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Original Communications.

ANTITYPHOID INOCULATION.

Observations on the Immunizing Properties and on the Manufacture of Typhoid Vaccine.

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Two recent papers emanating from the Vaccine Department of the Royal Army Medical College (Perry, Findlay and Bensted, 1933), have recorded (*a*) the assessment of the immunizing value of strains of *Bact. typhosum* in different phases by mouse protection tests, and (*b*) the antigenic variation of this organism by certain *in vivo* methods. Work on this subject of antityphoid inoculation has been continued with the object of obtaining some evidence that would correlate the results of these protection experiments in mice with the immunizing value of the vaccine in the human subject. Also to determine the most suitable modifications that should be introduced into the technique of manufacture of typhoid-paratyphoid vaccine to increase its efficiency. This communication is concerned with these further observations.

The Correlation of Immunity in the Human Subject with Protection in Mice following Inoculation with *Bact. typhosum* in Different Phases.

A field trial in endemic areas of the disease would, of course, supply the most satisfactory evidence of the immunizing power of any typhoid vaccine. The difficulties attendant on a test of this nature are very great and in the present circumstances cannot be overcome. To obtain unequivocal statistical evidence under conditions of natural infection would entail a standardized technique of laboratory diagnosis, a clinical and laboratory staff especially selected for this work, together with adequate controls. Opportunity may arise in the future to attain this ideal, but until such time as this is possible, field trials would only confuse the issue. In default, therefore, of this method of assessment of the value of the vaccine confirmation of the utility of mouse protection tests can only be obtained by recourse to laboratory tests that might be expected to yield evidence of any variation in response to the injection of different types of typhoid vaccine. The choice is restricted to methods by which more or less accurate quantitative estimation of immune substances can be made. Agglutination and bactericidal tests appear, therefore, the only alternatives available.

With regard to agglutination tests, it is agreed that flagellar agglutination is no measure of the resistance of the individual to infection (Felix, 1924, Arkwright, 1927, etc.). There is evidence, however, that the development of somatic agglutinins is some criterion of protection (Felix and Olitzki, 1926), but it is not universally accepted that the somatic titre has any quantitative relationship to the degree of immunity (Grinnell, 1932). Owing to the conflicting views that exist on the question it appeared desirable to obtain some further experimental evidence bearing on this subject.

For this purpose rabbits were injected with different types of typhoid vaccine ; the animals were bled after suitable intervals, the sera inactivated and stored in sterile ampoules until required. Bacillary suspensions were prepared from cultures of typhoid bacilli in different phases. In addition, vaccines were made from the soluble specific carbohydrate factor combined with various proteins. The hapten was prepared after the method described by Bruce White (1929), and the non-specific protein factors employed were normal human serum and artificially roughened *Bact. paratyphosum A*.

The rabbit antisera resulting from the inoculation of these vaccines, and normal horse-serum employed as a control, were mixed with one or two minimal lethal doses of the test organism and immediately injected intraperitoneally into mice.

Table I shows the results of these experiments together with the "O" and "H" titre of each serum used.

It would appear from the results of the above experiments that, within the limits of the test, there is evidence of relationship between the "O"

TABLE I.

TO DETERMINE THE RELATIONSHIP BETWEEN THE AGGLUTININ TITRE OF AN ANTI-TYPHOID SERUM AND ITS PROTECTIVE VALUE.

Rabbit anti-sera	Agglutinin titre		Result of injection of mixture of serum and 1 M.L.D. of Bact. typhosum.	Result of injection of mixture of serum and 2 M.L.D of Bact typhosum.
	H	O		
Anti-typhoid Si	1/125.	1/1500.	□ □ □ □ □	□ □ □ □ □ □ □ □ □ □
Anti-typhoid Sii	1/125	1/750	□ □ □ □ □	■ ■ ■ ■ ■
Anti-typhoid Rii.	1/30,000.	1/250	□ ■ ■ ■ ■	■ ■ ■ ■ ■
Anti-typhoid S hapten and rough para.A.	nil	1/25	□ □ ■ ■ ■	■ ■ ■ ■ ■
Anti-typhoid S hapten and human serum.	nil.	1/50.	□ ■ ■ ■ ■	■ ■ ■ ■ ■
Normal horse serum control.	nil.	nil.	□ ■ ■ ■ ■	/
Control without serum.	–	–	■ ■ ■ ■ ■	/

□ Mouse alive and quite fit 72 hours after injection.

■ Mouse dead within 48 hours from typhoid septicaemia

The amount of pure serum injected into each mouse was 0·5 cc. and the 1 M.L.D or 2 M.L.D of organisms was contained in 0·25 cc.

agglutinin titre and the protective power of the serum. Owing to the nature of the test it is not possible to judge the comparative values of sera having "O" titres of 1-250 and less.

That immunity may be present even though no agglutinins are detectable in the sera appears evident from mouse experiments. It has been noted during the course of this work that the agglutinin response of mice following the injection of the usual types of vaccine may be almost negligible. For example, when a pure smooth vaccine was used, mice were fully protected against 2 M.L.D. of the test organism, yet the "O" titre of the serum was only 1-12·5. Further, mice injected with two doses of the purified specific polysaccharide of a smooth typhoid bacillus combined with the non-specific nucleo-protein of a rough paratyphoid "A" bacillus were fully protected against 1 M.L.D. of *Bact. typhosum*, but no agglutinins could be demonstrated in the sera. This protection of mice with hapten mixtures against subsequent infection with typhoid bacilli is analogous to the work of Avery and Goebel (1931) on the pneumococcus.

Owing to the low "O" titres which normally follow inoculation of the human subject with the usual two doses of typhoid vaccine, agglutination tests cannot be expected to yield comparative evidence of any immunity that may be present.

Methods of estimating the bactericidal property of the blood following injection of the vaccine thus appear to be the sole laboratory tests that can be employed with any prospect of success.

Whilst it is realized that certain criticisms have been made regarding the interpretation of bactericidal tests (Hale, 1930), the concensus of opinion is that they afford valuable comparative evidence of immunity. Investigation was therefore directed to the determination of the comparative bactericidal values of human sera following the inoculation of killed cultures of *Bact. typhosum* of varying antigenic composition. The difficulties of selecting the most satisfactory method of carrying out this test are too well known to need any emphasis. It will suffice to mention that all the orthodox methods have been tried in detail. The technique that yielded the most consistent results was a slight modification of that described by Felix and Olitzki (1926) as the plate-culture method of Neisser and Weschberg. The essential features of this method consist in the use of a standardized complement and the visual determination by colony counts of the number of organisms surviving after contact with an immune serum for a given period.

Six individuals were employed in the investigation. As a preliminary, samples of blood were obtained for the estimation of the normal bactericidal power of the serum. Later, the men were inoculated with different typhoid vaccines prepared by the routine method and standardized to contain one thousand million organisms per cubic centimetre. The dosage of the vaccine was 0·5 c.c. followed by 1·0 c.c. after an interval of ten days, and samples of blood were taken ten days after the second injection. The sera

from the preliminary bleedings and those obtained subsequent to inoculation were heated to 52° C. in a water bath for half an hour (to inactivate complement) on each of two successive days, and were stored in the cold until required for use.

Three different cultures of *Bact. typhosum* were employed for the vaccines; the first being representative of the organism in its most pronounced rough phase—obtained artificially by growth in immune serum—the second consisting of a culture in the pure smooth phase, and a third intermediate in character between these two extremes. Two men (A and B) were inoculated with the vaccine prepared from the rough organisms, two (C and D) with that prepared from the partially rough organisms, and two (E and F) with that prepared from the pure smooth organisms.

TECHNIQUE OF THE TEST.

Reagents.—(1) Human sera—inactivated by heating at 52° C.

(2) Complement—normal rabbit serum. The animal was selected by preliminary titrations of the serum and the titrations were repeated at intervals to ensure the minimal bactericidal action on the test organism.

(3) Test organism—chosen by preliminary estimations as not being unduly susceptible to bactericidal action.

Method.—The details regarding dilutions of the sera, etc., will become evident on reference to the tables. The only points, however, that may usefully be mentioned are as follows :—

(1) Difficulty may be experienced in making shake cultures that yield a uniform distribution of colonies. This can be obviated by thoroughly agitating the tubes containing the melted agar and the test mixtures and allowing them to stand for a few minutes to enable the bubbles to dissipate before pouring the plates.

(2) The counting of the colonies can most easily be effected by the examination of the plates under a dissecting microscope with a magnification of thirty diameters. The result is expressed as the total number of colonies in the plates. This figure is obtained by multiplying the average number of colonies in each microscope field by a factor representing the ratio of the area of the microscope field to the area of the plate. If the colonies should be so numerous as to be uncountable the result is expressed as a single infinity figure, whilst complete overgrowth of the plate culture is represented by a double infinity figure. It was found that with the magnification employed over 60 colonies per field—corresponding to about 55,000 per plate—approached the limit of possible enumeration.

(3) The culture of *Bact. typhosum* used to estimate the bactericidal property of the serum in all the tests was selected after estimating its resistance against fresh rabbit serum. It was noted that organisms showing the slightest sign of roughness were very

TABLE II.

COMPARISON OF INCREASE OF BACTERICIDAL POWER OF HUMAN SERUM AFTER INOCULATION WITH VACCINES PREPARED FROM ROUGH AND SMOOTH CULTURES OF *Bact. typhosum.*

Individual.	Dose of Serum in c.c.	Dose of other reagents etc	Total number of colonies on plates.	
			Before inoculation.	After inoculation.
A Rough vaccine.	0·001 0·0005	To each dose of serum was added 0·025 c.c. of complement and 0·1 c.c. of a 1/100,000 dilution of a 24 hours broth culture of Bact. typhosum sufficient saline was added to each tube to make the final bulk 0·25 c.c. The mixture was left in contact for 4 hrs. at 37°C and shake plate cultures made from each tube.	28,000. 25,000.	20,000. 20,000.
B. Rough vaccine.	0·001 0·0005		35,000. 37,000.	25,000. 34,000
C. Semi-rough vaccine.	0·001 0·0005		17,500. 17,500.	18,000. 17,000
D. Semi-rough vaccine.	0·001 0 0005		31,000. 34,000.	25,000. 33,000.
E Smooth vaccine	0·001 0·0005		34,000 ∞	15,000. 25,000.
F Smooth vaccine	0·001 0·0005		33,000 ∞ ∞	10,000 26,000

Control plates — Immediate count 4,500.
Count after 4 hrs at 37°C ∞ ∞.

susceptible to the bactericidal action of this serum. This action was far less manifest on smooth organisms. The culture finally employed was a smooth type, and by conserving it in the pure smooth phase consistent results were obtained throughout the series of experiments.

Table II and the appended notes illustrate the results of this investigation.

It will be apparent from the above table that there is little or no difference between the increase in the bactericidal power of the serum of individuals injected with vaccines prepared from partially rough and markedly rough organisms. There is, however, a marked increase in the bactericidal value of the serum of the individuals inoculated with pure "smooth" vaccines.

Note.—It must be mentioned that the sera used in the above test have been stored in ampoules at a low temperature. The tests have been repeated on many occasions over a period of some months. The results have, in the main, been comparable, although occasionally a slight difference has been noted between the counts relating to the rough and semi-rough vaccines, as is shown below in the abridged table:—

Vaccine	Colony Counts	
	Before inoculation	After inoculation
Rough	6,400 5,900	6,600 6,600
Semi-rough	6,900 8,300	6,100 5,100
Smooth	7,800 9,200	2,600 1,200

The findings, have, however, without exception indicated the marked superiority of the smooth vaccine in raising the bactericidal power of the serum. It would appear that in the short immunizing process of two doses of vaccine to which individuals are usually subjected, the maximum increase in bactericidins is only produced by vaccines prepared from pure smooth organisms.

Confirmation of these results was obtained by mouse experiments. A series of bactericidal tests, employing the same technique, was made on the sera of mice immunized with rough and smooth vaccines. Forty mice were used in the test, twenty being inoculated with the smooth organisms and twenty with the rough. Ten mice from each group were retained for

protection tests, whilst the remaining ten were killed; the sera from each group were pooled and the bactericidins estimated. Table III illustrates the result of this test. Subsequent protection tests showed that the ten mice inoculated with the smooth organism were fully protected against 2 M.L.D. of *Bact. typhosum*, whereas the ten mice inoculated with the rough organism did not survive this test dose.

TABLE III.

COMPARISON OF INCREASE OF BACTERICIDAL POWER OF MOUSE SERUM AFTER INOCULATION WITH VACCINES PREPARED FROM ROUGH AND SMOOTH CULTURES OF *Bact. typhosum*.

Type of Vaccine	Dose of Serum in c c	Dose of other reagents etc	Total number of colonies on plates	
			Normal mouse serum	Immunized mouse serum
Rough	0·005	To each dose of serum was added 0.025 c.c. of complement and 0.1 c.c. of 1/100,000 dil. of 24 hrs broth culture of Bact typhosum Saline was added to make final bulk 0.25 c.c. The mixture was left in contact for 4 hrs.	∞	16,400
	0·0005		∞	5,800
Smooth	0·005		∞	1,100
	0 0005		∞	700
Control plates			Immediate count	3,100
			count after 4 hrs at 37°C	17,500

The results of bactericidal tests are greatly influenced by the details of the technique employed and it became evident during the course of this work that the essential factors, such as serum dilution, dose of organisms employed, and the period of contact of the mixture, require very delicate adjustment. It is not claimed that the figures that have been obtained are not open to criticism. It may, however, be stated that the reduction in colony counts, subsequent to inoculation, was not due to agglutination of the organisms, which has been one of the main criticisms of bactericidal tests employing colony counting methods. Investigation of this particular point has negatived such a possibility. The bactericidal tests were repeated with serum dilutions sufficiently high to exclude the influence of agglutination, the "H" and "O" agglutinin titres were estimated before and after inoculation, and results comparable with those given in Table II were obtained.

The foregoing investigations appear to prove that there is a definite correlation between the development of immune substances in the human subject and the results of mouse protection tests following the inoculation of typhoid vaccines. Smooth vaccines increase the bactericidal power of the blood and protect mice against subsequent lethal doses of living organisms, whereas vaccines made from rough organisms fail to produce either of these effects.

Preparation of Typhoid-Paratyphoid Vaccine.

In a previous communication (Perry, Findlay and Bensted, 1933) it has been mentioned that, as a result of mouse protection tests, the strain of *Bact. typhosum*, known as the Rawlings strain, had been discarded. This strain had been for many years included in the Army typhoid-paratyphoid vaccine, but deterioration in its protective properties had become obvious and substitution of a smooth culture of proved protective value was indicated. In addition, certain modifications in the method of preparation of the vaccine appeared desirable.

It may be mentioned, however, that since the above communication was published a note on work of a similar nature by Ahuja (1933), from the Central Research Institute, Kasauli, has appeared. This observer arrived at somewhat different conclusions, and states that he believes, as a result of his investigations, that a vaccine prepared from the "fixed" or "standard" type of the Rawlings strain of typhoid bacillus confers a degree of protection almost equal to that obtained with a vaccine prepared from a recently isolated strain of typhoid bacillus. It is not clear what the terms "fixed" or "standard" type of the Rawlings typhoid bacillus are meant to convey. Experience of this strain, which extends over many years, has made it evident that its behaviour under conditions of artificial culture is similar to that of any other strain of *Bact. typhosum*. The virulence and protective capacity of the organism are unstable factors, and depend upon the proportion of smooth surface antigens present in the culture. It is possible for these properties to vary in one or other direction according to the conditions of artificial environment. Whilst decrease in protective properties can be delayed by careful colony selection, the tendency is towards decadence and ultimate loss of efficacy as a vaccine, in spite of any cultural precautions that may be employed.

The first modification that was introduced in the preparation of the vaccine was the substitution of a smooth recently isolated strain of typhoid bacillus for the old Rawlings strain. The selection of this new strain was made on the basis of its proved efficacy in increasing the resistance of mice to infection and on the fact that its surface antigens were entirely smooth. Whilst this culture was salt stable undue importance was not attached to this property, which was emphasized in the article by Ahuja, as it has been shown by Grinnell (1932), amongst others, that stability in saline solution

is no criterion of the proportion of smooth antigens in the culture. Eleven out of twelve of the Rawlings rough cultures he worked with were salt stable.

Subsequently, certain *in vivo* methods (Perry, Findlay and Bensted, 1933) having shown that it is possible to produce pure smooth cultures of the Rawlings strain and its increased virulence and protective properties having been fully proved by mouse protection tests, it was decided to employ this rejuvenated Rawlings strain as the typhoid component. The suggestion is not made that it is superior to any other strain of typhoid bacillus in the same phase, but the choice has been made on its long association with typhoid vaccine. Obviously it is not the strain of the bacillus employed that is of importance, but that the emulsion from which the vaccine is manufactured should consist of pure smooth antigens only. The paratyphoid components of the vaccine (*Bact. paratyphosum* "A," Mears, and *Bact. paratyphosum* "B," Rowlands) remain unchanged. Animal experiments on the same lines as those undertaken with the typhoid bacillus have proved their suitability for inclusion in the vaccine. The numbers and relative proportions of the three organisms are also similar to that in the original vaccine.

An innovation has been introduced in the methods of preserving the strains. Previously, continuous subculture on inspissated egg-medium, to conserve as far as possible the smooth phase, was the rule. It has been found, however, that a more satisfactory method is to utilize the spleen of a mouse that has died of an experimentally produced typhoid or paratyphoid septicæmia. A mouse is inoculated intraperitoneally with a lethal dose of the organism, after its death the spleen is removed aseptically, dried *in vacuo* over calcium chloride, and stored in a sealed ampoule. It is uncertain, at present, how long the organism remains viable, but no trouble has been experienced in obtaining pure cultures of smooth organisms from this dried spleen after a period of four months.

The following are the details of the present method of manufacture of typhoid-paratyphoid vaccine :—

Typhoid Element.

Bact. typhosum, rejuvenated Rawlings strain.

(1) A small portion of the dried spleen, obtained in the manner described above, is inoculated into broth which is incubated at 37° C. for eight to twelve hours.

(2) The broth culture is plated out and the plates incubated for twenty-four hours at 37° C.

(3) The colonies that develop are invariably smooth, but in the event of the colonial appearance indicating a reversion, and loss of virulence, mouse passage, as described in the previous article (Perry, Findlay and Bensted, 1933), is undertaken. A typically smooth colony is marked by a ring.

(4) A tube of broth is inoculated from the marked colony and incubated for eighteen hours at 37° C.

(5) Three mice are inoculated intraperitoneally with 0·5 c.c. of a 1 in 5 dilution of this 18-hour broth culture. Employing the nutrient broth in use at the R.A.M. College, the bacterial content of this inoculum is approximately 50 millions, i.e. one minimal lethal dose. The inoculated mice should die within forty-eight hours from a typhoid septicæmia.

(6) A post-mortem examination is performed and plate cultivations are made from the heart blood.

(7) The plate should show a pure culture of smooth organisms and one discrete colony is selected. Subculture is made into broth tubes and also into the various carbohydrate media. The identity of the selected colony can thus be confirmed by its biochemical and serological reactions.

(8) A Roux bottle of unfiltered pea-flour tryptic-digest agar is seeded with the contents of one of the broth tubes and incubated for twenty-four hours.

(9) The growth is washed off in 300-400 c.c. of normal saline.

(10) The emulsion is sucked off by means of a vacuum pump into a sterile flask and provides inoculum sufficient for twenty or more Roux bottles.

(11) Each Roux bottle is inoculated with 15 c.c. of emulsion and incubated for forty-eight hours.

(12) Sterile saline to the amount of 80 c.c. is pumped into each bottle of medium and the growth washed off.

(13) A sample from each bottle is examined for purity by means of slide preparations.

(14) The emulsion from each bottle is sucked off by means of a vacuum pump into a three-litre graduated flask.

(15) The bulk of the emulsion is made up to the nearest multiple of 500 c.c. by pumping in sterile saline.

(16) Samples are removed for purity tests and bacterial count by the opacity method.

(17) The thick emulsion is heated for one hour at 53° C. allowing time for the centre of the emulsion to attain this temperature.

(18) Sufficient 5 per cent. carbol-saline is added to make a final concentration of 1 per cent. in the bulk. This is stored for forty-eight hours with occasional shaking.

(19) Sterility tests in fluid and solid media under aerobic and anaerobic conditions are made in the usual manner.

Paratyphoid Elements.

Bact. paratyphosum " A," strain Mears.
Bact. paratyphosum " B," strain Rowlands.
A similar procedure is followed.

(20) The three thick emulsions are mixed and diluted so that the finished vaccine contains :—

1,000 million *Bact. typhosum*,
750 million *Bact. paratyphosum* "A,"
750 million *Bact. paratyphosum* "B,"
in 0·5 per cent. carbol saline.

(21) The vaccine is filled into bottles or ampoules, and final sterility and toxicity tests, by mouse and guinea-pig inoculation, are carried out.

(22) The batch of vaccine is labelled with an expiry date which, at present, is calculated as one year from the date of manufacture of the thick emulsion employed.

Expiry Date of Vaccine.

In an earlier communication the desirability of ascertaining on some scientific basis the duration of potency of this vaccine was mentioned. It was noted that the expiry date of one year that had been affixed to the vaccine had been empirically selected. As recorded in this communication quantities of the vaccines used in the original investigations have been retained and stored both at ordinary temperatures and in the cold room. Mouse protection tests have now been undertaken at intervals of three months. Up to the present time—twelve months after the preparation of the emulsions—the vaccines have been proved to have fully retained their protective properties. It is possible, therefore, that further lapse of time will show that the present expiry date may be much extended. If this should be the case it will be evident that considerable economy would result in circumstances where it is essential that large reserves of vaccine should be available.

Reactions following Inoculation.

Some observations are desirable on the subject of the local and general reactions that are liable to follow the inoculation of typhoid-paratyphoid vaccine. In the earlier days, when this vaccine was first introduced into the Army, the severity of the symptoms following the injection of killed broth cultures proved a marked deterrent to this form of prophylaxis. With further experience, the intensity of these reactions was diminished by growing the organisms on an agar medium and employing a heavy broth inoculum. This method reduced the broth content in the finished vaccine to about five per cent. Notwithstanding this modification complaints were still received from time to time regarding severe general and local reactions.

Inoculation being on a voluntary basis in the British Army the importance of minimizing these post-inoculation effects was recognized. Special and detailed instructions are laid down in the Regulations for the Medical

Services of the Army (1932) regarding the precautions that should be taken after typhoid-paratyphoid inoculation. It became evident that indulgence in any severe form of exercise during the forty-eight hours following inoculation was one of the most important factors in producing undue reactions and this is particularly emphasized in these instructions. In addition, owing to the fact that intense reactions in animals followed the administration of broth vaccine, the technique of manufacture was further modified so that the final broth content of the suspension was reduced to a minimum. This procedure appeared to be justifiable as recent animal experiments have shown that saline suspensions of the organisms are as protective as broth emulsions.

It was considered possible that the severity of the after-effects might have been increased by the substitution of organisms more highly virulent than those previously employed in the vaccine. A number of experimental inoculations in the human subject were, therefore, undertaken to obtain some data bearing on this point before the issue of vaccines prepared from bacilli of a higher order of virulence.

Three vaccines were prepared, the paratyphoid elements being the same in each case, but the typhoid component in the first was the Rawlings strain of *Bact. typhosum* in its natural rough phase, in the second the Rawlings strain in its smooth phase and in the third a smooth recently isolated strain. Seventy-five men were used in the test and were divided into three batches. The inoculations were undertaken by an independent observer, the vaccines supplied were labelled with a serial number only and were without further indication of their exact nature. In this manner prejudice could not enter into the contrast of the resulting reactions. The vaccines were given in full doses, the temperature was taken before and after inoculation and careful observations were made of local reactions. The usual precautions advised after the injection of typhoid-paratyphoid vaccines were strictly observed and rest was enforced. In no case was there any general reaction that called for special comment, and the local reaction was not excessive. No difference was noted between the after-effects following the three different vaccines. It can, therefore, be safely assumed that the virulence of the organism injected—in the case of enteric vaccines—does not influence reaction to any extent, and that vaccines prepared from highly virulent organisms can be used for mass inoculation with safety, provided that the instructions issued with the vaccine are followed.

It must be noted that reactions of greater severity than usual appear to follow inoculations made during the warm seasons in the tropics. Ledingham and Balfour (1917) have emphasized that inoculations during the hot weather may be followed by excessively severe reactions and this has also been the opinion of those with experience of the vaccine under tropical conditions (Perry and Bensted, 1929). Administrative arrangements should, therefore, be made so that primary or reinoculations abroad are undertaken during the temperate season.

Reports have been received on occasion regarding severe reactions following the inoculation of troops on board ship and in consequence it seems advisable that all such injections should be completed before embarkation.

There remains to be mentioned a somewhat unusual delayed reaction that may not infrequently be observed. No untoward effect is noted at the usual period—six to eight hours after the administration of the vaccine—but malaise, rise of temperature and increased local reaction are complained of a few days later. This delayed reaction appears to be due to the liberation of toxins by rubbing or scratching the site of the injection as a result of the irritation induced by the inoculation.

Individuals will, however, be met with who display an especial sensitivity towards the inoculation of foreign proteins despite any precautions that can be taken. Apart from such individuals undue after-effects can in a large measure be attributed to neglect of the necessary after-care.

It is unfortunate that this neglect in the after-care of the inoculated individuals is still responsible for isolated reports of undue reactions. It is apparent that familiarity with the employment of the vaccine for many years has engendered carelessness in complying with the regulations that govern its use. One recent example may be quoted. A unit serving abroad carried out a series of reinoculations and reported, somewhat precipitately, intense general and local reactions. The same batch of vaccine had been employed in various stations at home and abroad without complaint. It subsequently transpired that the unit in question had undertaken these inoculations in the morning, during the hot weather, and that the individuals inoculated continued their ordinary duties, which entailed heavy exercise, throughout the day. It is not surprising, therefore, that the after-effects were unduly severe. A further example may be of interest. Amongst a batch of 15 men inoculated in a station at home one suffered from severe general reaction—rise of temperature with rigors—whilst the remaining 14 were not notably affected. This individual had, through a sense of misguided zeal, scrubbed a corridor a few hours after his inoculation.

It will be seen that every effort is made in the process of manufacture of the vaccine to produce a prophylactic which will yield the maximum possible protection with the minimum reaction. The latter desideratum can only be procured by the collaboration of the medical officer responsible for the injections.

Inoculation of Infants.

The fact that considerable numbers of children in the earlier years of life are still unprotected by inoculation when taken abroad is, no doubt, due to the mistaken idea of the severity of the reaction that may follow the inoculation and to the fact that the incidence of typhoid in this age category is not high. Experience both of the inoculation of children and of the occurrence of enteric fevers amongst infants abroad suggests

that these views have no real foundation. Provided that the dose is graduated, children from the age of two years onwards can be inoculated without anxiety, as the reactions are in no way unduly severe. For children from the ages of 2 to 4 years the most practical method is to dilute the ordinary vaccine ten times with normal saline, and of this diluted vaccine to administer two doses, 0·25 c.c. and 0·5 c.c., spaced by the usual interval. For older children—from 4 to 12 years—the ordinary vaccine should be diluted two and a half times, and two doses, 0·25 c.c. and 0·5 c.c., administered at the usual intervals.

Summary.

(1) An account is given of investigations made to compare the protective value of different typhoid vaccines by the correlation of mouse protection tests with the development of immune bodies in the human subject and in mice.

(2) Modifications introduced into the manufacture of the typhoid-paratyphoid vaccine are described.

(3) Some data bearing on the period over which the vaccine retains its potency are discussed.

(4) Comment is made on the local and general reactions following inoculation and also on the question of the employment of the vaccine for the immunization of children.

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