 (83%) had more than 0.1 U/ml and 100 (26%) even 1 to more than 10 U/ml anti-toxin. In this study a group of 67 children vaccinated four weeks before testing show no titer below Schick-negative level, 61 (88%) have a level of anti-toxin over 0.25 U/ml and 40 (62.5%) have 1 to 27 U/ml.

The responses to tetanus toxoids are also shown in table 3.

As there is no natural immunity or subclinical infection in tetanus, the presence of anti-toxin is a direct result of active immunity.

113 children vaccinated 5-6 years ago with crude formol toxoid and had an average titer of 0.035 U/ml.

The response with PTAP is much better. In fact, as it is shown in table 3, children vaccinated 1-4 years ago with PTAP have mostly titers between 0.07 and 0.7 A.U./ml. But between 43 not vaccinated controls 40 (93%) have no detectable tetanus antitoxin.

Summary and Conclusions

1. The results of active immunization program conducted by the American Joint Distribution Committee in Iran amongst children of some kindergarten and elementary schools of Tehran are studied.

2. The high rate Schick negative (94 to 100%) shows the value of active immunization and the natural boosting.

3. The immune response of 683 children is analyzed. It is found sufficient amounts of Diphtheria and Tetanus antitoxins in quite a big number of children under investigation.

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TABLE 2

Diphtheria antitoxin value 1 month to 6 years after immunization.

Date of the last boosting	Vaccine injected	Total children over under	Antitoxin U/ml															
			0.01	0.01 0.02	0.02 0.1	0.1 0.25	0.25 0.5	0.5 0.75	0.75 1.0	1.0 1.5	1.5 2.0	2.0 2.5	2.5 3.0	3.0 4.0	4.0 5.0	5.0 10	10 30
6 years before bleeding	F.T.	12	1	—	1	5	—	3	1	—	—	1	1	1	1	1	—
5 years before bleeding	F.T.	103	2	7	20	13	19	16	18	2	2	2	2	—	—	—	—
4 years	A.P.T.	64	2	6	8	11	13	10	9	3	2	—	—	—	—	—	—
3 years	P.T.A.P.	181	9	7	26	30	22	27	26	12	3	3	6	2	3	5	—
2 years	P.T.A.P.	148	1	11	14	23	20	17	22	16	6	2	5	—	2	3	6
1 year	P.T.A.P.	60	1	1	5	4	9	8	6	12	4	1	3	1	2	3	—
1 month	P.T.A.P.	67	—	5	1	1	9	2	9	10	1	3	2	—	—	14	10
Controls no vaccinated		48	13	15	8	9	3										
TOTAL CHILDREN		683															

TABLE 3

Tetanus Antitoxin value one month to 6 years after immunization.

Date of the last boosting	Vaccine injected	Total children	Antitoxin U/ml over	under 0.002	0.002 0.004	0.004 0.01	0.01 0.02	0.02 0.10	0.10 0.50	0.50 1.0	1.0
6 years before bleeding	F.T.	12	5	1		2	2	2			
5 years	F.T.	101	24	11	22	23	15	6			
4 years	P.T.A.P.	69	8	6	12	13	12	5	2		1
3 years	P.T.A.P.	164	32	15	30	23	37	20	7		1
2 years	P.T.A.P.	133	5	10	11	15	24	45	21		2
1 year	P.T.A.P.	57	6	3	2	4	15	21	3		3
1 month	P.T.A.P.	49		4	1	3	6	9	23		3
Controls, no vaccinated		43	40	3							
TOTAL	**CHILDREN**	**683**									

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