A COMPARATIVE STUDY OF PHENOLIZED AND ALCOHOLIZED T.A.B. VACCINES

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As the Stack Medical Research Laboratories at Khartoum are responsible for the issue of T.A.B. vaccine to both military and civil services in the Sudan, its manufacture is normally on a considerable scale which has been greatly increased during the war years. The technique of preparation has closely followed the methods used, until recently, for the standard phenolized T.A.B. vaccine for the British Army and so need not be detailed.

Felix (1941) described a new type of alcoholized vaccine and since then it has been emphasized that its protective value is greater than that of the standard phenolized vaccine, also that the general reactions are less severe. As the newer vaccine has now been adopted as the standard for the British Army it was of considerable importance to determine whether the Sudan should follow suit. In consequence the following trials of the comparative value of the two vaccines in man and animals have been carried out.

PREPARATION OF VACCINE

The strains used in the preparation of T.A.B. vaccine in the Sudan are *Bact. typhosum* (Ty 2), *Bact. paratyphosum* A 'O' (No. 38), *Bact. paratyphosum* B 'O' (No. 62); the latter two strains were locally isolated.

A pooled heavy suspension in saline from a series of culture bottles of each organism was first prepared. Each suspension was divided into two lots, one being alcoholized according to the technique of Felix (1941) and the other heat killed and phenolized (0.5 %) in the usual way. The three suspensions (T.A.B.) of each lot were subsequently diluted, standardized and mixed in the usual proportions. In this manner it was possible to ensure that both suspensions (alcoholized and phenolized) were immunogenically identical before the respective chemical treatment.

TESTS FOR POTENCY OF VACCINES

After consideration the following tests were selected:

- (a) Response of agglutinins in volunteers following the standard two doses of vaccines.
- (b) Response of agglutinins in rabbits.
- (c) Degree of active immunity in mice tested with virulent cultures.

(a) Response of agglutinins in volunteers

200 recruits of the Sudan Defence Force from the Nuba Mountains area, none of whom had any history of previous T.A.B. inoculation, were divided into two batches; one received the standard phenolized vaccine and the other alcoholized vaccine.

Samples of blood were withdrawn from all these recruits before inoculation and again a fortnight after the second dose of vaccine. All doses were administered at a weekly interval, that of the phenolized vaccine being 0.5 and 1.0 c.c. respectively, while the alcoholized vaccine was administered according to the recommended dosage of Felix, Rainsford & Stokes (1941); it was not practicable, however, to have a 3-week interval between the two doses.

The men were instructed to report if any symptoms interfered with their military duties; but only one, who had received phenolized vaccine, did so. However, all those inoculated with phenolized vaccine complained of the usual fever and malaise while those inoculated with alcoholized vaccine had no complaints apart from a transitory stinging sensation immediately following inoculation.

The results of the agglutination tests are summarized in Tables 1, 2 and 3.

Comments. The chief difference observed is the marked response of the 'Vi' agglutinins, and to a lesser degree the 'O' to the alcoholized as compared with the phenolized vaccine. The 'H' agglutinin response to the phenolized vaccine is, as would be expected, more marked. A feature of some interest is the very poor response of the Para A 'O' agglutinins to either vaccine.

(b) Response of agglutinins in rabbits

Twenty-four healthy animals were selected and their sera tested. All showed negative agglutination results against *Bact. typhosum* 'H', 'O', 'Vi'; *Bact. paratyphosum* A 'O'; and *Bact. paratyphosum* B 'O'.

The rabbits were divided into four groups of six and each given two standard doses of vaccine consisting of (1) T 100 million, A 50 million, B 50 million; and (2) T 200 million, A 100 million, B 100 million administered at a 7-day interval either by the subcutaneous or intravenous routes. The animals

Table 1. *Agglutination titres before inoculation*

Serum dilution	T			Para A	Para B
	'H'	'O'	'Vi'	'O'	'O'
1/12.5	—	—	7	—	—
1/25	8	7	2	—	—
1/50	8	6	—	—	—
1/125	8	—	—	—	—

— = no agglutination.

Table 2. *Phenolized vaccine. Agglutination titres after inoculation*

Serum dilution	T			Para A	Para B
	'H'	'O'	'Vi'	'O'	'O'
Negative	—	—	29	36	—
1/12.5	—	—	27	—	—
1/25	—	—	22	55	—
1/50	—	—	14	5	—
1/125	—	36	3	—	11
1/250	—	53	1	—	82
1/500	2	7	—	—	3
1/1250	2	—	—	—	—
1/2500	92	—	—	—	—

Note. Four of this batch did not receive a second injection owing to either illness or desertion.

Table 3. *Alcoholized vaccine. Agglutination titres after inoculation*

Serum dilution	T			Para A	Para B
	'H'	'O'	'Vi'	'O'	'O'
Negative	—	—	—	30	—
1/12.5	—	—	—	—	—
1/25	—	—	2	54	—
1/50	—	—	14	8	—
1/125	9	7	45	—	—
1/250	14	55	26	—	84
1/500	25	23	5	—	8
1/1250	22	7	—	—	—
1/2500	22	—	—	—	—

Note. Eight of this batch did not receive a second injection owing to either illness or desertion.

were bled 7, 14 and 21 days after the second dose of vaccine and their agglutinin response tested.

The results are summarized in Table 4.

Comments. The results show a slightly less response of *Bact. typhosum* 'H' agglutinins in the alcoholized groups than in the corresponding phenolized groups but no difference in the 'O'; there was a very poor response of 'Vi' agglutinins in the phenolized vaccine, five of the twelve rabbits showing no response, while all the animals receiving the alcoholized vaccine showed appreciable titres.

(c) Active immunity tests with virulent cultures

As the number of mice was limited it was decided to carry out only the active immunity test. It has been, of course, recognized that this test is regarded

as being the least sensitive (Felix 1941); on the other hand, the active immunity test in mice is used in the Army Medical School, Washington, in the assessment of potency of T.A.B. vaccine prepared for the U.S. Army (Siler and Others, 1941).

Sixty mice were divided into two groups and inoculated with phenolized and alcoholized vaccines respectively. To eliminate the possibility of a local immunity of the peritoneum following intraperitoneal administration the doses were given subcutaneously as follows, T 50 million, A 25 million, B 25 million followed after a week by double these amounts. The challenging dose was given subcutaneously 10 days after the second dose. An equal number (sixty) of controls also received the subcutaneous dose. The results of the tests with *Bact. paratyphosum* A 'O' are not included as the test strain was of such low virulence that there were no deaths in the controls.

The results are summarized in Table 5.

Comments. The results are clear-cut; no difference could be detected between the protective values of the respective vaccines against *Bact. typhosum*. In the case of *Bact. paratyphosum* B, in the 10 M.L.D. group, the difference is not statistically significant. Thus, each afforded complete protection against 2 M.L.D., almost complete against 5 M.L.D. and partial against 10 M.L.D. of each organism.

In discussing these results with Col. H. T. Findlay, D.D.P., M.E.F. in 1945, he pointed out that in the mouse protection experiments the vaccines were administered subcutaneously whereas the usual practice was to use the intraperitoneal route and that this difference in technique might possibly affect the results.

In view of this criticism it was decided to carry out further experiments.

120 mice were divided into four groups, all receiving the same doses of vaccine as in the previous mouse experiment. Group I was given phenolized vaccine subcutaneously, group II phenolized vaccine intraperitoneally, group III alcoholized vaccine subcutaneously and group IV alcoholized vaccine intraperitoneally. Ten days afterwards a challenging dose of virulent *Bact. typhosum* (strain Ty2) was given. As the number of mice was limited it was decided not to repeat the experiment with *Bact. paratyphosum* B.

Control mice (60) were given a challenging dose by the respective routes.

The results are summarized in Table 6.

Comments. These results fully confirm the previous findings that the protective values of the vaccines against *Bact. typhosum* are identical. There appears to be a slightly greater degree of protection in the groups inoculated by the intraperitoneal route but the total numbers are too small for statistical evaluation.

Table 4

Rabbit	7 days after						14 days after						21 days after					
	T			A 'O'	B 'O'		T			A 'O'	B 'O'		T			A 'O'	B 'O'	
	'H'	'O'	'Vi'				'H'	'O'	'Vi'				'H'	'O'	'Vi'			
	Phenolized vaccine. Subcutaneous inoculation																	
1	1/2500	1/2500	0	1/250	1/2500		1/2500	1/2500	0	1/250	1/2500		1/2500	1/2500	0	1/250	1/2500	
2	1/1250	1/500	1/12.5	1/125	1/2500		1/1250	1/500	1/12.5	1/125	1/2500		1/1250	1/500	1/12.5	1/125	1/2500	
3	1/2500	1/2500	0	1/125	1/2500		1/2500	1/2500	0	1/125	1/2500		1/2500	1/2500	0	1/125	1/2500	
4	1/1250	1/250	0	1/250	1/2500		1/500	1/250	0	1/250	1/2500		1/500	1/250	0	1/250	1/2500	
5	1/1250	1/250	1/25	1/250	1/2500		1/1250	1/250	1/25	1/250	1/2500		1/1250	1/250	1/25	1/250	1/2500	
6	1/1250	1/2500	1/12.5	1/250	1/2500		1/1250	1/1250	1/12.5	1/250	1/2500		1/1250	1/1250	1/12.5	1/250	1/2500	
	Phenolized vaccine. Intravenous inoculation																	
1	1/1250	1/2500	1/12.5	1/250	1/1250		1/2500	1/2500	1/12.5	1/250	1/1250		1/2500	1/2500	1/12.5	1/250	1/1250	
2	1/2500	1/500	1/25	1/125	1/250		1/2500	1/1250	1/50	1/50	1/250		1/2500	1/1250	1/50	1/50	1/250	
3	1/2500	1/1250	1/12.5	1/125	1/250		1/2500	1/1250	1/25	1/125	1/500		1/2500	1/1250	1/25	1/125	1/500	
4	1/2500	1/2500	0	1/125	1/2500		1/2500	1/2500	0	1/125	1/2500		1/2500	1/2500	0	1/125	1/2500	
5	1/2500	1/2500	1/12.5	1/250	1/2500		1/2500	1/2500	1/12.5	1/250	1/2500		1/2500	1/2500	1/12.5	1/250	1/2500	
6	1/2500	1/500	0	1/250	1/2500		1/2500	1/500	0	1/250	1/2500		1/2500	1/500	0	1/250	1/2500	
	Alcoholized vaccine. Subcutaneous inoculation																	
1	1/250	1/500	1/25	1/125	1/1250		1/250	1/500	1/50	1/125	1/1250		1/250	1/500	1/50	1/125	1/1250	
2	1/1250	1/2500	1/12.5	1/250	1/2500		1/1250	1/2500	1/25	1/250	1/2500		1/1250	1/2500	1/50	1/250	1/2500	
3	1/500	1/2500	1/50	1/250	1/1250		1/500	1/2500	1/125	1/250	1/1250		1/500	1/2500	1/125	1/250	1/1250	
4	1/125	1/1250	1/12.5	1/250	1/250		1/125	1/1250	1/25	1/250	1/500		1/125	1/1250	1/25	1/250	1/500	
5	1/250	1/1250	1/25	1/250	1/250		1/250	1/1250	1/50	1/250	1/1250		1/250	1/1250	1/50	1/250	1/500	
6	1/250	1/500	1/25	1/125	1/250		1/250	1/500	1/50	1/125	1/250		1/250	1/500	1/50	1/125	1/250	
	Alcoholized vaccine. Intravenous inoculation																	
1	1/500	1/2500	1/25	1/250	1/2500		1/500	1/2500	1/25	1/250	1/2500		1/500	1/2500	1/25	1/250	1/2500	
2	1/500	1/2500	1/50	1/250	1/2500		1/500	1/2500	1/50	1/250	1/2500		1/500	1/2500	1/50	1/250	1/2500	
3	1/1250	1/2500	1/12.5	1/125	1/2500		1/1250	1/2500	1/25	1/250	1/2500		1/1250	1/2500	1/25	1/250	1/2500	
4	1/250	1/2500	1/12.5	1/250	1/2500		1/250	1/2500	1/25	1/250	1/2500		1/250	1/2500	1/25	1/250	1/2500	
5	1/250	1/2500	1/12.5	1/250	1/2500		1/250	1/2500	1/25	1/250	1/2500		1/250	1/2500	1/25	1/250	1/2500	
6	1/250	1/2500	1/12.5	1/250	1/2500		1/250	1/2500	1/25	1/250	1/2500		1/250	1/2500	1/25	1/250	1/2500	

Emulsion control against stock agglutinating sera:

Bact. typhosum 'H' 1/25000*Bact. paratyphosum* A 'O' 1/2500*Bact. typhosum* 'O' 1/5000*Bact. paratyphosum* B 'O' 1/1250*Bact. typhosum* 'Vi' 1/2000

In the meantime, Col. Findlay carried out a small preliminary comparative study, in mice, of the protective values of alcoholized and phenolized vaccines as used by the British Army and he has kindly given the writer permission to state that he obtained very comparable results.

Table 5

	2 M.L.D.	5 M.L.D.	10 M.L.D.
Alcoholized vaccine			
<i>Bact. typhosum</i> (Ty 2)	0/5	1/5	3/5
<i>Bact. paratyphosum</i> B (No. 62)	0/5	1/5	2/5
Phenolized heat-killed vaccine			
<i>Bact. typhosum</i> (Ty 2)	0/5	1/5	3/5
<i>Bact. paratyphosum</i> B (No. 62)	0/5	1/5	3/5
Controls			
<i>Bact. typhosum</i> (Ty 2)	5/5	5/5	5/5
<i>Bact. paratyphosum</i> B (No. 62)	5/5	5/5	5/5

Numerator = deaths. Denominator = total.

Bact. typhosum (Ty 2) 1 M.L.D. = 40 millions.

Bact. paratyphosum B (No. 62) 1 M.L.D. = 20 millions.

Table 6

Subcutaneously			Intraperitoneally		
2 M.L.D.	5 M.L.D.	10 M.L.D.	2 M.L.D.	5 M.L.D.	10 M.L.D.
Phenolized vaccine					
0/10	1/10	6/10	0/10	0/10	4/10
Alcoholized vaccine					
0/10	2/10	5/10	0/10	0/10	4/10
Controls					
10/10	10/10	10/10	10/10	10/10	10/10

Numerator = deaths. Denominator = total.

DISCUSSION

The contention of Felix and others that with alcoholized vaccine the general reactions are less severe than with the standard phenolized vaccine is borne out in the present study. From the results of the agglutination tests of the human and rabbit sera it is also agreed that the 'Vi' response is greater with alcoholized vaccine than with the phenolized vaccine.

Judged, however, from the two mouse protection tests carried out there is no evidence that the immunogenical potency of the alcoholized vaccine is superior to that of the standard phenolized heat-killed vaccine.

Special emphasis has been laid on the fact that

the vaccines used for these experiments were all prepared from the same pooled suspension of the respective organisms. Felix *et al.* (1941) and Climie (1942) in their comparative studies used vaccines of entirely different sources, the assumption apparently being, that the various suspensions of the organisms used for the preparation of the vaccines were immunologically identical (before treatment) but this is not necessarily the case.

It has been observed that alcoholized vaccine on standing for sometime in the refrigerator tends to form a firm deposit of organisms on the bottom of the bottle, whereas phenolized vaccine remains in a fairly even suspension. While it is standard practice to have printed instructions on each bottle to 'shake well' it is by no means easy to resuspend the clumped bacteria and hence to be certain of administering the correct number of organisms, especially as the initial dose is of small volume (0.25 c.c.). This fact has also been noted by Col. Findlay who suggests, that to assist the resuspension of the bacteria, glass beads be introduced into the bottle.

Further it has been found in a dusty country like the Sudan, the risks of contamination during the preparation of the alcoholized vaccine in bulk, with the larger number of necessary manipulations, are considerably greater than in the case of the standard phenolized vaccine.

After due consideration of the above points it was finally decided that the change over to the alcoholized vaccine was not justified in the Sudan.

SUMMARY

1. A comparative study of alcoholized and phenolized T.A.B. vaccines has been made.
2. No difference could be detected in the immunological potency of the two vaccines.
3. The contentions of Felix and others that the reactions with alcoholized vaccine are less severe and 'Vi' response greater than with phenolized vaccine are confirmed.
4. The change over of large scale production in the Sudan from phenolized to alcoholized vaccine is not considered justifiable.

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