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Intradermal TAB immunization against enteric infections

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INTRODUCTION

The value of TAB vaccine in the prevention of enteric fevers is established. Ordinarily the vaccine is given subcutaneously, though the intradermal route has been recommended by Tuft (1931), Perry (1937), Longfellow & Luippold (1940). However, Luippold (1944), following further work, suggested that subcutaneous inoculations gave satisfactory enough results; he, however, added that in elderly persons or in others where allergy was suspected, the intracutaneous method was to be preferred. Observations recently made in India include those by Karani, Jadeja & Ganguli (1957), Kalra, Ganguli & Bardhan (1960) and Karani, O'Leary & Luxa (1960). The problem has also been studied among British troops by Barr, Sayers & Stamm (1959). The present study is an attempt to evaluate the antibody response and the clinical reactions to TAB vaccine administered subcutaneously or intradermally, as a primary immunization procedure amongst Indian troops.

MATERIALS AND METHODS

The vaccine

(i) The intradermal vaccine was prepared by Central Research Institute, Kasauli. It was designated 'Special TAB Vaccine', containing 5000 million organims per ml. (typhoid-2500 millions; paratyphoid A and partyphoid B 1250 millions each).

(ii) The vaccine given subcutaneously was the ordinary TAB vaccine prepared by C. R. I. Kasauli and used by the Armed Forces in India. It contains 2000 million organisms per ml. (typhoid 1000 millions, paratyphoid A and paratyphoid B 500 millions each).

Both the vaccines were heat killed and preserved with phenol.

The strains used in both vaccines were:

(i) Armed Forces Medical College strain *Salmonella paratyphi* A, no. 1564/X/156/P, isolated at the Department of Pathology of the College from a case (this strain is fairly common in this locality).

(ii) *S. paratyphi* B, Findley.

(iii) *S. typhi* Vi, Pillay.

(iv) *S. typhi* (Rawlings).

Strains (ii), (iii) and (iv) are well known, while strain (i) occurs frequently in India.

There was no difference apart from the concentration between the special and the ordinary vaccines.

The following doses were adopted, the interval between the doses being 14 days:

Intradermal group (i.d.): 1st dose—0·1 ml. of special TAB vaccine (500 million organisms). 2nd dose—0·2 ml. of special TAB vaccine (1000 million organisms).

Subcutaneous group (s.c.): 1st dose—0·5 ml. of the ordinary vaccine (1000 million organisms). 2nd dose—1·0 ml. of the ordinary vaccine (2000 million organisms).

It will be seen that the number of organisms introduced subcutaneously is twice the number of organisms given intradermally. Volumes, of course, are less in the intradermal method.

Trial subjects

The trial was carried out on fresh recruits of an Army Engineer group. These young healthy recruits, of age-group 17–19 years, were drawn from various parts of the country, and all had good physical and nutritional standards. They had had no previous TAB inoculation. A group of volunteers numbering 257 was divided by a method of random sampling into two groups. The trial group of 128 persons was given the special TAB vaccine by the intradermal route and the control group of 129 persons the ordinary TAB vaccine by the subcutaneous route. The intradermal injections were all given on the forearm and the subcutaneous injections in the deltoid region.

Techniques

Before inoculation the recruits were bled to determine the basal antibody levels. Agglutinin levels against AO, BO, TO, Vi, AH, BH and TH were determined by Felix's method for 'O' agglutination (Cruickshank, 1960) and by Dreyer's method for 'H' agglutination (Cruickshank, 1960). The bacterial suspensions used in these tests were the standard products of the Department of Pathology, Armed Forces Medical College, Poona.

The investigation was divided into two parts, clinical and immunological.

Clinical studies

Local and general reactions were recorded under five headings at 24, 48 and 72 hr. after inoculation: (i) temperature over 99° F.; (ii) inability to work; (iii) severe local pain; (iv) severe headache; (v) redness and swelling of diameter over 10 cm.

Immunological studies

Serological investigations were carried out before inoculation to determine the basal titre, then at 6 weeks, between 21 and 22 weeks, and finally at 59–60 weeks after the first TAB inoculations.

RESULTS

Clinical studies

The data on clinical reactions are given in Table 1. As regards rise of temperature there was no significant difference between the two groups after the first inoculation, but after the second inoculation the intradermal group showed a greater

incidence. There was no significant difference between the two groups regarding inability to work. The incidence of severe local pain, severe headache, and redness and swelling was generally higher in the intradermal group.

Immunological studies

The results in Table 2 indicate that:

(*a*) The basal titre is generally of the same order in both groups, but in subsequent determinations the titre is more often higher in the intracutaneous group.

(*b*) A distinct antibody rise is noticed at 21–22 weeks and 59–60 weeks; however, in the subcutaneous group, the titre at 60 weeks marks a reduction from a

Table 1. *Clinical reactions with subcutaneous and intradermal* TAB *inoculation*

Reaction	Dose	Period of observation (hr.)	No. of subjects examined		No. of positive reactions		Percentage		Remark
			s.c.	i.d.	s.c.	i.d.	s.c.	i.d.	
Temp. 99° F. and over	1st	24	129	128	23	16	17·8	12·5	—
		48	129	128	3	5	2·3	3·9	—
		72	129	128	0	2	0·0	1·6	—
	2nd	24	125	123	10	17	8·0	13·8	—
		48	125	123	1	12	0·8	9·8	**
		72	125	123	0	1	0·0	0·8	—
Inability to work	1st	24	129	128	20	12	15·5	9·4	—
		48	129	128	1	2	0·8	1·6	—
		72	129	128	0	0	0·0	0·0	—
	2nd	24	125	123	19	22	15·2	17·9	—
		48	125	123	2	3	1·6	2·4	—
		72	125	123	0	0	0·0	0·0	—
Severe local pain	1st	24	129	128	85	101	65·9	78·9	*
		48	129	128	58	86	45·0	67·2	**
		72	129	128	16	34	12·4	26·6	**
	2nd	24	125	123	53	107	42·4	87·0	**
		48	125	123	44	50	35·2	40·6	—
		72	125	123	10	19	8·0	15·4	—
Severe headache	1st	24	129	128	45	46	34·9	35·9	—
		48	129	128	13	39	10·1	30·5	**
		72	129	128	1	11	0·8	8·6	**
	2nd	24	125	123	38	55	30·4	44·7	*
		48	125	123	15	23	12·0	18·7	—
		72	125	123	1	4	0·8	3·3	—
Redness and swelling of diameter over 10 cm.	1st	24	129	128	47	65	36·4	50·7	*
		48	129	128	64	105	49·6	82·0	**
		72	129	128	10	85	7·8	66·4	**
	2nd	24	125	123	22	77	17·6	62·6	**
		48	125	123	60	104	48·0	84·6	**
		72	125	123	25	56	20·0	45·5	**

s.c. = Subcutaneous. i.d. = Intradermal.

* Difference significant as tested by χ^2 ($P < 0{\cdot}05$).

** Difference significant as tested by χ^2 ($P < 0{\cdot}01$).

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Table 2. *Agglutinin responses to* TAB *vaccine*

Titre	Basal s.c.	Basal i.d.	6 weeks s.c.	6 weeks i.d.	21–22 weeks s.c.	21–22 weeks i.d.	60 weeks s.c.	60 weeks i.d.
			(*a*) TO agglutinin					
—	12	22	0	1	2	0	4	2
10	22	15	4	1	0	0	0	0
20	49	38	43	21	4	2	6	2
40	25	33	42	33	7	5	10	4
80	20	20	30	25	26	11	22	5
160	1	0	2	23	34	23	21	11
320	0	0	0	8	24	33	17	27
640	0	0	0	2	11	29	11	24
1280	0	0	0	1	2	12	2	18
2560	0	0	0	0	0	1	0	0
Total	129	128	121	115	110	116	93	93
Geometric mean	21·6	19·9	36·3	65·3	149·0	297·8	120·9	333·9
Significance	—		$P < 0{\cdot}01$		$P < 0{\cdot}01$		$P < 0{\cdot}01$	
No. with titre of 80 or more*	21	20	32	59	97	109	73	85
Percentage	16·3	15·6	26·4	51·3	88·2	94·0	78·5	91·4
			(*b*) AO agglutinin					
—	120	120	110	93	52	56	49	18
10	8	8	11	18	0	0	8	8
20	1	0	0	3	38	40	25	44
40	0	0	0	1	14	13	10	16
80	0	0	0	0	4	3	1	6
160	0	0	0	0	2	4	0	1
Total	129	128	121	115	110	116	93	93
Geometric mean	3·4	3·4	3·5	4·1	10·0	9·9	7·8	16·6
Significance	—		—		—		$P < 0{\cdot}01$	
No. with titre of 20 or more*	1	0	0	4	58	60	36	67
Percentage	0·8	0·0	0·0	3·5	52·7	51·7	38·7	72·0
			(*c*) BO agglutinin					
—	101	97	45	31	17	20	19	12
10	24	25	38	38	0	0	6	5
20	4	5	35	38	24	21	28	25
40	0	1	3	7	38	28	22	22
80	0	0	0	1	14	22	14	17
160	0	0	0	0	13	17	4	11
320	0	0	0	0	4	8	0	1
Total	129	128	121	115	110	116	93	93
Geometric mean	4·1	4·3	8·2	10·2	32·2	36·7	20·8	30·4
Significance	—		—		—		$P < 0{\cdot}01$	
No. with titre of 40 or more*	0	1	3	8	69	75	40	51
Percentage	0·0	0·8	2·5	7·0	62·7	64·7	43·0	54·8

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Table 2 (*cont.*)

Titre	Basal s.c.	Basal i.d.	6 weeks s.c.	6 weeks i.d.	21–22 weeks s.c.	21–22 weeks i.d.	60 weeks s.c.	60 weeks i.d.
				(*d*) Vi agglutinin				
—	128	127	121	98	88	97	87	82
10	1	1	0	12	1	0	2	1
20	0	0	0	5	21	15	4	10
40	0	0	0	0	0	3	0	0
80	0	0	0	0	0	1	0	0
Total	129	128	121	115	110	116	93	93
Geometric mean	3·2	3·2	3·2	3·9	4·5	4·4	3·5	3·9
Significance	—		—		—		—	
No. with titre of 10 or more*	1	1	0	17	22	19	6	11
Percentage	0·8	0·8	0·0	14·8	20·0	16·4	6·5	11·8
				(*e*) TH agglutinin				
—	114	99	0	1	2	4	1	2
25	5	16	4	3	9	6	9	0
50	3	7	20	12	21	9	13	3
125	4	5	43	30	34	28	22	7
250	3	1	37	48	21	19	19	21
500	0	0	15	20	15	28	10	23
1250	0	0	2	1	4	16	13	27
2500	0	0	0	0	2	6	6	10
5000	0	0	0	0	2	0	0	0
Total	129	128	121	115	110	116	93	93
Geometric mean	6·8	8·1	155·3	183·6	146·9	240·7	203·5	502·5
Significance	—		—		$P < 0{\cdot}01$		$P < 0{\cdot}01$	
No. with titre of 50 or more*	10	13	117	111	99	106	83	91
Percentage	7·8	10·2	96·7	96·5	90·0	91·4	89·2	97·8
				(*f*) AH agglutinin				
—	119	101	1	2	1	7	7	2
25	3	18	6	8	5	8	18	1
50	2	4	22	25	30	11	11	4
125	2	2	33	33	32	26	14	12
250	3	3	49	34	18	26	15	24
500	0	0	6	13	20	24	20	28
1250	0	0	3	0	3	9	8	17
2500	0	0	1	0	1	4	0	5
5000	0	0	0	0	0	1	0	0
Total	129	128	121	115	110	116	93	93
Geometric mean	6·2	7·8	146·4	124·3	138·6	179·8	118·4	358·0
Significance	$P < 0{\cdot}01$		—		$P < 0{\cdot}05$		$P < 0{\cdot}01$	
No. with titre of 50 or more*	7	9	114	105	104	101	68	90
Percentage	5·4	7·0	94·2	91·3	94·5	87·1	73·1	96·8

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Table 2 (*cont.*)

Titre	Basal s.c.	Basal i.d.	6 weeks s.c.	6 weeks i.d.	21–22 weeks s.c.	21–22 weeks i.d.	60 weeks s.c.	60 weeks i.d.
				(*g*) BH agglutinin				
—	123	112	6	3	13	7	13	2
25	1	8	19	9	18	10	16	0
50	3	2	48	35	38	23	17	6
125	2	3	28	26	18	31	14	11
250	0	3	14	26	16	24	20	40
500	0	0	6	14	5	12	10	23
1250	0	0	0	2	2	7	3	9
2500	0	0	0	0	0	2	0	2
Total	129	128	121	115	110	116	93	93
Geometric mean	5·6	6·8	66·8	110·5	58·8	120·4	74·2	278·2
Significance	—		$P < 0{\cdot}01$		$P < 0{\cdot}01$		$P < 0{\cdot}01$	
No. with titre of 50 or more*	5	8	96	103	79	99	64	91
Percentage	3·9	6·3	79·3	89·6	71·8	85·3	68·8	97·8

s.c. = Subcutaneous. i.d. = Intradermal.

* These or less are the titres ordinarily found in India among uninoculated population.

peak which had been reached earlier, in contrast with the intracutaneous group in which the titre at 60 weeks is generally higher than the earlier values.

It appears that the intradermal method of inoculation, with a lesser number of bacteria, has given better immunity, as judged by agglutination titres, than the routine subcutaneous method.

The results also indicate that the response to Vi antigen was low in all cases and that the response to AO antigen was generally lower than that to TO and BO antigens.

The high titres shown by an appreciable number of men at the basal level may mean that they had enteric fever or some other fever with common antigenic property sometime before the trial began.

DISCUSSION

In this study, clinical features such as raised temperature, pain and swelling and subjective symptoms like headache are more marked with the intradermal method. It was reported earlier by Karani *et al.* (1960) that the intradermal method produced less local and general reactions than the subcutaneous route; they gave 0·1 ml. (200 million organisms) to 115 Service personnel as an annual booster dose. The British trial by Barr *et al.* (1959), showed that the reaction pattern was milder with the intradermal route. Our observations, however, do not confirm those findings.

The results of agglutinin responses to the TAB vaccine over a year (Table 2) show that the antibody response by the intradermal route is generally higher and

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perhaps lasts a little longer than that given by the subcutaneous route. This agrees with the findings of Tuft, Yagle & Rogers (1932) who noticed that the intradermal route produced a better and more lasting response than all the other routes except the intravenous, but by the intravenous route the titres were maintained for shorter periods. They used one-tenth of the dose; the number of organisms was not mentioned. These workers also reported that the antibody response was not associated with constitutional reactions, a finding which is not corroborated by the present study. This may be due to the fact that the number of organisms employed in the present trial by the intradermal route is more than that given by Tuft *et al.* (1932).

The British trial (Barr *et al.* 1959) employing 500 million organisms of TAB vaccine by the intradermal route and 1250 million organisms by the subcutaneous route showed approximately the same serological responses to the TAB components using either route. The trial of Kalra *et al.* (1960) also showed poor antibody rise with the intradermal route; the number of organisms introduced by these workers were 200 and 400 million organisms by the intradermal and 1000 and 2000 million organisms by the subcutaneous route, with an interval of one week between the two doses. Neither of these trials was of sufficient duration; the antibody responses were determined a fortnight after the last injections.

In the present study, it is noticed that agglutinin response to AO antigen is poor in both the groups as compared to those to TO and BO antigens. The reason for this poor antibody response to *Salm. paratyphi* A in the vaccine is not known. Whether increasing the number of this organism in the TAB vaccine will stimulate better antibody response, remains to be determined. This is important from the preventive aspect of immunization in India, where paratyphoid A is considered to be the most common enteric infection after typhoid.

SUMMARY

To compare the effects of subcutaneous and intradermal methods of administration of TAB vaccine for primary immunization against enteric infections an investigation was carried out on 257 Army recruits.

The general and local reactions were more unfavourable by the intradermal method. The agglutinin responses were better with the intradermal route.

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