Serologic Response in Man to Varying Amounts of Inactivated Epidemic Typhus Vaccine.* (32263)

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The immunization regimen until 1962 for personnel in the Armed Forces of the United States against epidemic typhus fever consisted of 2 doses of vaccine given 7 to 10 days apart (primary immunization regimen); thereafter, single booster injections were given at yearly intervals. In collaborative studies with Dr. Charles L. Wisseman, Jr., University of Maryland, we demonstrated in 1960 that 92 of 100 medical students developed detectable levels of complement fixing antibody after 2 doses of an experimental epidemic typhus vaccine; 87% of the students responded after only 1 dose of vaccine (unpublished observations). These findings suggested that the amount of antigen contained in the 2.0 ml of vaccine required in the primary immunization regimen for man might be more than was needed to accomplish satisfactory antibody responses.

In the work reported here which was carried out in 1960-1963 our objectives were to determine the minimum dose of epidemic typhus vaccine which would elicit demonstrable antibody in the majority of recipients after a single dose of vaccine and to determine the minimum primary dose necessary to prepare an individual to respond in a booster fashion when given additional vaccine at a later date. On the basis of the results of work reported in this paper the immunization regimen in 1963 was changed for military personnel and now consists of a single 0.5 ml dose of epidemic typhus vaccine which is administered on entrance into the military service and another 0.5 ml dose of vaccine which is given at the time of overseas travel(1).

Materials and methods. Vaccine. A commercially produced lot of epidemic typhus vaccine was used first for the primary course of immunization and 6 months later for the booster dose. This single vaccine lot was assayed for potency by the manufacturer in toxin neutralization (TN) tests employing 2½ units of toxin. The vaccine was shown to be of satisfactory potency in the standard assay procedure which requires that a 1:32 dilution of pooled serum from vaccinated guinea pigs neutralize only 2 units of epidemic typhus toxin(2). A sample of the same lot of vaccine was assayed for potency in tests performed in our laboratory and it was found that a 1:256 dilution of pooled serum obtained from guinea pigs vaccinated by the standard procedure(2) neutralized 2 units of toxin. When examined by standard complement fixation (CF) technique(3) employing 2 antigen units this serum pool also had a titer of 1:256. Additional information which indicated that this was a highly potent vaccine was obtained in the mouse protection test(4). The value obtained in this test was 4.1 or, based on calculations made according to the methods of Reed and Muench(5), 1.0 ml of 1:12,000 dilution of vaccine when injected into mice, would be expected to protect 50% of the test animals against an intravenous challenge of 2 toxin units.

Volunteers and inoculation and bleeding schedules. The Marine population and the Marine Corps Recruit Depot, Parris Island, S. C., have been described(6). Three-hundred and sixty-nine Marine recruits at Parris Island who were initially without epidemic typhus CF and TN antibodies were studied to determine their antibody response to sub-

* The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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TABLE I. Serologic Responses in Marines to Different Amounts of Typhus Vaccine.

Vaccinees					
Group	No. of persons	Vaccine dilution	Number 1 ml doses	Antigenic mass*	% of volunteers developing antibodies†
A	46	Undil.	2	100	100
B	60	Undil.	1	50	98
VII	23	1/2	1	25	100
C	65	1/4	1	12	91
D	56	1/16	1	3	38
E	60	1/64	1	0.8	37
F	59	1/256	1	0.2	15

* Expressed as percent of basic course.
† Either 1 or the other or both CF and TN antibodies.

cutaneous injection of varying doses of epidemic typhus vaccine. The schedules of inoculation employed in the volunteers is shown in Table I, first 4 columns. Pre-vaccination blood specimens were collected at the time of injection and post-vaccination specimens were obtained 7, 14, 21, 28 and 150 days later. The specimens collected at 150 days were obtained just prior to administration of the booster dose vaccine; other blood specimens were obtained at 7, 14 and 21 days after the booster dose.

Serology. CF tests for epidemic typhus antibody were performed according to the standard procedure(3) employing 2 units of antigen and 2 full units of complement with overnight incubation at 4°C before the addition of the hemolytic system. The TN tests were carried out by the method described earlier(4). Briefly, 2.1 ml of diluted serum were mixed with an equal volume of epidemic typhus toxin diluted to contain 4 units. The serum-toxin mixtures were incubated at room temperature for 1 hour, after which each of 6 mice was injected by the intravenous route with 0.5 ml of the mixture. The serum at the test dilution was considered to be free of TN antibody, if all 6 mice were dead at 24 hours after inoculation. The test specimen was considered positive for presence of such antibody if any of the mice were alive 24 hours after inoculation.

Results. Serologic responses to different amounts of typhus vaccine. Ninety-one to 100% of the individuals immunized according to any of the first 4 schedules listed in Table I developed either CF or TN typhus antibodies or both. In contrast, only about ⅓ of the volunteers who received 1 ml of a 1:16 or 1:64 dilution of vaccine developed measurable amounts of antibody. A single 1 ml dose of a 1:256 dilution of vaccine elicited demonstrable antibody in 9 of 59 men (15%). The results suggested that when a potent vaccine is employed, only a fraction of the typhus antigen contained in the old standard typhus immunization course (7) is needed to elicit antibody formation in man.

The height of the antibody response was directly proportional to the mass of typhus antigen administered. As shown in Table II, geometric mean CF antibody titers on the 28th day (expressed to $\log_2$) for individuals in Group A, B and C were 4.7, 3.3 and 2.3, respectively. Only about ⅓ of the men in Groups D and E and 1/6th in Group F developed antibody; the geometric mean titers were less than 1 in these instances.

Serologic response to booster injection. Table II also shows the CF antibody response of a sample of each group of volunteers following a booster dose of 0.5 ml of undiluted typhus vaccine. The 23 men in Group VII served as controls for the booster part of the study. All but one of the 23 men developed CF and TN antibodies; one individual developed only TN antibody. The mean CF antibody titer at post-booster day 14 in men in Group VII was 1:10 ($\log_2$ 3.3).

Unfortunately, the booster dose contained too large an antigenic mass. Ideally, the booster injection should have contained the smallest amount of antigen which would elicit low levels of antibody in ¼ to ⅓ of persons without prior experience with typhus antigen. Thus, it might have been more appropriate to have used a booster dose con-

TABLE II. Booster Effect of 0.5 ml of Epidemic Typhus Vaccine in Recruit Marines.

Group	A	B	C	D	E	F	VII
Dose	2 × 1 ml	1 × 1	1/4 × 1	1/16 × 1	1/64 × 1	1/256 × 1	1/2 × 1
After primary dose	(46 men)	(60)	(65)	(56)	(60)	(59)	
Day 0	0	0	0	0	0	0	0
" 28	4.7*	3.3	2.3	—	—	—	No basic
" 150	1.9	2.0	2.0	.5	.6	—	course
After booster	(10 men)	(6)	(20)	(22)	(25)	(18)	(23)
Day 7	3.0	2.0	3.0	3.1	2.1	1.4	2.1
" 14	3.6	2.5	3.5	4.4	2.7	2.0	3.3

* Geometric mean CF titer, log 2.

taining an antigen mass comparable to that given Group D or E in their primary course of immunization which resulted in geometric mean titers of less than 1 on day 150 after the primary dose.

Possible relation of vaccine assay value to antibody response in man. It is possible to establish a hypothetical relation between the vaccine assay value and the expected antibody response in a group of individuals (Table III). The vaccine employed in the current study had an assay value of 1:256. An assay value of 1:32 meets the potency requirements for epidemic typhus vaccine(2). The test vaccine diluted 1:4 and given in a dose of 1 ml evoked an antibody response in 91% of the recipients (Table III, column 6). Using these data it is possible to extrapolate the expected percentage of men who would develop antibody to typhus vaccine. For example, an epidemic typhus vaccine with an assay value as determined in our laboratory of 1:128 or half that of the vaccine used in this study would be expected to elicit an antibody response in more than 90% of the recipients when administered in a 0.5 ml dose on a single occasion. A vaccine with an assay value of 1:64 would have to be used in a proportionate larger amount to evoke an equivalent response. Similarly, a vaccine with an assay value of 1:32 would have to be used according to the old standard regimen, *i.e.*, 2 doses of 1 ml each, in order to induce antibody in 90% of vaccinees.

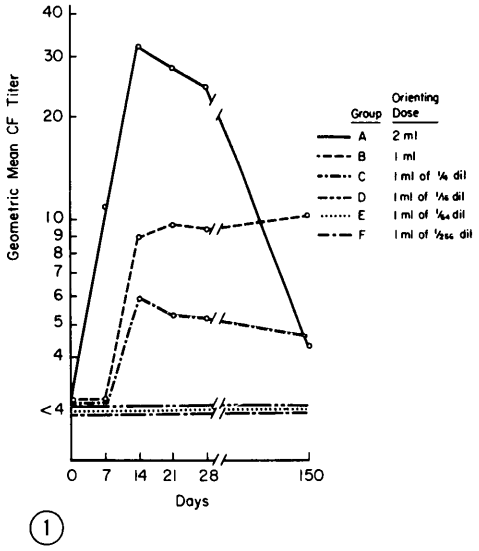
TABLE III. Possible Relation of Vaccine Assay Value (DBS) to Antibody Response in Man.

Dose*	% with antibody	Expected % of men developing AB after 1 dose of typhus vaccine with DBS assay value of			
		1:32	1:64	1:128	1:256
1 X 2	100	91	100	100	100
1 X 1	98	38	91	100	100
1/2 X 1	100	37	38	91	100
1/4 X 1	91	16	37	38	91
1/16 X 1	38	<16	16	37	38
1/64 X 1	37	<16	<16	16	37
1/256 X 1	16	<16	<16	<16	16

* Inoculum in each instance was 1.0 ml.
DBS assay value of vaccine employed: 1:256.

Serological response of normal and "immunologic prepared" persons without typhus antibodies to undiluted vaccine. The antigenic mass contained in the booster dose in this study was too large to determine the size of the primary dose of vaccine necessary to prepare an individual so that he would later respond in a booster fashion (Table II). Serological tests performed on paired sera taken before and after the booster dose of vaccine from groups of 6 to 23 members in each group who were without demonstrable antibodies 28 days after the primary dose of vaccine, and from groups of 22 and 23 controls who received typhus vaccine for the first time, showed that the CF and TN antibody responses were somewhat higher in the persons who had previously received undiluted vaccine or vaccine diluted as much as 1:16 than in the unprepared group. These findings are presented graphically in Fig. 1 and 2 for CF antibody and in Fig. 3 and 4 for TN antibody. As little as 1 ml of a 1:16 or 1:64 dilution of typhus vaccine "prepared" the recruits to respond in a booster fashion even though these small antigenic masses elicited either no, or very little demonstrable antibodies.

Booster response in relation to prior antibody titer. In general, those men who had antibodies a month after the initial course had the same or a higher titer following the booster (Table IV). Forty-two of the 51 persons (82%) who had no detectable CF



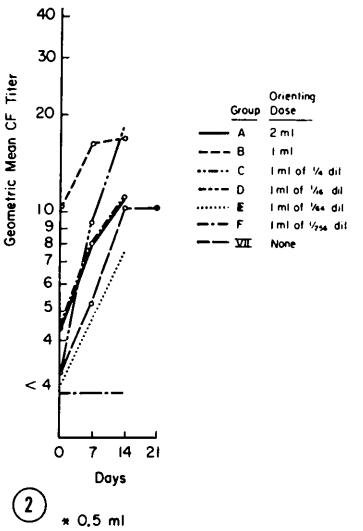
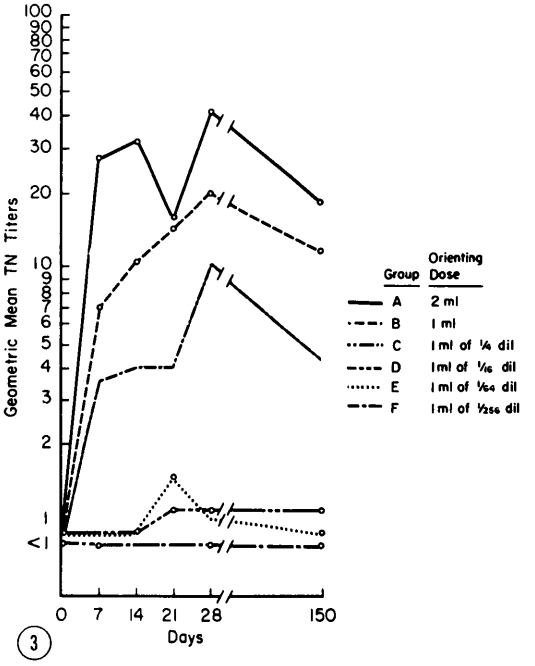


FIG. 1. Complement-fixing antibody response in Marine recruits to orienting dose of epidemic typhus vaccine.

FIG. 2. Complement-fixing antibody response in Marine recruits to booster dose of 0.5 ml of epidemic typhus vaccine.



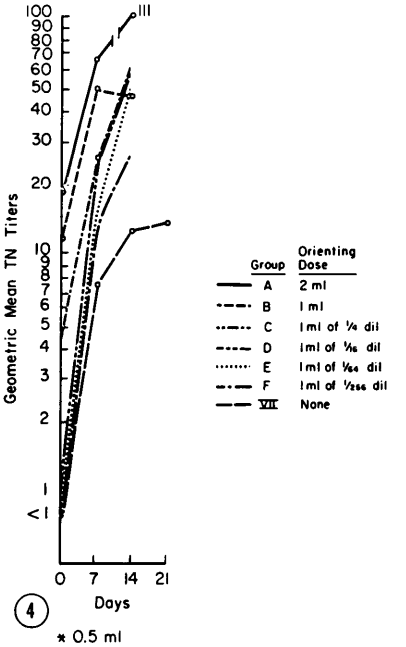


FIG. 3. Toxin neutralizing antibody response in Marine recruits to orienting dose of epidemic typhus vaccine.

FIG. 4. Toxin neutralizing antibody response in Marine recruits to booster dose of 0.5 ml of epidemic typhus vaccine.

TABLE IV. C.F. Booster Response in Marine Recruits in Relation to Prior C.F. Titer.

Post-booster Titer	Pre-booster Titer					
	0	4	8	16	32	Total
0	9*	–				9*
4	16	3	–			19
8	10	8	2			20
16	9	4	6	2		21
32	5	1	1		1	8
64+	2	4	1			7
Total:	51	20	10	2	1	84

▨ = Recruits whose post-booster antibody level was higher than pre-booster level.

* These 9 distributed as follows: Group A, 0; B, 0; C, 1; D, 2; E, 3; F, 3.

antibody following the basic course became positive after the booster. The sero-conversion rate is high because 0.5 ml of undiluted vaccine which constituted the booster dose was capable of eliciting CF antibody in almost all individuals who had not previously received typhus vaccine.

Discussion. There is at present no particular urgency for immunologic control of epidemic typhus fever in most areas of the world. Nevertheless, the disease remains a potential danger because of silent carriers of *R. prowazeki* who under certain catastrophic conditions might serve as foci from which epidemic typhus might spread(4). In case of such an event, stores of vaccine and knowledge of their most efficient use would certainly be desirable.

The findings presented here indicate that when a highly potent vaccine is employed in the immunization procedure, only a fraction of the typhus antigen contained in the standard typhus immunization course(7) is needed to elicit antibody in man.

As might have been anticipated, the height of the antibody response in the volunteers varied with the mass of antigen administered. It was possible to establish a positive relation between the vaccine assay value as determined in our laboratories and the antibody response one might expect in man. This finding was of first importance in considerations concerned with the modification of the vaccination schedule employed in epidemic typhus immunization. In 1963, on the basis of the work reported here, the primary course of immunization for military personnel in the United States was changed from 2 ml doses of vaccine given 7 to 10 days apart to a single injection of 0.5 ml of highly potent (assay value of 1:128 or higher) vaccine(1). It is emphasized, however, that with use of the new dosage schedule, careful attention must be paid to the exact potency of the vaccine employed since the dosage schedule is related directly to vaccine potency (Table III). Barker and his coworkers recently reemphasized the importance of the direct relationship between dosage schedule and potency value in the use of inactivated epidemic typhus vaccine(8,9).

In this study the booster dose of vaccine contained too large an antigenic mass to serve as a minimal stimulus. Retrospectively, it might have been better to have used a booster containing just enough antigen to elicit low levels of antibody in ¼ to ⅓ of recruits without prior experience with typhus antigen. Of the 51 recruits who had no detectable CF antibody following the basic course, all but 9 became positive after the booster. This high conversion rate is of limited significance, however, since 0.5 ml of undiluted vaccine which constituted the booster dose, was itself capable of eliciting CF antibody in almost all of those who had not previously received typhus vaccine. Nevertheless, from the standpoint of the projected development of a polyvalent rickettsial vaccine which has been under study (Morris *et al*, in preparation) the information gathered in this investigation will be useful. A measurement is now available of the mass of epidemic typhus antigen needed to immunize young adults in the basic course of immunization and this amount of epidemic typhus antigen presumably represents the minimal amount which should be incorporated in an experimental polyvalent rickettsial vaccine.

Summary. Three hundred and sixty-nine Marine Corps recruits, initially without either

complement fixing or toxin neutralizing epidemic typhus antibodies, were studied to determine their antibody response to subcutaneous injection of decreasing doses of commercially produced epidemic typhus vaccine administered in 1 or 2 doses of 1 ml each. When immunized with either 1 or 2 doses of undiluted vaccine or with 1 dose of vaccine diluted 1:2 or 1:4 91 to 100% of the 194 marines responded by developing either complement fixing or toxin neutralizing antibodies, or both. About ⅓ of 116 marines who received 1 dose of vaccine diluted 1:16 or 1:64 developed these antibodies. A single dose of vaccine diluted 1:256 elicited demonstrable antibodies in 15% or 9 of 59 men. These results suggested that the amount of antigen contained in 2.0 ml of vaccine required in the basic course of the immunization regimen was more than was needed to accomplish reasonable antibody responses in man. Accordingly, the primary immunization regimen for epidemic typhus for military personnel in the United States was changed in 1963 from 2 1-ml doses of vaccine given 7 to 10 days apart to a single 0.5 ml dose on entrance into military service and another 0.5 ml dose of vaccine at the time of overseas assignment.

1. U. S. Dept. of the Army, the Navy and the Air Force, Immunization Requirements and Procedures, BUMED Instruction 6230. ID. (9 July) 1963.

2. U. S. Dept. of H.E.W., Minimum Requirements: Typhus Vaccine, 6th revision, July 22, 1954.

3. Smadel, J. E., Diagnostic Procedures for Virus and Rickettsial Diseases, Am. Publ. Hlth. Assn., 2nd ed., 1956, 513.

4. Smadel, J. E., Jackson, E. B., Campbell, J. M., Arch. de l'Inst. Pasteur de Tunis, 1959, v36, 481.

5. Reed, L. J., Muench, H., Am. J. Hyg., 1938, v27, 493.

6. Chanock, R. M., Mufson, M. A., Bloom, H. H., James, W. D., Fox, H. H., Kingston, J. R., J. Am. Med. Assn., 1961, v175, 213.

7. U. S. Dept. of the Army, the Navy and the Air Force, Immunization Requirements and Procedures, BUMED Instructions (5 Sept) 1962.

8. Barker, L. F., Patt, J. K., Edmondson, W. P., Gundelfinger, B. F., Bloom, H. H., Jackson, E. B., Smadel, J. E., in press.

9. Barker, L. F., Patt, J. K., in press.

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