IMMUNIZATION AGAINST TYPHOID AND PARATYPHOID WITH ALCOHOL-KILLED, ALCOHOL-PRESERVED AND HEAT-KILLED, PHENOL-PRESERVED, VACCINE

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In 1941, Felix (1941) described a new type of typhoid and paratyphoid vaccine in which the organisms were killed by, and preserved in, alcohol. The advantages claimed for this vaccine were that it produced Vi antibody of full functional efficiency in a proportion of cases inoculated, and that the local and general reactions to its use were milder than with ordinary vaccine.

An opportunity was offered whilst immunizing a group of nurses and Civil Defence workers, of comparing this vaccine in a small series of cases with ordinary typhoid-paratyphoid vaccine. Of one hundred and six individuals inoculated, forty-four were done with ordinary T.A.B.C. vaccine, and sixty-two with alcohol T.A.B.C. vaccine. Both groups were composed mainly of females, but the proportion of males in each case was the same. The ages ranged from 16 to 50. The age distribution in both was similar, and each individual was asked to keep a note of the local and general reactions following injection.

The vaccines used in the experiment differed widely in age. The ordinary T.A.B.C. vaccine was prepared by the Lister Institute in 1938, and was between 2½ and 3 years old at the time of its use having been kept till then in cold storage. The organisms in this vaccine are killed by heat (at 56° C.) and preserved in 0·5 % phenol. The strain of *B. typhosus* from which it is made (Ty 2) is well known for its ability to produce Vi antigen (Felix, 1938).

The vaccine was given subcutaneously into the arm, at the insertion of the deltoid, in doses which varied for the sexes, males having 0·5 and 1·0 c.c., and females 0·4 and 0·8 c.c. The interval between the doses was a week in each case.

The other T.A.B.C. vaccine was also prepared by the Lister Institute, but was between 6 and 9 months old when used. The strains from which it is prepared are the same as those in the previous vaccine (with the exception of *B. paratyphosus* C), but the organisms are killed by 75 % alcohol and preserved in 22·5 % alcohol (Felix, 1941). Its composition is the same as that of the previous vaccine, namely 1000 million *B. typhosus* and 500 million of each of the other components per c.c.

This vaccine was also given subcutaneously, but the dosage for males was 0·25 and 0·5 c.c., and that for females 0·2 and 0·4 c.c., as recommended by Felix, Rainsford & Stokes (1941). The interval between the doses was 2 weeks in each case.

Blood was withdrawn from each subject 3 weeks after the second injection and agglutination tests carried out against suspensions of *B. typhosus* H, O and Vi, and *B. paratyphosus* B, H and O. Standard suspensions, supplied by the Standards Laboratory, Oxford, were used throughout, and agglutination tests were incubated at 37° C. for 2 hr., those for H agglutination being read at the end of that time, and those for O and Vi agglutination after standing at room temperature for a further 22 hr. The end-titre was taken as that tube which showed definite agglutination visible to the naked eye.

The Vi agglutination tests were carried out according to the technique laid down in the Standards Laboratory leaflet, and a positive reading was taken as being one in which granular agglutination was clearly visible to the naked eye over the base of the tube, the supernatant fluid being practically clear. For purposes of comparison a suitable dilution of Standards Laboratory Vi serum to produce such a reading was put up with each test.

The results in the two series of cases are shown in Tables 1 and 2. As the O suspension of *B. paratyphosus* B was not of Standard agglutinability, but Standard × 0·5, there are no readings recorded in the columns showing a serum dilution of 1 in 20.

Table 1. *Heat-killed, phenol-preserved, T.A.B.C. vaccine*

	No. of sera with end-titres of									
Antigen:	0	20	40	80	160	320	640	1280	2560	Total
B. typhosus H	0	0	0	0	6	11	12	10	5	44
B. typhosus O	6	7	11	14	4	2	0	0	0	44
B. paratyphosus B: H (sp.)	1	0	2	8	10	11	7	4	1	44
B. paratyphosus B: O	11	—	10	13	9	1	0	0	0	44

Vi agglutinins: No serum produced a positive agglutination in a dilution of 1 in 5 or over.

It will be seen that, as might be expected, the readings for H agglutination were either absent, or very low, in the alcohol-vaccine group. With regard to O agglutination, however, there was not a great deal of difference except that the highest alcohol-vaccine titre for *B. typhosus* was 160, whilst that of ordinary vaccine was 320. Fewer cases showed no O agglutination in the alcohol-vaccine group than in the other.

The average O titres showed surprisingly little difference, those for *B. typhosus* being 57·0 in the alcohol-vaccine group, and 67·7 in the ordinary vaccine group. For *B. paratyphosus* B the figures were 65·4 and 72·7 respectively.

Table 2. *Alcohol-killed and preserved T.A.B.C. vaccine*

	No. of sera with end-titres of									
Antigen:	0	20	40	80	160	320	640	1280	2560	Total
B. typhosus H	40	13	5	2	1	1	0	0	0	62
B. typhosus O	3	10	23	22	4	0	0	0	0	62
B. paratyphosus BH (Sp.)	43	9	6	1	2	0	0	1	0	62
B. paratyphosus BO	10	—	20	25	6	1	0	0	0	62

	No. of sera with end-titres of					
Antigen	0	5	10	20	40	Total
B. typhosus Vi	53	6	1	2	0	62

A marked dissimilarity was seen when the Vi agglutination results were examined, as no serum in the ordinary group showed positive agglutination in a dilution of 1 in 5, or over, though several showed a trace. On the other hand, nine of the sixty-two sera in the alcohol vaccine group showed titres varying from 5 to 20.

Alcohol vaccine on injection caused a sharp stinging sensation which lasted about ¼ min. The local reaction was rather less than that caused by the ordinary vaccine used in this experiment as can be seen in Table 3 where the results are summarized. Redness and swelling around the site of injection in the alcohol-vaccine group was less marked than with the other, but lasted about the same length of time, ultimately leaving a small palpable nodule which lasted several weeks. It must be pointed out, however, that ordinary T.A.B.C. vaccine tends to become less toxic as it becomes older, and that this

vaccine was nearly at the end of its official life of 3 years. Despite this, it can be seen, when the summarized general reactions are studied (Table 3), that alcohol-vaccine caused much less inconvenience, with the single exception that tender axillary lymph glands appeared to be more common.

Both vaccines had been used intravenously for the production of protein shock in cases of chronic gonorrhoea and syphilis. As the transient appearance of Vi agglutinins has been reported in human subjects inoculated subcutaneously with heat-killed, phenolized T.A.B. vaccine (Bensted, 1940), patients so treated were bled every 4 days and their sera examined for the presence of these agglutinins.

One patient only (a girl of 10 years) was given alcohol T.A.B.C. vaccine, and her Vi titre, after the last four intravenous injections, ranging from 0·01 to 0·1 c.c., was 320.

Table 3. *Reactions to alcohol-killed and preserved, and heat-killed, phenol-preserved vaccine, expressed in percentages*

Local reactions

Group	No. of injections	Negative	Reaction lasting for 24 hr.	48 hr.	More than 48 hr.
Alcohol vaccine	124	27·4	45·9	15·3	11·4
Ordinary vaccine	88	22·7	51·1	17·0	9·2

General reactions

Group	No. of injections	Headache	General malaise	Gastro-intestinal upset	Adenitis	Myalgia
Alcohol vaccine	124	4·0	7·2	4·0	5·6	2·4
Ordinary vaccine	88	20·4	15·9	6·8	1·1	5·6

Eleven other patients who had the ordinary T.A.B.C. vaccine have been similarly followed up, and the sera of five of these has shown Vi agglutinins, the titres varying from 5 to 10. None of these cases showed Vi agglutinins before inoculation, and in four cases they first appeared 4 days after the second injection. In the fifth case they were noted 10 days after the first injection. The doses varied from 0·05 to 0·2 c.c., and were given at intervals of 4 days. Only one of these patients had previously been inoculated with T.A.B. vaccine, but another was said to have had typhoid fever in childhood. Vi agglutinins were only present for 8–12 days.

In a further four cases in which other batches of T.A.B.C. or T.A.B. vaccine were given intravenously, and whose sera were examined before and after treatment, Vi agglutinins were present in one. This man received a dose of 0·2 c.c. of a 2 months old T.A.B. vaccine prepared in the same way and from the same strains as the heat-killed, phenolized T.A.B.C. vaccine mentioned above. His response was good, the temperature reaching 106° F. Eight days later Vi agglutinins appeared in his serum to a titre of 40. Four days after this the titre reached 160 and thereafter slowly fell. This man gave no history of previous immunization or infection.

Thus, of fifteen cases given heat-killed, phenolized vaccine intravenously, Vi agglutinins made a transient appearance in six.

DISCUSSION

The results in this small series support the conclusions reached by Felix *et al.* (1941). Local reactions to alcohol-vaccine are milder, and general reactions very much milder than with ordinary T.A.B.C. vaccine. Despite this, the precautions usually taken in giving

antityphoid-paratyphoid vaccines cannot be disregarded. A general reaction with rigors, and repeated vomiting, has been seen in an individual who performed hard physical exertion ½ hr. after receiving 0·5 c.c. of alcoholized vaccine. This man's first injection caused him no inconvenience, but he was off work for 3 days after his second.

Felix *et al.* (1941) reported the occurrence, in three cases out of one hundred and forty given the customary large doses of T.A.B.C. alcohol-vaccine, of a peculiar kind of delayed local reaction which appeared about the fourth day after injection, commencing with local induration which generally became fluctuant by the sixth day after injection and persisted for several weeks. This occurred after the first injection (0·5 c.c.) in two cases, and after the second injection (1·0 c.c.) in the other case. No such reactions were seen in subjects given the reduced scale of doses later recommended by Felix and his co-workers. In the present series of sixty-two cases, who all received the smaller dosage, one showed this reaction. It appeared on the fourth day after the first dose (0·2 c.c.), was painless, did not interfere with the use of the arm, and lasted for nearly 4 weeks. As in the cases referred to above, there were no constitutional symptoms.

Though it is generally recommended that such susceptible subjects should receive only half the amount of vaccine for a second dose, this girl received the same amount (0·2 c.c.) 2 weeks after the first dose, but this time the reaction was that normally seen with such an injection.

An interesting point is the slight difference in average O titres between the two groups especially as the heat-killed, phenolized vaccine used was nearly 3 years old, whilst the alcoholized vaccine was less than 9 months old. Opinions vary as to the loss of potency that occurs during the storage of vaccines and most commercial vaccines are given a life of 18 months in cold storage. Alcohol T.A.B.C. vaccine is known to be potent for at least 9 months after storage in the cold (Felix, 1941), and Schutze (1930) could demonstrate that there was no deterioration in the prophylactic value of *B. paratyphosus* B vaccine stored for 9 months in a cold room. He quotes Konrich (1929), who found that storage of typhoid vaccines for 3 years under these conditions did not damage their immunogenic properties. Mishulow, Mowry & Stocker (1937) came to a similar conclusion after investigating, by mouse protection and agglutination tests, the potency of typhoid-paratyphoid vaccines preserved with 0·5 % tricresol and stored for 2½–3 years at 8 and 10° C.

There would not seem to be much deterioration of potency, in the phenolized T.A.B.C. vaccine used here, so far as ability to produce O agglutinins is concerned. This vaccine was possibly less toxic than others, as some from the same batch was used in Southampton during 1940, when the number of severe reactions was surprisingly low (Williams, 1941).

Typhoid-paratyphoid vaccines are said to become less toxic with age, and that seems likely, as it became increasingly difficult to produce protein shock reactions with this vaccine during the last few months of its official life of 3 years.

The proportion of cases in which there was a demonstrable increase of Vi agglutinins (14·4 %) in the alcohol-vaccine group was rather lower than the majority of the findings of Felix and his co-workers. No Vi agglutinins were demonstrable in the heat-killed, phenolized vaccine group, but this is not really surprising because of the known deleterious effect of phenol on Vi antigen (Felix & Bhatnagar, 1935), and because of the susceptibility of the latter to the action of heat (Felix, Bhatnagar & Pitt, 1934). Why the administration of such a vaccine intravenously should cause the appearance, though transient, of Vi agglutinins is difficult to decide, unless it be that there is a reversal of the reaction between

the antigen and the phenol, which is known to occur (Felix & Petrie, 1938). Bensted (1940) found a similar occurrence of Vi agglutinins in nine of twenty-five persons who had severe reactions with ordinary subcutaneous T.A.B. vaccine.

It may be that this phenomenon occurs in every case inoculated with ordinary T.A.B. vaccine, but is only occasionally strong enough to produce circulating Vi antibody.

SUMMARY AND CONCLUSIONS

1. A comparison of the agglutinogenic response and reactions, both local and general, between a series of sixty-two persons immunized with alcohol-killed, alcohol-preserved, T.A.B.C. vaccine, and forty-four persons immunized with a much older heat-killed, phenol preserved, T.A.B.C. vaccine is described.

2. It was found that there was comparatively little difference between the two series in regard to O agglutinin response.

3. Vi agglutinins were found in 14·4 % of the alcohol-vaccine cases, but none appeared in the heat-killed, phenolized vaccine cases.

4. There was no significant difference in the frequency of local reactions, but the general reactions were markedly fewer in the alcohol-vaccine cases.

5. The occurrence of Vi agglutinin response in six out of fifteen cases given heat-killed, phenol-preserved, T.A.B.C. or T.A.B. vaccines intravenously is recorded.

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