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Duration of Immunity Conferred by Typhoid Vaccine*

Results of Re-vaccination by Intracutaneous Injection of Typhoid Vaccine

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TWO progress reports have been published on the studies being made of typhoid vaccine in the laboratories of the Army Medical School.[1, 2]

During the past two years we have had the opportunity to undertake experimental studies with a view to estimating the duration of the protection afforded by the vaccine. Also, a study has been made of the value of re-vaccination with a single small dose of vaccine intracutaneously injected. This paper is a progress report of these two phases of our studies of typhoid vaccine.

DURATION OF IMMUNITY

The presence of protective antibodies in the blood stream after vaccination with typhoid vaccine is one indication of immunity, and the persistence of such antibodies over a period of time may be utilized to estimate the duration of the immunity conferred by the vaccine. In conducting these studies, we have used the mouse protection test to measure the antibody content of the blood serum. The mouse protection test measures the passive immunity conferred on mice by the inoculation of blood serum of an immunized individual. This test, as we have used it in our work, has been described in a previous communication.[2]

Preliminary to the experimental

* Presented by Lieut. Colonel G. C. Dunham, M.C., before the Laboratory Section of the American Public Health Association at the Sixty-seventh meeting in Kansas City, Mo., October 26, 1938.

studies regarding duration of immunity in vaccinated individuals, a study was made of a group of individuals who had never been vaccinated and who presented no history of having had typhoid fever. In all tests made before vaccination a constant dose of 10,000 organisms was used against 0.1 c.c. of individual's serum, on the assumption that if 0.1 c.c. of the blood serum of an individual afforded mice no protection against 10,000 live virulent typhoid organisms, the individual, arbitrarily, could be considered non-immune. This method was used as a screening test to eliminate those who were highly immune, and to conserve mice; also, because of the difficulty of determining the end point where the protective antibody content of the serum is very low.

All such tests were controlled by injection into groups of mice of 10, 100, and 1,000 organisms without serum, to determine the minimum lethal dose of the culture being used. In this series of observations, it was found that the minimum lethal dose for the control mice was either 100 or 1,000 organisms. Consequently, where, for example, an individual's serum did not protect mice against the dose of 10,000 organisms, the number of m.l.d. against which that serum, theoretically, would afford no protection might be as low as 10 or 100, depending on the virulence of the culture used. In Table I, the results of the tests are expressed as the number of m.l.d. of the test organism (Strain No. 63 *infra*) against which the serum protected or failed to protect mice.

A constant dose of 10,000 organisms was used in testing sera before vaccination. In some of the cases included in this study, 10,000 organisms of the culture used represented 100 m.l.d. for the control mice, while in other cases 10,000 organisms represented only 10 m.l.d. The former are placed in the first group and the latter in the second group in Table I. It is possible that the blood sera of many of the 176 persons in the first group which failed to protect mice against 100 m.l.d., would also have failed to protect against 10 m.l.d. had 1,000 instead of 10,000 test organisms been used.

Apparently, in many instances, the adult individual possesses some immunity to typhoid fever, even though he has not had typhoid fever in a diagnosable form and has never been immunized by vaccination. Our studies indicate that the protective antibody content of the blood sera of a large proportion of these individuals is relatively low, as compared with that of vaccinated persons. As shown in Table I, the blood sera of 79 per cent of the first group of 222 persons failed to protect mice against 100 m.l.d. of the test

TABLE I

Protective Antibody Content of Blood Sera of Individuals Not Vaccinated. Results Are Expressed as the Number of Minimum Lethal Doses of the Test Organism Against Which the Serum of the Individual Tested Protected, or Failed to Protect, Mice

	*First Group 100 * m.l.d.*		*Second Group 10 * m.l.d.*		*Total*	
	Number	*Percentage*	*Number*	*Percentage*	*Number*	*Percentage*
Protected	46	21	51	24	97	22
Failed to protect	176	79	163	76	339	78
Total	222	100	214	100	436	100

* The test dose consisted of 10.000 organisms and represented either 10 or 100 minimum lethal doses, depending upon the virulence of culture used.

organism, and 76 per cent of the second group of 214 failed to protect against 10 m.l.d.

In this study, the blood sera of 21 per cent of the first group and 24 per cent of the second group protected mice against more than 100 m.l.d. and 10 m.l.d., respectively. It is quite probable that the group contained some individuals who had had undiagnosed typhoid fever, in which case their blood sera would have protected mice against a much higher number of m.l.d. Obviously, the use of a single fixed dose of organisms, the technic first adopted for all preliminary titrations, even though it amply sufficed to pick out the non-immunes, would give no definite information concerning immunes. In our more recent work we are expanding the dosage in such manner as will enable us to secure the end point in all preliminary titrations.

A group of 200 individuals was studied with a view to determining the protective antibody content of the blood serum shortly after immunization. In each instance, the blood was taken on the 14th day after the third inoculation of typhoid vaccine. No one of this group had received more than the one course of vaccine, and none gave a history of having had typhoid fever. The results are shown in Table II, and are expressed in multiples of the m.l.d. of the test organism against which 0.1 c.c. of the individual's serum completely protected mice.

The results of the tests, as shown in Tables II, III, and IV, are expressed in terms of complete protection afforded mice against multiples of the m.l.d. for mice of a virulent strain of the typhoid bacillus suspended in mucin (5 per cent). A strain designated in our laboratories as Strain No. 63 was used for this purpose.

It will be noted in Table II that 2 weeks after vaccination the blood sera protected mice against from a minimum of 1,000 to a maximum of 1,000,000 m.l.d. of the test organism. In 80 per cent of the observations, the sera protected against at least 10,000 m.l.d.

TABLE II

Protective Antibody Content of Blood Sera of 200 Persons 2 Weeks After Vaccination. Results Are Expressed as Number of Minimum Lethal Doses of the Test Organism Against Which the Serum of the Immunized Individual Protected Mice

Minimum Lethal Doses	*Number of Persons*	*Percentage*
1,000,000	4	2.0
100,000	35	17.5
10,000	121	60.5
1,000	40	20.0
Total	200	100.0

Three groups of individuals have been studied with regard to the duration of immunity for varying periods of time. In one group, consisting of 56 persons, the blood sera were retitrated from 12 to 17 months after the initial vaccination. In another group of 30 individuals, the blood sera were retitrated after an interval of from 24 to 29 months subsequent to initial vaccination. In all instances, the individuals comprising these two groups were immunized with vaccine made from a virulent strain (Strain No. 58) and the blood sera had been titrated 2 weeks after initial vaccination. None of those included in this study gave a history of previous vaccination or of having had typhoid fever. The results obtained are shown in Table III.

It is apparent that the number of observations in Table III are too few to permit drawing definite conclusions on a statistical basis. However, the results obtained, while subject to confirmation by additional studies, do indicate the trend in the persistence of immunity as conferred by immunization with typhoid vaccine, in so far as

TABLE III

Protective Antibody Content of Blood Sera of 56 Persons 12 to 17 Months, and of 30 Persons 24 to 29 Months Subsequent to Vaccination with Typhoid Vaccine. Results Are Expressed in Minimum Lethal Doses of the Test Organism Against Which the Serum of the Individual Protected Mice *

Minimum Lethal Doses	12 to 17 Months After Vaccination		24 to 29 Months After Vaccination	
	No. of Persons	Percentage	No. of Persons	Percentage
10,000	12	21.4	1	3.3
1,000	27	48.2	13	43.4
100	17	30.4	9	30.0
10	..	....	6	20.0
1	..	....	1	3.3
Total	56	100.0	30	100.0

* Two weeks subsequent to vaccination these blood sera protected mice against from 1,000 to 1,000,000 m.l.d. of the test organism.

the mouse protection test can be used as a measure of the immunity possessed by the individual.

Twelve to 17 months after immunization the blood sera protected mice against from 100 to 10,000 m.l.d. of the test organism. Twenty-four to 29 months after vaccination the blood sera protected mice against from 1 to 10,000 m.l.d. (Table III).

During a period of 12 to 17 months subsequent to vaccination, the blood sera of 39 of the 56 individuals, or 69 per cent of the group, afforded mice complete protection against at least 1,000 m.l.d. of the test organism. Two years after vaccination the blood sera of 14 of the 30 individuals, or 46 per cent of the group, protected mice against 1,000 lethal doses of the test organism.

Despite the relatively great decrease in the protective antibody content of the blood in from 1 to 2 years subsequent to immunization, as measured by the mouse protection test, the average member of the groups observed, and the majority of the individuals involved, still have considerable immunity 1 year and 2 years after vaccination, as is indicated by the persistence of protective antibodies sufficient to protect mice against a relatively large number of minimum lethal doses of the test organism.

The third group consists of 311 individuals who had been vaccinated from one to several times, and among whom the time interval since the last vaccination ranged from 1 year to more than 10 years (Table IV). No data are available relative to the protective antibody content of the blood sera 2 weeks after vaccination, as for the groups discussed above (Tables II and III).

The blood sera of the members of the group who received their last course of vaccine 12 to 24 months previously protected mice against from 1 to 10,000 m.l.d. of the test organism. The blood sera of 62 per cent of this group protected mice against at least 1,000 m.l.d. These findings are comparable to those shown in Table III.

The blood sera of the members of the group vaccinated from 24 to 36 months before they were tested also protected mice against from 1 to 10,000 m.l.d., and 35 per cent of the sera protected mice against at least 1,000 m.l.d.

Thereafter, the protective antibody content of the blood sera of individuals

TABLE IV

Protective Antibody Content of Blood Sera of Previously Immunized Individuals at Different Periods of Time Subsequent to Last Vaccination. Results are Expressed in Minimum Lethal Doses of the Test Organism Against Which the Serum of the Individual Protected Mice

	Time Since Last Vaccination									
	12–24 Months		*25–36 Months*		*4–5 Years*		*6–10 Years*		*More than 10 Years*	
Minimum Lethal Doses	*No. of Persons*	*Per cent*	*No. of Persons*	*Per cent*	*No. of Persons*	*Per cent*	*No. of Persons*	*Per cent*	*No. of Persons*	*Per cent*
10,000	19	16.5	2	2.2	..	....	..	....	..	....
5,000	4	3.5	3	3.2	22	52.4	9	29.0	11	36.7
1,000	48	41.7	28	30.1	15	35.7	17	54.8	14	46.6
100	42	36.5	37	39.8	5	11.9	3	9.7	5	16.7
10	1	0.9	19	20.4	..	....	2	6.5	..	....
1	1	0.9	4	4.3	..	....	..	....	..	....
Total	115	100.0	93	100.0	42	100.0	31	100.0	30	100.0

last vaccinated from 4 to 5 years, 6 to 10 years, and more than 10 years prior to the time of testing decreased gradually and more slowly. While the numbers of persons involved in the 4 to 5 years, 6 to 10 years, and more than 10 years groups are relatively small, the results suggest that some degree of immunity persists for a long period of time—more than 10 years—after immunization with typhoid vaccine. It should be noted, however, that most of the members of these groups had been vaccinated two or more times.

COMMENT

Determination of the protective antibody content of the blood subsequent to immunization with typhoid vaccine, through the ability of the blood serum to confer passive immunity to mice, cannot be considered as measuring the total potential defense response of the immunized individual to typhoid fever. However, the production of immune substances and their presence in the blood, as shown by the mouse protection test, is a part of the response of the defense mechanism of the tissues to the inoculation of typhoid vaccine, and is demonstrable evidence of immunity to infection with *E. typhosa*.

As shown in Table II, the degree of immunity present in different individuals 2 weeks after vaccination varies widely. At this time all of the blood sera tested protected mice against at least 1,000 m.l.d. of the test organism, and 80 per cent protected against at least 10,000 doses, 20 per cent against 100,000 doses, and 2 per cent against one million m.l.d. Immediate protective antibody concentration in the blood serum sufficient to protect mice against 1,000 minimum lethal doses of virulent organisms appears to represent the minimum immunity conferred by vaccination with typhoid vaccine, when given in three subcutaneous injections of 0.5 c.c., 1 c.c., and 1 c.c. at weekly intervals. As epidemiological evidence indicates that immunization with typhoid vaccine protects against infection for some time after vaccination, it is believed the assumption is justified that if the blood serum will protect against 1,000 m.l.d., the individual has suffi-

cient immunity to protect him against dosages of the organism ordinarily encountered in nature. Undoubtedly some, if not a large majority, of immunized persons whose blood sera will not protect mice against less than 1,000 m.l.d. are still immune to typhoid fever.

The data presented in Tables III and IV indicated that during the second year after immunization the blood sera of 69 per cent of a group of 56 persons, and 62 per cent of a group of 115 persons protected mice against 1,000 to 10,000 m.l.d. Also, that during the third year after vaccination the blood sera of 46 per cent of a group of 30 persons, and 35 per cent of a group of 93 persons protected mice against 1,000 or more m.l.d.

Not only does the degree of immunity produced by immunization with typhoid vaccine vary greatly in different individuals, in so far as can be judged by the mouse protection tests, but the duration of the immunity conferred by vaccine is also subject to wide variations. Because of these variations, it would be impossible to determine if any one person was immune at any given time, without testing his blood serum. However, it appears from the results of these studies that a large proportion of immunized persons are protected for at least 2 to 3 years after vaccination.

SUMMARY

Several groups of individuals have been studied with a view to determining, by means of the mouse protection test, the concentration of protective antibodies in the blood at intervals of time subsequent to immunization with typhoid vaccine.

Prior to vaccination, the sera of 79 per cent of one group of individuals tested failed to protect mice against 100 m.l.d. of a live virulent test organism, and 76 per cent of another group failed to protect against 10 m.l.d. of the test organism.

Within 2 weeks after vaccination the antibody content of the blood sera tested increased rapidly to a comparatively high level. There was a relatively rapid decrease in circulating antibodies during the 2 year period subsequent to vaccination. Thereafter, the decrease was more gradual and 10 or more years after the last vaccination the antibody concentration in the blood was sufficient to suggest that a large proportion of the individuals tested still possessed considerable immunity to typhoid fever.

During the second year after vaccination, 69 per cent of one group of immunized individuals and 62 per cent of another group protected mice against 1,000 m.l.d. of the test organism. During the third year subsequent to immunization, 46 per cent of the blood sera of one group and 35 per cent of another group protected mice against 1,000 m.l.d. of the test organisms.

CONCLUSIONS

1. Experimental evidence based on mouse protection tests indicates that there is a material decrease in protective antibodies in the blood during the first and second years subsequent to immunization with typhoid vaccine.

2. The decrease in the protective antibody content of the blood is the greatest during the first 2 years after vaccination, and is comparatively slow thereafter.

3. The protective antibody content of the blood of the average individual for as long as 10 years subsequent to immunization with typhoid vaccine is sufficient to indicate that he still possesses considerable immunity to typhoid fever. The results obtained by this study suggest that persons who have been immunized with typhoid vaccine should be re-vaccinated from 2 to 4 years after initial vaccination.

RE-VACCINATION BY INTRACUTANEOUS INJECTION

Where immunization is employed to control typhoid fever in civilian com-

munities, or among troops, re-vaccination is usually considered necessary. It has been customary in re-vaccination against typhoid fever to use the same dosage of vaccine and the same methods of administering the vaccine as in original vaccination. That is, three subcutaneous injections of vaccine at about weekly intervals. This method is time consuming, and, not infrequently, in persons who have been vaccinated several times, causes rather severe reactions.

It is known that in immunizing against certain diseases other than typhoid fever, one dose of antigen will elicit an immunological response in previously immunized persons. In view of this, we have undertaken a study to determine if one dose of typhoid vaccine would stimulate the defense mechanism in previously vaccinated persons and, if so, the degree of response that results from such a stimulus.

A group of 100 individuals were each given one dose of 0.1 c.c. of typhoid vaccine (100 million organisms) intracutaneously in the arm or forearm. All had been immunized with typhoid vaccine from 2 to 10 years, or more, prior to re-vaccination. Some had received two or more courses of the vaccine.

Blood specimens were taken immediately before and 14 days after re-vaccination. The blood sera were titrated by the mouse protection test, using 0.1 c.c. of the individual's serum against multiple m.l.d. of the test organism (Strain No. 63 *supra*) for normal mice. The results are shown in Table V.

Before re-vaccination, the blood sera of members of this group protected mice against from 1 to 10,000 m.l.d. of the test organism. In 71 per cent of the observations the blood sera failed to protect mice against 1,000 m.l.d. and 21 per cent failed to protect mice against 100 m.l.d. (Table V-A).

TABLE V

Protective Antibody Content of Blood of 100 Persons Before Vaccination and 2 Weeks After Vaccination with 0.1 c.c. of Typhoid Vaccine Intracutaneously. The Protection Expressed as the Number of Minimum Lethal Doses of the Test Organism Against Which the Sera Protected Mice

A

Before Vaccination

Protection in m.l.d.	*Number of Persons*
10,000	1
5,000	2
1,000	26
500	10
100	40
10	16
1	5
Totals	100

B

Two Weeks After Vaccination

Protection in m.l.d.	*Number of Persons*
100,000	7
50,000	3
10,000	66
5,000	3
1,000	21
Totals	100

After re-vaccination, the blood sera protected mice against from 1,000 to 100,000 m.l.d. In 76 per cent of the cases, the blood sera protected mice against at least 10,000 m.l.d. (Table V-B).

COMMENT

The results of a study of the protective antibody content of the blood sera of 200 individuals 2 weeks after original vaccination with a series of three injections of typhoid vaccine have been tabulated in Table II. It will be seen that in this group 20 per cent of the sera protected mice against at least 1,000 m.l.d., and 80 per cent of the sera protected against at least 10,000

m.l.d. In comparison, in the re-vaccination of a group of 100 persons with one dose of vaccine (0.1 c.c.) administered intracutaneously (Table V), 21 per cent of the blood sera obtained 2 weeks after re-vaccination protected mice against 1,000 m.l.d. of test organisms, and 76 per cent protected mice against at least 10,000 m.l.d. Three per cent protected mice against 5,000 m.l.d.

The results of these two studies are comparable, and indicate that the immediate immunological response to re-vaccination with one intracutaneous injection of 0.1 c.c. of typhoid vaccine is approximately the same as that which occurs after the first vaccination with three subcutaneous injections of 0.5 c.c., 1 c.c., and 1 c.c., respectively.

Re-vaccination with one small intracutaneous dose of typhoid vaccine has the advantage that it is, obviously, a much simpler procedure than is a method requiring three subcutaneous injections at weekly intervals. Also, the reaction is, almost without exception, much milder than that produced by subcutaneous injection of 0.5 c.c. or 1 c.c. of the vaccine.

In this study the reactions produced by the vaccine have been consistently mild and of short duration. The local reaction consisted of an area of redness at the site of the injection, and in a few instances slight swelling of the tissues. The most severe systemic reactions observed consisted of mild malaise, headache, and myalgia. Involvement of the axillary lymph nodes was observed in 46 per cent of the cases, headache in 20 per cent, and rise in temperature in 8 per cent. The highest temperature noted was 101°. In many instances, those presenting mild reactions following the intracutaneous injection of the vaccine had a history of relatively severe reactions after subcutaneous injections of the vaccine.

Data are not yet available which would permit the comparison of the immediate or long range immunological response to re-vaccination with one intracutaneous dose of typhoid vaccine with that produced by re-vaccination with three subcutaneous injections at weekly intervals. This phase is being given further study and will be made the subject of a future report.

The duration of immunity following re-vaccination with one intracutaneous injection of 0.1 c.c. of the typhoid vaccine has not been determined, but will be investigated in the future.

SUMMARY

A group of 200 persons who had never been vaccinated were given three subcutaneous injections of 0.5, 1 c.c. and 1 c.c. respectively of the typhoid vaccine. A group of 100 individuals who had received from one to several courses of the typhoid vaccine from 2 to 10 years or more prior to the time this study was made were re-vaccinated with one intracutaneous dose of 0.1 c.c. of typhoid vaccine.

Two weeks after vaccination, the blood sera of those re-vaccinated with a single dose of 0.1 c.c. of the vaccine intracutaneously and of those vaccinated for the first time with three subcutaneous injections of the vaccine protected mice against a virulent strain of *E. typhosa* to approximately the same degree.

The results obtained indicate that the immunological response of the individual to re-vaccination with one dose of 0.1 c.c. of vaccine intracutaneously parallels that following the initial vaccination with three subcutaneous injections of 0.5 c.c., 1 c.c., and 1 c.c. of the vaccine at weekly intervals.

The reactions observed following re-vaccination with 0.1 c.c. of typhoid vaccine are mild as compared with those frequently produced by subcutaneous injection of the vaccine.

CONCLUSIONS

1. Re-vaccination against typhoid fever of previously immunized persons with one intracutaneous dose of 0.1 c.c. of typhoid vaccine produces an immediate immunological response which is comparable to that following original vaccination with three subcutaneous injections of the vaccine.

2. As compared with re-vaccination with three subcutaneous injections of the vaccine, re-vaccination with one small intracutaneous dose of vaccine is a simple and time saving procedure.

3. The reaction following the intracutaneous injection of 0.1 c.c. of typhoid vaccine is relatively mild as compared with that frequently produced by the subcutaneous injection of 0.5 c.c. or 1.0 c.c. of the vaccine.

REFERENCES

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The Initiation of Social Medicine

STATISTICS indicate that professional, economic, and social factors influence mortality rates—a fact that has long been recognized, but until recently neglected by the medical profession.

Figures, quoted by Dr. René Sand in a paper presented to the Medical Society of Geneva, show that although mortality rates have been decreasing since the second half of the 19th century, the decrease has been proportional in all the social classes so the difference between the classes has varied little.

Public health and social medicine are becoming an increasingly important part of the medical curricula, as is shown by a survey of such courses in medical schools. Eventually, it is believed, the medical course will be built around the normal person; the first year will be devoted to a study of normal physiology, followed in the succeeding years by courses in public health and by clinical work in which the social aspects of disease will be emphasized. The relationship of the medical profession to social medicine is yet to be established, but if the medical profession has had this training, the solution of the problem will be easier, happier, and quicker, and the individual members of the medical profession will be able to adapt themselves to the new situation more readily. — Dr. René Sand: *Revue Medicale de la Suisse Romande,* 18. 12:761–772 (Oct. 25), 1938.