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Immunization of man with typhoid and cholera vaccine. Agglutinating antibodies after intracutaneous and subcutaneous injection

By H. A. L. CLASENER*
Institute of Tropical Hygiene, Leiden, The Netherlands
and B. J. W. BEUNDERS
Director Royal Netherlands Army and Airforce Health Department Medical Service, The Hague, The Netherlands

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INTRODUCTION

Tuft, the originator of intracutaneous typhoid immunization (Tuft, Yagle & Rogers, 1932), found that, in man, intracutaneous immunization with one-fifth of the normal dose resulted in as much antibody as subcutaneous immunization with the normal dose, and that the reaction to the vaccination was lower after intracutaneous immunization. These observations were confirmed for both re-vaccinations (Siler & Dunham, 1939; Longfellow & Luippold, 1940) and primary vaccinations (Valentine, Park, Falk & McGuire, 1935; Perry, 1937; Chiang & Ch'en, 1958). On the other hand, Morgan, Favorite & Horneff (1943) and Luippold (1944) found less antibody after intracutaneous immunization. The reduction of vaccination reactions obtained with intracutaneous immunization, even with a ten times concentrated vaccine (Shi, 1958), has been generally recognized, but Bardhan, Dutta & Krishnaswami (1963), using only twice the subcutaneous dose for intracutaneous vaccination, observed more severe reactions in the intracutaneous group. These more severe reactions were accompanied by higher antibody titres.

The intracutaneous method also proved satisfactory for vaccination with cholera vaccine (Singer, Weis & Hoa, 1948; Noble, 1964) and a combination of cholera and typhoid vaccine (Noble & Fielding, 1965). After preliminary investigations (Barr, Sayers & Stamm, 1959), the intracutaneous vaccination method was adopted by the British Army for routine immunization with a combined vaccine containing enteric and tetanus antigens (Noble, 1963).

In an attempt to improve the standard immunization schedule used for recruits of the Dutch Army, it was decided to investigate not only the possibilities for a simpler time schedule but also the question of intracutaneous vaccination.

* Present address: Institute for Rheumatism Research, University Hospital, Leiden, The Netherlands.

MATERIAL AND METHODS

Subjects

About 1000 healthy 19- to 20-year-old men were randomly divided into eight groups. All the subjects were military recruits of the same draft and were stationed in the same army camp.

Vaccines

The cholera, typhoid, paratyphoid A, and paratyphoid B (CTAB) vaccine was a heat-killed, phenol-preserved suspension of bacteria, containing per ml.: 8000×10^6 *Vibrio cholerae* bacteria, 1000×10^6 *Salmonella typhi*, 750×10^6 *Salmonella paratyphi A* and 750×10^6 *Salmonella paratyphi B* bacteria. The same batch of this vaccine was used throughout the experiment.

The tetanus-diphtheria (TD) vaccine contained per ml.: 10 Lf tetanus toxoid and 30 Lf diphtheria toxoid, adsorbed with aluminium phosphate. The vaccines were standard commercial preparations obtained from the National Institute of Public Health, Utrecht, The Netherlands.

Dosage

All subjects received two 0·5 ml. doses of the tetanus-diphtheria vaccine in the deltoid muscle, with a 4- or 5-week interval (Table 1).

The dose of the CTAB vaccine differed for the eight groups according to the time schedule used, but chiefly according to the location of the injections. The subcutaneous injections were given in 0·5 or 1·0 ml. doses in the lower deltoid region. The intracutaneous injections were given in 0·1 or 0·2 ml. doses in the extensor surface of the forearm (Table 1). Opposite arms were used for the TD and CTAB vaccines.

At the time of the experiment the routine schedule of immunization of recruits was that of group 1.

Sera

Three weeks after the last injection with CTAB vaccine, blood was taken by venepuncture. Sera were stored at $-20°$ C.

Agglutination tests

Twofold serum dilution series beginning with 1/10 were made in volumes of 0·5 ml. in 0·9 % NaCl solution, in round-bottomed tubes of 50×7 mm. To these serum dilutions, equal volumes of bacterial suspensions were added forcibly to achieve good mixing. The first serum dilution thus became 1/20. The salmonella antigens were the standard commercial preparations for the determination of O- and H-agglutinins of the National Institute of Public Health, Utrecht, The Netherlands. Since a suitable cholera antigen for the agglutination test was not commercially available, the cholera antigen was freshly prepared each day by suspending in 0·9 % NaCl solution the 18 hr. growth on nutrient agar slopes of *Vibrio cholerae* of the Ogawa and Inaba strains (see Vella & Fielding, 1963). The

Table 1. *Time schedules, type of injection (subcutaneous = s.c., or intracutaneous = i.c.), and doses of the CTAB vaccine*

Week 1		2	3	4	5	6	7	8
Date .....6. iv. 60		13. iv. 60	21. iv. 60		3. v. 60	10. v. 60	17. v. 60	27. v. 60
Group								
1	TD	—	—	—	TD	— CTAB s.c. 0·5 ml.	— CTAB s.c. 1·0 ml.	— CTAB s.c. 1·0 ml.
2	TD	—	—	—	TD	— CTAB i.c. 0·1 ml.	— CTAB i.c. 0·2 ml.	— CTAB i.c. 0·2 ml.
3	TD	— CTAB s.c. 0·5 ml.	— CTAB s.c. 1·0 ml.	—	— CTAB s.c. 1·0 ml.	TD	—	—
4	TD	— CTAB i.c. 0·1 ml.	— CTAB i.c. 0·2 ml.	—	— CTAB i.c. 0·2 ml.	TD	—	—
5	TD CTAB s.c. 0·5 ml.	—	— CTAB s.c. 1·0 ml.	—	TD CTAB s.c. 1·0 ml.	—	—	—
6	TD CTAB i.c. 0·1 ml.	—	— CTAB i.c. 0·2 ml.	—	TD CTAB i.c. 0·2 ml.	—	—	—
7	TD CTAB s.c. 1·0 ml.	—	—	—	TD CTAB s.c. 1·0 ml.	—	—	—
8	TD CTAB i.c. 0·2 ml.	—	—	—	TD CTAB i.c. 0·2 ml.	—	—	—

Table 2. *Means (m) and standard deviations (sd) of the transformed titres (log_2 of 1/10 of the reciprocal of the serum dilution)*

		antigen													
		TO		TH		AO		AH		BO		BH		V. CHOL.	
Group	No. of persons	*m*	*sd*	*m*	*sd*	*m*	*sd*	*m*	*sd*	*m*	*sd*	*m*	*sd*	*m*	*sd*
1	50	4·1	1·5	5·3	1·6	3·2	1·9	7·0	1·2	4·0	1·3	6·6	1·5	5·0	1·3
2	50	3·8	1·5	5·8	1·9	2·8	2·0	7·2	1·7	3·9	1·4	7·5	1·8	4·7	1·4
3	50	3·7	1·2	5·2	1·2	2·6	2·0	6·7	1·4	3·8	1·3	6·8	1·4	4·8	1·5
4	50	3·7	1·1	5·6	1·2	2·9	2·0	6·9	1·3	3·9	1·4	6·8	1·7	4·6	1·0
5	50	4·1	1·4	4·9	1·4	3·0	2·0	6·4	1·3	4·3	1·3	6·2	1·8	4·7	1·3
6	50	3·8	1·6	5·3	1·4	3·1	2·0	6·7	1·3	3·9	1·4	7·0	1·5	4·6	1·1
7	50	3·9	1·3	4·6	1·4	2·7	1·8	6·4	1·4	4·2	1·1	6·3	1·4	4·8	1·1
8	50	4·0	1·4	5·3	1·3	2·9	2·0	6·9	1·3	4·1	1·4	6·9	1·5	4·7	1·0
		Addition to Table 2 from the literature: agglutination titres from field trials, expressed in the same transformations as our data													
Acetone	217	0·7	0·9	6·7	1·4										
Heat-phenol	207	0·9	0·9	6·1	1·9										
Alcohol	200	2·3		5·6											
Heat-phenol	200	2·6		5·8											

Data from Ashcroft *et al.* (1964)

Data from Yugoslav typhoid commission (1962)

resulting suspension contained about 5×10^8 bacteria per ml. Strains were selected for smoothness by their resistance to complement (Singh & Ahuja, 1951).

After 18 hr. incubation at 37° C. in a water-bath, the agglutinations were read with the naked eye by the pattern on the bottom of the tube, without shaking the tubes. The serum dilution in the last tube showing agglutination as compared to the control tube, was taken as the titre of the serum. This corresponds to the 'last trace' reading of Gardner (1937), Felix (1938), and Vella (1963).

To equalize possible daily variation, care was taken to investigate equal numbers of sera from each of the eight groups each day. To avoid bias in reading the agglutination tests, sera were coded so that the investigator would be unaware of the group number of the sera when reading. All agglutination tests were read by the same investigator. The investigations were terminated when, for each group, the sera of 50 persons who had completed the immunization schedule had been examined.

RESULTS

For convenience of calculation and presentation, the reciprocals of the titres were divided by 10 and the logarithms to the base 2 of these numbers were taken. The values of the sera thus became the same as the numbers of the last tubes in which agglutination was observed. These values will be called transformed titres. For each antigen and each group, the cumulative frequency distribution of the transformed titres, plotted on normal probability paper, approached a straight line sufficiently closely for the frequency distribution of the transformed titres to be considered normal. Therefore, the results could be evaluated by comparing the means and standard deviations of the transformed titres (see Table 2). Differences in the means of groups 1 and 8, evaluated with the *t*-test, did not reach the 1 % level of significance.

An attempt to compare the incidence of reactions after intracutaneous and subcutaneous injection failed, because the number of reactions recorded by the doctors (nine out of 550 subcutaneous injections and five out of 550 intracutaneous injections) was too low to show significant differences. Calculations according to Lidwell (1963) and Kheifetz & Khazanov (1959) showed that at least 150 persons per group (i.e. 1650 injections of each kind) would have been necessary to obtain a significant 50 % reduction.

No local reactions were reported.

DISCUSSION

As shown by field trials, the mouse is not a good experimental model for the selection of typhoid vaccines for use in man (Standfast, 1964). Probably no better guide for the selection of an effective typhoid vaccine is provided by the titres of agglutinating antibodies in human sera after immunization (see addition from the literature in Table 2), although differences between agglutination titres produced by more effective and less effective vaccines are statistically significant (Benenson, 1964). Nevertheless, once a vaccine is chosen, we think that the agglutination test has some place in solving minor problems such as we were dealing with in this study.

The special role of the skin in immunologic processes was stressed long ago (Zinn & Katz, 1927; Tuft, 1931). For inducing allergic processes, the effect of injecting intracutaneously has even been called equal to that of using adjuvants (Waksman, 1956; see also Leskowitz & Waksman, 1960). The particular effectiveness of the cutis as an immunization route is possibly connected with the abundance of the lymphatic network of the corium as compared to that of the subcutis (Rusznyak, Foldi & Szabo, 1960). Intracutaneous injection has been described as an intralymphatic injection (Hudack & McMaster 1933), but the fate of the injected fluid under the influence of such variables as tissue pressure and inflammation is not clear (McMaster & Parsons 1950; Rusznyak, Foldi & Szabo 1960). In any case, the absorption of an antigen differs according to whether it concerns a primary or a secondary intracutaneous injection (Birkhaug & Boe, 1946; Korngold *et al.* 1953).

When no definite information on the essential difference between the subcutis and the cutis as a route of immunization could be obtained from the literature, another aspect of this problem suggested itself, namely that of the antigen-dose/antibody-response relationship. The literature survey done by Stevens (1956) (see also Stille, 1960) gives a general impression of this relationship, but we still did not feel certain that with one-fifth of the normal dose of CTAB vaccine injected subcutaneously we would not have obtained the same titres as with the full dose. Tuft (1931) injected the same dose intracutaneously, subcutaneously and intramuscularly, but the results led him to remark that 'it is rather difficult to give subcutaneous and intramuscular injections without involvement of the skin'. This same difficulty was mentioned by Waksman & Morrison (1951). The failure in our experiments to try the effect of the smaller intracutaneous doses given subcutaneously made it necessary to undertake further investigation of the antigen-dose/antibody-response relationship with typhoid vaccine in man (Clasener, 1967).

CONCLUSION

No reason was found, with respect to agglutinating antibody titres or vaccination reactions, for not simplifying the rather laborious immunization schedule of group 1 into the much simpler schedule of group 8 for the routine immunization of recruits.

SUMMARY

Young men were immunized against cholera, typhoid, paratyphoid A, and paratyphoid B. Agglutinating antibodies were measured 3 weeks after completion of the immunization schedule. Two injections separated by a 4-week interval were found to be as efficient as three injections with 1-week intervals.

Intracutaneous immunization with one-fifth of the standard subcutaneous dose was just as efficient as the standard subcutaneous immunization and did not cause greater general or local reaction.

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