ANTIGENICITY OF T.A.B.C. VACCINE AFTER ADMIXTURE WITH TETANUS TOXOID FOR VARIOUS PERIODS

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(With 1 Figure in the Text)

Introduction

When, in December 1940, it was decided to introduce a combined anti-enteric and anti-tetanus prophylactic vaccine for the immunization of naval personnel two problems arose in connexion with the keeping properties of the mixed vaccine, the extent of deterioration in its immunogenic power, and the possible undesirable interaction of the formalized tetanus toxoid with the heat-killed phenolized suspension of the typhoid and paratyphoid organisms.

Some degree of loss of potency was, of course, to be expected, as in all other biological products, but it was obvious that this could not be investigated until sufficient time had elapsed from the date of preparation. For this reason it was not feasible to give any definite, or, at that time, approximate 'expiry date' to the vaccine when issued for use, but official instructions were promulgated to the effect that the vaccine was to be used as soon as practicable after receipt and that stocks of vaccine in excess of ordinary requirements were not to be demanded. From the monthly inoculation reports forwarded from all ships and establishments using the vaccine, it is evident that this is being done and that the vaccine is being used within a short period after manufacture.

The first of the two problems concerned the tetanus toxoid element, the second the anti-typhoid element. The research described here deals solely with the latter question and was designed to determine by direct experiment on men the degree of loss of potency, on keeping under ordinary conditions of temperature, of the anti-typhoid element of the combined vaccine.

Owing to the obvious impossibility of carrying out a decisive 'field trial' on human beings, the degree of the protective power of the vaccine has been assessed, as is the generally accepted practice, by the estimation of its agglutinogenic value. The first problem, that is to say the effect of the T.A.B.C. component of the vaccine on the formalized tetanus toxoid, is not dealt with in the present paper but will be the subject of a further one.

The investigation detailed below gives a reassuring answer to doubts expressed as to the antigenic stability of the anti-typhoid elements of the combined vaccine, and it would appear quite safe to allow an expiry time of 18 months for the standard combined vaccine.

Methods

T.A.B.C. vaccine

The vaccines used were those prepared in the R.N. Medical School for routine use in naval personnel. The same strains of typhoid and paratyphoid bacilli were used throughout, namely:

Bact. typhosum (Rawlings), R.A.M.C.
Bact. paratyphosum A (Mears).
Bact. paratyphosum B (IHB_3 Felix).
Bact. paratyphosum C (original Tidy), non-flagellated strain.

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These strains were maintained by subculture and passaged regularly in mice. Before being used for the preparation of a fresh batch of vaccine each strain was fully identified and agglutinated to titre with its homologous antiserum. The vaccines were heat-killed and preserved by the final addition of 0·25% phenol.

Tetanus toxoid

This formalized product was supplied by Parke, Davis and Co. To every 4 l. of toxoid, the required amount (approx. 200 c.c.) of strong bacillary emulsion was added to give the necessary mixture required for each dose of vaccine.

Volunteers

Three groups of adult males of the 18–25-year age group, previously uninoculated, were obtained in three training centres. Each volunteer was given the usual course of two doses separated by an interval of 4 weeks. The first dose of 1 c.c. contained the necessary amount of tetanus toxoid $+500 \times 10^6$ *Bact. typhosum* and 250×10^6 each of *Bact. paratyphosum* A, B and C. The second dose of 1 c.c. contained the same amount of tetanus toxoid $+1000 \times 10^6$ *Bact. typhosum* and 500×10^6 each of *Bact. paratyphosum* A, B and C.

Blood samples were taken immediately before the first injection and 10 days after the second injection. The batches of vaccine were distributed as follows:

Establishment	Vaccine dose	Batch no.	Date of vaccine	Age of vaccine
Training centre G	1st	3	10. xi. 40	16 months
,, ,, G	2nd	24	20. i. 41	16 months
,, ,, C	1st	47	28. iii. 41	12 months
,, ,, C	2nd	49	31. iii. 41	13 months
,, ,, R	1st	75	19. ii. 42	6 weeks
,, ,, R	2nd	82	9. iii. 42	7 weeks

Serological technique

Antigens. Oxford Standard emulsions were used.

Dilutions of serum made throughout with separate pipettes for dilutions ranging from 1/5 to 1/1280.

Temperature and time of agglutination:

O reactions in 2 hr. at 54° C. in water-bath.
H reactions in 2 hr. at 37° C. in water-bath.
Vi reactions in 2 hr. at 37° C. in incubator.

Readings made after standing 18 hr. at room temperature—interpreted according to Oxford Laboratory standards.

Results

The results are summarized in Tables 1 and 2, and in Fig. 1.

'H' agglutinin response

With rare exceptions, 'H' agglutinins were absent in the sera before inoculation from all three groups of volunteers even at a dilution of 1/10. As shown in Fig. 1 the response to inoculation with the 6–7 weeks vaccine was very good in the case of *Bact. typhosum*, *Bact. paratyphosum* A and B, high titres being reached in the majority of individuals. The absence of *Bact. paratyphosum* C 'H' agglutinins observed throughout the investigation was due to the absence of 'H' agglutinogen in the strain used in the vaccine.

The response to inoculation with the 16 months vaccine was also satisfactory, although its effect was somewhat reduced as compared with fresh vaccine.

Unexpectedly the response to *Bact. typhosum* 'H' was markedly reduced after the use of the 12–13-months vaccine, although responses to *Bact. paratyphosum* A and B were slightly reduced as compared with those due to the fresh vaccine.

'O' agglutinin response

At low titres a considerable proportion of sera before inoculation reacted with *Bact. typhosum* 'O' and *Bact. paratyphosum* B 'O', but relatively few with *Bact. paratyphosum* A 'O' or C 'O'. As shown in Fig. 1, the distribution of pre-inoculation titres was approximately the same in all three groups of volunteers except that the initial titres were relatively lower in those receiving the oldest batch of vaccine.

Table 1. *To show the H agglutinin titre in previously uninoculated subjects before and after inoculation with mixtures of antityphoid-paratyphoid vaccine and tetanus toxoid of different ages*

Age of vaccine-toxoid mixture	No. of sera tested	Stage	Percentage of sera showing standard* agglutination at dilutions of with *Bact. typhosum* 'H' 1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	with *Bact. paratyphosum* A 'H' 1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
6–7 weeks	24	Pre-inoculation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Post-inoculation	100	100	100	100	95·8	91·7	58·3	25·0	100	100	100	100	100	95·8	75·0	29·1
12–13 months	18	Pre-inoculation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Post-inoculation	38·8	33·3	11·1	5·5	0	0	0	0	100	100	94·4	94·4	94·4	72·2	33·3	16·6
16 months	22	Pre-inoculation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Post-inoculation	100	100	100	100	77·3	18·1	4·5	4·5	100	100	100	100	90·9	36·4	9·1	4·5

Age of vaccine-toxoid mixture	No. of sera tested	Stage	Percentage of sera showing standard* agglutination at dilutions of with *Bact. paratyphosum* B 'H' 1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	with *Bact. paratyphosum* C 'H' 1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
6–7 weeks	24	Pre-inoculation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Post-inoculation	100	100	100	95·8	95·8	91·7	83·3	58·3	0	0	0	0	0	0	0	0
12–13 months	18	Pre-inoculation	5·5	5·5	5·5	0	0	0	0	0	0	0	0	0	0	0	0	0
		Post-inoculation	100	100	97·4	97·4	88·8	61·1	27·7	16·6	0	0	0	0	0	0	0	0
16 months	22	Pre-inoculation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Post-inoculation	100	100	100	90·9	86·4	63·6	27·2	4·5	0	0	0	0	0	0	0	0

* Standard agglutination as defined by Standards Laboratory, Oxford.

Table 2. *To show the O and Vi agglutinin titre in previously uninoculated subjects before and after inoculation with mixtures of antityphoid-paratyphoid vaccine and tetanus toxoid of different ages*

Age of vaccine-toxoid mixture	No. of sera tested	Stage	Percentage of sera showing standard* agglutination at dilutions of with *Bact. typhosum* O 1/10	1/20	1/40	1/80	1/160	1/320	*Bact. paratyphosum* A 'O' 1/10	1/20	1/40	1/80	1/160	1/320	*Bact. paratyphosum* B 'O' 1/10	1/20	1/40	1/80	1/160	1/320
6–7 weeks	24	Pre-inoculation	91·7	41·2	0	0	0	0	8·3	0	0	0	0	0	59·1	8·3	0	0	0	0
		Post-inoculation	100	100	91·7	75·0	25·0	4·2	37·5	8·3	0	0	0	0	100	100	87·5	41·7	0	0
12–13 months	18	Pre-inoculation	94·4	72·2	5·5	0	0	0	5·5	0	0	0	0	0	66·6	38·8	5·5	0	0	0
		Post-inoculation	100	94·4	27·7	5·5	0	0	44·4	16·6	0	0	0	0	94·4	88·8	38·8	11·1	0	0
16 months	22	Pre-inoculation	72·7	27·3	4·5	0	0	0	18·2	4·5	0	0	0	0	36·4	13·6	4·5	0	0	0
		Post-inoculation	95·4	95·4	54·5	36·4	4·5	0	40·9	4·5	0	0	0	0	100	100	63·6	22·7	4·5	0

Age of vaccine-toxoid mixture	No. of sera tested	Stage	Percentage of sera showing standard* agglutination at dilutions of with *Bact. paratyphosum* C 'O' 1/10	1/20	1/40	1/80	1/160	1/320	*Bact. typhosum* 'Vi' 1/5	1/10	1/20	1/40	1/80	1/160
6–7 weeks	24	Pre-inoculation	4·2	0	0	0	0	0	8·3	0	0	0	0	0
		Post-inoculation	70·8	16·6	0	0	0	0	62·5	16·6	4·2	0	0	0
12–13 months	18	Pre-inoculation	0	0	0	0	0	0	5·5	0	0	0	0	0
		Post-inoculation	27·7	11·1	0	0	0	0	50·0	5·5	0	0	0	0
16 months	22	Pre-inoculation	13·6	4·5	0	0	0	0	27·3	0	0	0	0	0
		Post-inoculation	63·6	27·2	9·1	0	0·	0	68·2	18·1	0	0	0	0

* Standard agglutination as defined by Standards Laboratory, Oxford.

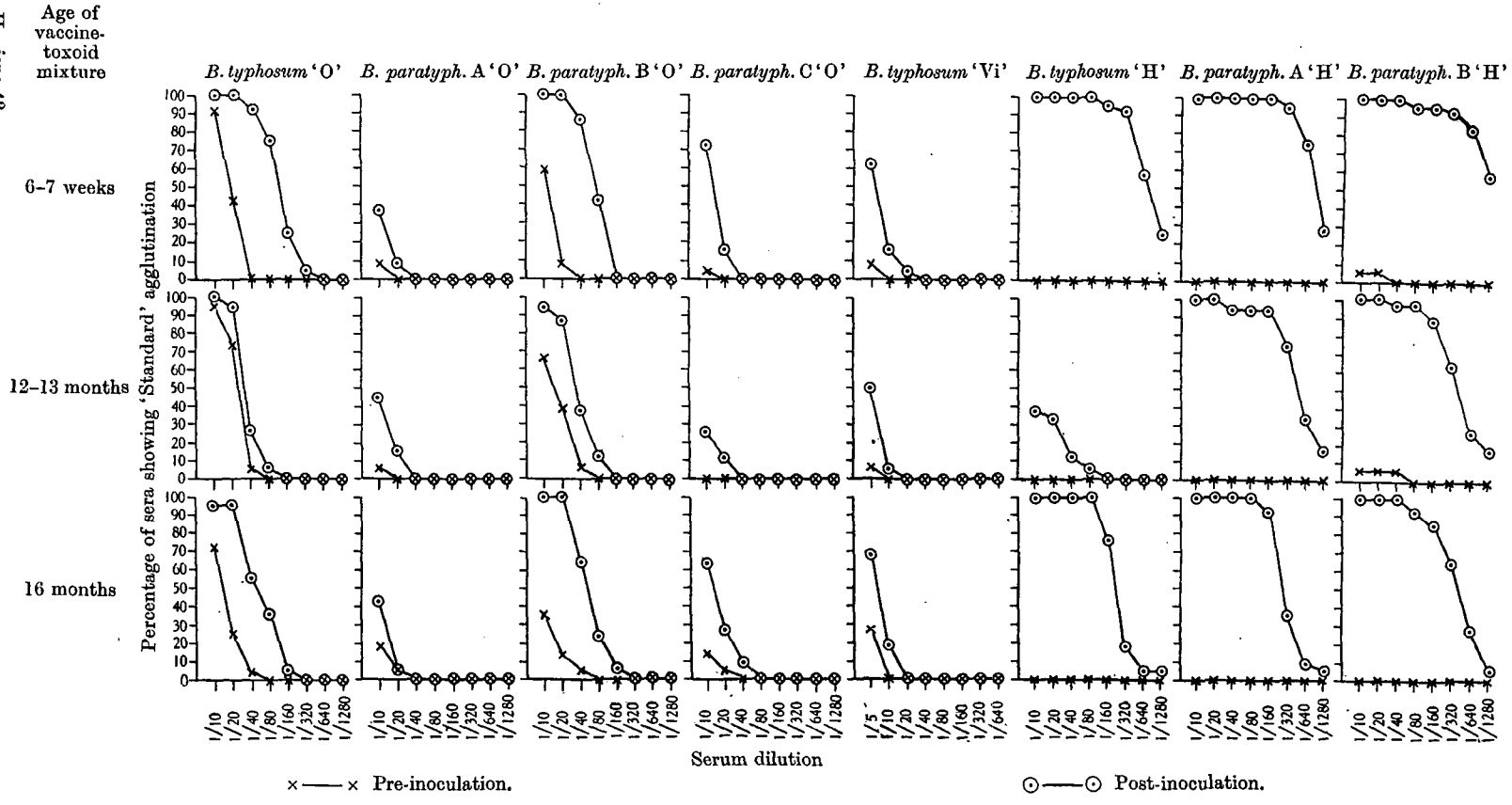

Fig. 1. To illustrate the distribution of titres to Oxford Standards Laboratory suspensions in previously uninoculated subjects before and after inoculation with fresh and aged mixtures of T.A.B.C. heat-killed, phenolized vaccine and tetanus toxoid.

After inoculation with fresh vaccine the 'O' titres against all four organisms were raised, but those for *Bact. typhosum* and *Bact. paratyphosum* B were much higher than for the other two organisms.

The curves showing the post-inoculation distribution of 'O' titres following the use of the 16 months vaccine were similar in shape to those produced by fresh vaccine although reduced in degree.

The corresponding curves following the use of the 12–13 months vaccine were also similar in shape although the titres were still further reduced in degree.

'Vi' agglutinin response

Whereas only 8·3% of pre-inoculation sera in the group given fresh vaccine showed standard agglutination with *Bact. typhosum* 'Vi' antigen at a dilution of 1/5 serum and none at a dilution of 1/10 or higher, the percentages of post-inoculation sera giving this degree of agglutination at dilutions of 1/5, 1/10 and 1/20 were 62·5, 16·6 and 4·2% respectively. The 16 months vaccine had practically the same effect, while there was still a demonstrable though reduced increase following the use of the 12–13 months vaccine.

Discussion

The figures obtained are of value in indicating the range of titre to be expected immediately after the routine course of anti-typhoid-tetanus inoculation with R.N. Medical School vaccine-toxoid using Oxford Standard emulsions for titration. With a few exceptions the pre-inoculation titres were within the limits expected in normal sera. The relatively high proportion of normal sera reacting with 'O' antigens at low dilutions was not an exceptional finding. It is probable that such reactions were due to the presence of normal, non-specific agglutinins. It is interesting that these reactions were more frequent in the case of *Bact. typhosum* and *Bact. paratyphosum* B, the only two organisms of importance in this country.

The marked reduction in the response to *Bact. typhosum* 'H' following the use of the 12–13 months vaccine raised the possibility that the strain of *Bact. typhosum* possessed relatively little 'H' antigen at the time of manufacture of the vaccine. That possibility cannot be entirely excluded with the data available, although it was an extremely unlikely occurrence. In any case, minute amounts of 'H' antigen are sufficient to produce a good response.

The extent to which the observed loss was due to 'ageing' of the vaccine or to the action of the toxoid and its phenol content could not be ascertained. The ideal experiment required to provide the fullest information on this point is the division of one batch of vaccine into two portions, one being mixed with toxoid and the other retained without toxoid. Groups of volunteers should then be given the two portions of vaccine at certain intervals after preparation. For preference the experiment should be triplicated with vaccine kept at 2–4, 18 and 37° C.

The 'Vi' agglutinin response to the vaccine-toxoid mixture was no worse than that reported by Felix, Rainsford & Stokes (1941) following the use of vaccine alone. It is unlikely, therefore, that the hypothesis advanced by Rainsford (1942) 'that the ability of heat-killed-phenolized vaccine to sensitize the tissues to Vi antigen when given alone may be lost when it is mixed with tetanus formol-toxoid' will be substantiated.

Conclusions

1. The antigenicity of batches of T.A.B.C. vaccine after mixture with tetanus toxoid and held at 18–25° C. for 6–7 weeks, 12–13 months and 16 months was tested in groups of adult males in whom the pre-inoculation titres for the various antigens were strictly comparable.

2. The batch used within 6–7 weeks of production produced the greatest effect, but the older batches were not greatly reduced in potency.

3. Within the limitations imposed by the restricted investigation, the more effective result following the use of the 16 months vaccine as compared with the 12–13 months vaccine indicates that factors in addition to that of time were concerned in the deterioration of antigenic power.

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