Typhoid Vaccine Studies

Revaccination and Duration of Immunity*

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FOLLOWING the work of Grinnell,[1,2] who used intraperitoneal injections into mice to study the relationship between the killing power of various strains of the typhoid bacillus to their effectiveness as immunogenic agents, and the subsequent extension of this line of research by Perry, Findlay, and Bensted,[3–6] who applied this same method to a similar study, an investigation along comparable lines was begun in the laboratories of the Army Medical School during the latter part of the year 1934.

A statistical study of the effectiveness of typhoid vaccines had been made earlier in the same year by Hawley and Simmons.[7] Since then, four progress reports of laboratory studies of typhoid vaccination have emanated from the laboratories of the Army Medical School.[8–11]

Early in this work, methods of technic were improved more nearly to validate interpretation of results obtained by such experimental work with mice, due to the introduction of gastric mucin as a vehicle for the test organism in mouse immunization experiments by Nungester *et al.*,[12] and by Miller,[13] and finally by its application to the mouse protection test for passive immunity to the typhoid bacillus by Rake.[14]

The third report emanating from the laboratories of the Army Medical School[10] in February, 1939, introduced a study of revaccination by intracutaneous injection of typhoid vaccine. Prior to this, Perry[15] reported "O" and "H" agglutinin responses in previously vaccinated individuals who were restimulated by intracutaneous injections of 0.1 cc. of vaccine; and recently, Tuft[16] reported a satisfactory response in 4 similar individuals similarly revaccinated, as evidence by both agglutination tests and serum protection tests—but adds that the "group was too small to warrant definite conclusions."

The report which follows is a continuation of the study announced by the laboratories of the Army Medical School in February, 1939,[10] and, as did this preliminary publication, includes a study of duration of immunity subsequent to vaccination along with further observations following revaccination by the intracutaneous injection of typhoid vaccine.

REVACCINATION

The usual method of revaccination is the same as for initial vaccination; that is, three subcutaneous doses—0.5 cc., 1.0 cc., and 1.0 cc., respectively—at approximately weekly intervals. (The

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TABLE 1

Protective antibody content of blood sera of 189 individuals before revaccination and 14 days after revaccination with 0.1 cc. of typhoid vaccine intracutaneously. All had received only one course of vaccine from 2 to 3 years previously. Protection expressed as the number of minimum lethal doses of the test organism against which the serum protected mice.

Minimum Lethal Doses	Before Revaccination		14 Days After Revaccination	
	Number	Per cent	Number	Per cent
100,000			15	7.9
10,000	7	3.7	95	50.3
1,000	25	13.2	70	37.0
100	65	34.4	9	4.8
10	58	30.7		
1	34	17.0		
Total	189		189	

dosage cited is for vaccine containing 1,000 million organisms per cc.) This series of 3 doses constitutes a " standard course " of vaccination, and will hereafter be referred to as such. The " standard course " is prescribed for the military service, and military personnel are required to be revaccinated at 3 year intervals. Exemptions are granted after 2 courses at a 3 year interval, and to individuals over 45 years of age. This method is time consuming, requiring a minimum of 2 weeks and 3 separate visits to the doctor. Because of this, studies were undertaken to determine the efficacy of a single dose of vaccine administered to previously vaccinated individuals.

Intracutaneous administration — A group of 189 young adult males who had received one " standard course " of typhoid vaccine from 2 to 3 years previously, were selected. Each of these individuals was given 0.1 cc. of vaccine (100 million organisms) into the skin of the arm or forearm. The antibody content of the blood serum in each case was titrated by means of the mouse protection test immediately before and 14 days subsequent to this revaccination. (This test, as employed in this work, has been described in a previous communication.[9]) The results of this study are shown in Table 1.

The blood sera of all members of this group, after revaccination, protected

TABLE 2

Protective antibody content of blood sera of 51 individuals before revaccination and 14 days after revaccination with 0.5 cc. of typhoid vaccine subcutaneously. This group had received from 1 to 7 courses of vaccine, the last of which had been given from 2 to 20 years previously. Protection expressed as the number of minimum lethal doses of the test organism against which the serum protected mice.

Minimum Lethal Doses	Before Revaccination		14 Days After Revaccination	
	Number	Per cent	Number	Per cent
100,000				
10,000	1	2.0	23	45.1
1,000	5	9.8	24	47.1
100	27	52.9	4	7.8
10	12	24.0		
1	6	12.0		
Total	51		51	

mice against at least 100 m.l.d., and in 95 per cent of the cases against at least 1,000 m.l.d. of the test organisms.

Subcutaneous administration—In another group of 51 individuals, each was given 0.5 cc. of vaccine (500 million organisms) subcutaneously. Members of this group had received from 1 to 7 " standard courses " of vaccine, the last of which had been given from 2 to 20 years previously. The blood sera were titrated immediately before and 2 weeks subsequent to revaccination. The results of this study are shown in Table 2.

It will be seen that the blood sera of all members of this group, after revaccination, protected mice against at least 100 m.l.d., and in 92 per cent of the cases against at least 1,000 m.l.d. of the test organism.

In order to compare the results obtained in these two groups with the usual method of revaccination, 50 individuals who had received from 1 to 5 " standard courses " of vaccine were selected. In each case the last course had been given at least 2 years previously. Each member of this group was given a " standard course " of vaccine, and the blood serum of each individual was titrated immediately before and 2 weeks after administration of the 3rd dose of vaccine. The results of this study are shown in Table 3.

It will be seen that in all cases, after revaccination, the blood serum protected mice against at least 100 m.l.d., and in 96 per cent of the group the blood serum protected mice against at least 1,000 m.l.d. of the test organism.

The immunological response, as measured by the mouse protection test, is very much the same in all three methods of revaccination. The blood serum of all individuals in the three groups protected mice against at least 100 m.l.d. 2 weeks after revaccination, while 95 per cent of the first group (0.1 cc. intracutaneously), 92 per cent of the second group (0.5 cc. subcutaneously), and 96 per cent of the third group (" standard course "), protected against at least 1,000 m.l.d. of the test organism.

Reactions following the administration of 0.1 cc. of typhoid vaccine intracutaneously are relatively much less severe than those following subcutaneous administration. A considerable number of individuals who had been vaccinated two or more times previously, in addition to those who had been vaccinated only once before, have been revaccinated by the intracutaneous method (0.1 cc.). No severe reactions have been observed.

TABLE 3

Protective antibody content of blood sera of 50 persons before revaccination and 14 days after revaccination with 3 doses of typhoid vaccine. Members of this group had received from 1 to 5 courses of vaccine, the last of which was given 2 or more years prior to revaccination. Protection expressed as the number of minimum lethal doses of the test organism against which the serum protected mice.

Minimum Lethal Doses	*Protection Before Revaccination*		*Protection 2 Weeks After Revaccination*	
	Number	*Per cent*	*Number*	*Per cent*
100,000			8	16.0
10,000			31	62.0
1,000	14	28.0	9	18.0
100	16	32.0	2	4.0
10	13	26.0		
1	7	14.0		
Total	50		50	

The most severe systemic reactions noted consisted of some fever, mild malaise, headache and myalgia. In a study of 100 consecutive cases, involvement of the axillary lymph nodes, consisting of slight swelling and tenderness without suppuration, was observed in 46 per cent of the cases, headache in 20 per cent, and an elevation of body temperature in 8 per cent. The highest temperature noted was 101°. In many instances, those presenting these relatively mild reactions following the intracutaneous administration of vaccine gave a history of severe reactions following subcutaneous administration of the vaccine. The local reaction has usually consisted of an area of redness at the site of vaccination and in a few instances some swelling of the tissues.

DURATION OF IMMUNITY

No test is available which will determine definitely the amount of immunity to typhoid fever possessed by a given individual. The mouse protection test measures the circulating antibody content of the blood serum and to this extent measures the degree of immunity to natural infection. With this as the only available method of detecting the quality of the individual's immunity, an attempt was made to determine the duration of immunity subsequent to vaccination. Results of such determinations indicating an increase or a decrease in the antibody content of the blood serum may, for present purposes, be interpreted as a corresponding increase or decrease in the individual's immunity.

It is not known what degree of immunity, as measured by the mouse protection test, an individual must possess in order to protect him from natural infection by typhoid organisms. In studying this phase of the problem, a group of young adult males, who gave a negative history of typhoid fever and of typhoid vaccination, and were therefore, presumably, non-immune, was selected. The blood sera of 107 such individuals were titrated by the mouse protection test in such a way that both the lower and upper end points were determined—that is, the dosage of test organisms against which the serum protected all mice, and against which the serum *failed* to protect any mice. Of this group, the sera of 89, or 83 per cent, protected mice against 1 m.l.d. of the test organism, but not against 10 m.l.d.; the sera of 12, or 11 per cent, protected mice against 10 but not against 100 m.l.d.; and the sera of 6, or 6 per cent, protected mice against 100 m.l.d. of the test organism. It is probable that in any group of 100 such individuals, some would have acquired a certain degree of immunity due to casual contact with the infection, or by a mild, undiagnosed attack of typhoid fever. However, in 94 per cent of this group, the blood serum would not protect mice against 100 m.l.d. of the test organism.

It seemed probable that young children who have not had typhoid fever, who have not been vaccinated against the disease, and who, because of their age, have had little opportunity for casual contact with the infection, would be non-immune. With this in mind, the blood sera of 21 children were titrated by the mouse protection test. The ages of these children varied from 10 months to 7 years. The sera of 20 of the children failed to protect mice against 1 m.l.d. dose of the test organism; the serum of one child (3½ years of age) protected mice against 1, but not against 10 m.l.d., of the test organism.

Individuals convalescent from an attack of typhoid fever, and typhoid carriers, should have sufficient immunity to protect them from natural infection. The blood sera of 9 typhoid convalescents were titrated by the mouse protection test. In each instance, the nature of the infection was positively diagnosed as typhoid fever. The results

TABLE 4

Protective antibody content of blood sera of 9 typhoid fever convalescents. Protection expressed as the number of minimum lethal doses of the test organism against which the serum protected mice.

Minimum Lethal Doses	*No. of Persons*	*Per cent*
100,000	2	22.2
10,000	4	44.4
1,000	2	22.2
100	1	11.1
Total	9	

TABLE 5

Protective antibody content of blood sera of 10 typhoid carriers. Protection expressed as the number of minimum lethal doses of the test organism against which the serum protected mice.

Minimum Lethal Doses	*No. of Carriers*	*Per cent*
100,000	1	10.0
10,000	2	20.0
1,000	6	60.0
100	1	10.0
Total	10	

of this study are shown in Table 4.

In all of the 9 convalescents, the blood serum protected mice against at least 100 m.l.d., and the sera of 8 of them protected against at least 1,000 m.l.d. of the test organism.

The blood sera of 10 known carriers of *Eberthella typhosa* were titrated by the mouse protection test to determine the degree of immunity to typhoid fever possessed by this class of individuals. The results of this study are shown in Table 5.

The sera of all 10 of these carriers protected mice against at least 100 m.l.d., and in 9 of them the serum protected mice against at least 1,000 m.l.d. of the test organism.

The number of cases used in the studies of presumably non-immune children, typhoid convalescents, and typhoid carriers, is too small to justify conclusions of a statistical nature, but they do serve to indicate differences in the degree of immunity possessed by presumably non-immune individuals and by those who are immune.

In view of the results obtained in the studies described above, as well as those shown in Tables 6 and 7, it is believed that, as a working basis, an individual may be regarded as immune to ordinary typhoid infection when his blood serum will protect mice against 100 m.l.d. of a virulent organism. However, in the absence of more definite data, it is believed that this degree of immunity should be considered as the minimum necessary to protect against typhoid fever. Using this assumption, a study was made to determine the duration of immunity following the vaccination of individuals who had never been previously vaccinated and who gave no history of typhoid fever. All members of this group were young adult males. The results of this study are shown in Table 6.

This study shows that 12 to 18 months subsequent to the initial vaccination the blood sera of 20 per cent of the group did not protect mice against 100 m.l.d. of the test organism; that 2 years after vaccination the blood sera of 40 per cent of the group did not protect mice against 100 m.l.d.; and that 2½ to 3 years after vaccination the blood sera of 57 per cent of the group failed to protect mice against 100 m.l.d.

The studies on duration of immunity as described above concerned individuals who had been vaccinated only once. A study has also been made of the duration of immunity wherein two or more courses of typhoid vaccine had been given previously and wherein from 2 to 10 years or more had elapsed since the last revaccination. The results of this study are shown in Table 7.

TABLE 6

Protective antibody content of blood sera at different periods of time after vaccination. Members of this group had received one course of vaccine. Protection expressed as the number of minimum lethal doses of the test organism against which the serum protected mice.

Minimum Lethal Doses	2 Weeks After Initial Vaccination		12–18 Months After Initial Vaccination		2 Years After Initial Vaccination		2.5 to 3 Years After Initial Vaccination	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
100,000	39	26.17						
10,000	65	43.62	23	14.3	6	6.38	1	1.05
1,000	37	24.83	53	33.0	12	12.77	13	13.68
100	8	5.37	52	32.3	38	40.43	27	28.42
10			26	16.1	22	23.40	36	37.89
1			7	4.3	16	17.02	18	18.95
Total	149		161		94		95	

The number of cases in this study is too small to justify definite conclusions. Apparently, where the individuals have received two or more courses of vaccine, the group immunity tends to remain at a higher level for a longer time than when only one course of vaccine has been administered, but there is still a tendency toward relative decrease of immunity with time, even after several revaccinations.

The severity of reactions following the administration of typhoid vaccine when given subcutaneously tends to increase with the number of times the individual is revaccinated by this method, until severe reactions are a frequent occurrence among those who have been revaccinated a number of times. These studies indicate that when typhoid vaccine is given intracutaneously in a dose of 0.1 cc., severe reactions are extremely rare, even when individuals vaccinated by this method have been previously vaccinated a number of times. The data shown in Tables 6 and 7 indicate that while the decrease in immunity after several vaccinations is not as marked as that following one course only, nevertheless there is a considerable decrease

TABLE 7

Protective antibody content of blood sera at different periods of time subsequent to the last vaccination. Members of this group had received 2 or more courses of vaccine, and from 2 to 10 years or more had elapsed since the last vaccination. Protection expressed as the number of minimum lethal doses against which the serum protected mice.

Minimum Lethal Doses	Time Subsequent to Last Vaccination									
	2 Years		3 Years		4–5 Years		6–10 Years		10 Years+	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
10,000	2	7.69	3	9.38	4	11.11	4	7.70	1	2.94
1,000	7	26.92	12	37.50	16	44.44	18	34.62	8	23.53
100	8	30.77	11	34.37	8	22.22	17	32.69	11	32.36
10	7	26.92	6	18.75	8	22.22	10	19.23	9	26.47
1	2	7.69					3	5.77	5	14.7
Total	26		32		36		52		34	

in group immunity even though the members of the group have been vaccinated several times.

CONCLUSIONS

The following conclusions are based on results obtained by mouse protection tests, and on the assumption that these tests yield a reliable index to an individual's quality or degree of immunity.

1. Revaccination with a single dose of 0.1 cc. of vaccine intracutaneously administered constitutes a reliable method of renewing immunity to typhoid fever, and should be the method of choice.

2. Revaccination with a single dose of 0.5 cc. of vaccine subcutaneously administered also produces a satisfactory renewal of immunity to typhoid fever. This procedure should be considered as an alternative method when conditions preclude intracutaneous administration.

3. In order to maintain a high degree of immunity to typhoid fever, as indicated by humoral antibodies, revaccination at 1 year periods appears to be an advisable procedure. Certainly, it appears that the interval between revaccinations should not exceed 2 years. It may be added that revaccinations at the intervals recommended should not be discontinued because of age nor because of any number of previous revaccinations.

We wish to emphasize the fact that, at this time, we do not advocate any change in the method of *initial* vaccination—that is, with a " standard course " of vaccine. A study of methods of initial vaccination is now in progress in these laboratories.

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