SENSITIZED VERSUS NON-SENSITIZED TYPHOID BACTERIA IN THE PROPHYLAXIS AND TREATMENT OF TYPHOID FEVER

WITH A REPORT OF SEVENTEEN CASES TREATED WITH SENSITIZED VACCINE

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Sensitization means the mixing of an antigen (bacteria, red blood cells, proteins, etc.) with its specific antibodies. These antibodies are found in the serum of an animal that has been previously immunized with the particular antigen. If, for example, a rabbit or goat receives injections of typhoid bacteria, the usual antibodies (agglutinins, bacteriolysins, bacteriotropins, (opsonins), complement fixation bodies, etc.) appear in its serum. If an emulsion of typhoid bacilli be now mixed with this serum, inactivated, the bacteria become sensitized; that is, during the process of sensitization the bacteria unite with the specific immune bodies present in the serum. Sensitized bacilli may be dead or living, depending on whether the bacteria have or have not been killed previous to being mixed with the immune serum.

I was the first definitely to demonstrate, in 1910, that the immunity attained by injections of non-sensitized typhoid bacteria was actively bacteriolytic and only slightly bacteriotropic in character, while that with sensitized bacteria was mainly bacteriotropic. Intravenous inoculations of rabbits and guinea pigs with emulsions of non-sensitized bacteria (ordinary vaccines) produced a serum rich in agglutinins, precipitins, bacteriolysins and complement fixation bodies, and but poor in bacteriotropins. Similar doses of completely sensitized bacilli produced hardly any agglutinins, precipitins or complement fixation bodies, few bacteriolysins, but a very strong bacteriotropic (opsonic) serum, which was also curative for mice. Broughton-Alcock corroborated this finding in man.

The following explanation was offered for these differences: By means of sensitization the typhoid bacteria and their immune bodies (amboceptors) are combined in the test tube so that when injected into an animal the bacteria are ready for immediate attack and destruction by the complement of the blood. With the injection of ordinary non-sensitized bacteria, the tissue cells first manufacture the antibodies (bacteriolysins, etc.) which can unite with the bacteria, that is, sensitize them, before the latter can be broken up. Whenever typhoid bacteria are disintegrated, their central portion, the so-called endotoxin, is liberated. From his experiments the author believes that this central substance can probably act as an antigen and stimulate its own antibody, which is bacteriotropic in character (the anti-endotoxin, for purposes of designation). This sensitization is a biologic means of preparation for attaining the rapid destruction of the typhoid bacteria, liberating their central substance and allowing the latter to stimulate their own antibodies, bacteriotropins in nature.

Naturally, some bacteriotropins (opsonins) will appear also with injections of ordinary bacteria, especially if repeated for a long period of time; as then sensitization occurs in vivo after sufficient bacteriolysins have been formed.

When working with sensitized bacteria one must be certain that all the bacilli are saturated with antibodies (completely sensitized), otherwise immune bodies will be stimulated by the bacteria which were left unsensitized (incomplete sensitization). This probably explains the reports of some observers who find numerous agglutinins, complement fixation bodies, etc., after injection of sensitized bacteria (Négre). If, too, it is remembered that individual strains of typhoid bacteria so frequently differ from each other in their antibodies, a further possibility for incomplete sensitization is realized (Garbat, Raskin). With these preliminary remarks, the following discussion of the relative value of ordinary typhoid vaccine and sensitized typhoid vaccine in the prophylaxis and treatment of typhoid fever will be more readily understood.

The inoculation of dead typhoid bacteria (ordinary vaccine), as a prophylactic measure against typhoid fever is now well recognized. The efficiency of this procedure is easily explained on the basis of the biologic indications to be met. The injection of bacteria into a normal individual is usually followed by the production of a great number of various antibodies: bacteriolysins, agglutinins, bacteriotropins (Bischoff, Harrison, Leishman, Levy, Shoemaker, Thomas, Wollstein) and occasionally complement fixation bodies. Some of these immune agents are probably capable of destroying the typhoid bacilli. If the inoculation

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lated individual is exposed to infection, that is, if live bacteria find their way into his body, they are readily destroyed and will not multiply. Naturally, if the army of invaders is excessive, or the defense too slight, infection will arise in spite of the prophylactic measure. Fortunately, such instances are exceptional. The protection is therefore explained on a bacteriolytic, and to a less degree, on a bacteriotropic basis. From clinical experiences it has been shown that immunity persists even after these immune bodies cannot be demonstrated any longer (Cole13).

The prophylaxis attained by the injection of Besredka's living sensitized typhoid vaccine, cannot, if sensitization is complete, be explained on identically the same principles, for, as soon as the sensitized bacteria enter the normal system, they combine with the complement of the blood and are broken up. The agglutinating and bacteriolytic antibodies and the complement fixation bodies are therefore either not produced at all or only in very small quantities; consequently, the reliance for protection must be placed on the bacteriotropic or phagocytic activity, stimulated by the liberated central substance of the broken-up bacteria, the so-called endotoxin. The fact that the bacteria are alive does not necessarily mean that they are harmful, because, as was mentioned above, they are destroyed by the organism immediately after injection.

The relative excellence of the sensitized method over the original Wright method has not been definitely established, but the fact that Besredka and Metchnikoff strongly advocate it, is an important point in its consideration.

It may be in place here to consider a problem which has suggested itself to me, but which I have thus far been unable to carry out. If the injection of ordinary vaccine causes mainly a bacteriolytic reaction, and the administration of sensitized vaccine mainly a bacteriotropic reaction, would it not be advisable in prophylactic immunization to inoculate individuals with both sensitized and non-sensitized vaccines? In this way a combined form of immunity would be attained. As experimental basis for this suggestion I have shown that if the serum from rabbits immunized with non-sensitized bacteria is mixed in equal quantity with the serum from rabbits immunized with sensitized bacteria, this mixed serum was more curative for mice infected with typhoid bacteria than equal quantities of either serum alone. Naturally it will be necessary to establish the best method for such mixed immunization; whether the two vaccines should be administered separately and at different times or whether they should be given at the same time, as would be accomplished also by an incompletely sensitized vaccine.

In the therapy of typhoid fever the use of the ordinary non-sensitized vaccines has not met with as favorable results as for purposes of prophylaxis. Up to the present time several thousand cases have been treated (Watters,15 Horner16), but the beneficial effects in the majority of instances are not sufficiently marked to warrant the employment of the vaccines as a routine procedure. Occasionally a striking crisis in the course of the disease is observed, but just as frequently an increase in the severity of the symptoms develops. Reports of instances in which the effects have been distinctly harmful (or even fatal) must also be considered.

When the biologic basis for the possible therapeutic value of vaccines in typhoid fever is asked, the answer is much more difficult and hypothetical than in the question of prophylaxis. Several factors are to be kept in mind. First, typhoid fever is a self-limited disease, running a typical clinical course and probably associated with definite phases of immunity to account for its characteristic picture and self-limitation. Second, the number of bacteria existing in a typhoid patient is very great; they circulate everywhere and stimulate the tissue cells continually, resulting in the formation of agglutinins, bacteriolysins, bacteriotrops, complement fixatives, etc.; these antibodies are apparently produced very slowly and are either not of sufficient number or of a nature suitable to overcome the infection quickly. It usually takes four weeks or more to ultimately accomplish this. The reason for this prolonged period may possibly be ascribed to the structure of the typhoid bacillus. As was said above, this bacterium belongs to a class of micro-organisms whose central substance, the so-called endotoxin, is liberated only after the bacillus has been broken up. This central substance also stimulates the formation of antibodies (antiendotoxins for purposes of designation) which are bacteriotropic in action and probably important elements in the curative process of typhoid fever.

Were it permissible to divide the phases of typhoid fever from an immunological point of view, as is usually done from the clinical aspect, one would assume that the first stage consists of the multiplication of the invaded typhoid bacilli. Then as a defensive reaction, the tissue cells stimulate the formation of antibodies (bacteriolysins, agglutinins and but few bacteriotrops). The next phase sees the numerous bacteriolysins attack the bacteria, sensitize them and with the aid of the complement the micro-organisms are broken up and their endotoxins liberated. The latter thus freed further stimulate the tissue cells with the result that other protective bodies (antiendotoxins) are produced in sufficient numbers to prevent any harmful effects and finally to help overcome the infection. Naturally, these very schematic stages are not sharply limited and do not fall within definite periods of time. Sluggishness or absence of the proper reaction on the part of the tissue cells at any stage is followed by a protraction of the disease or even death; death by infection if the bacteria multiply and are not broken up due to insufficient response by the bacteriolysins, or death by intoxication if marked bacteriolyis has occurred, or has occurred so quickly that the liberated endotoxins are not in turn neutralized by sufficient antiendotoxins.

Keeping this explanation in mind, one can readily observe that the injection of ordinary vaccines in typhoid fever aims at nothing more than what the body is already doing with all its power, namely, the production of antibodies for the breaking up of the bacteria, the liberation of their endotoxins and the ultimate manufacture of antiendotoxins. There may be cases in which the body cells are inactive and are stimulated to activity and production of antibodies only after the inoculation of the vaccine. Here the ordinary bacteria are of undoubted aid.

As a general rule, however, it is best to relieve the sick body as much as possible of any active reaction. During an infection the tissue cells are less responsive
than during health, especially if the disease be a severe and prolonged one. That is why an efficient serum (passive immunity) would be the ideal form of specific therapy. Sensitized vaccines possibly hold a position between serum therapy and ordinary bacterin treatment. In the first place, they might save the system from the strain of producing the primary antibodies for the destruction of the bacteria, and second, this provision might hasten the stage of liberation of the endotoxins and the stimulation of the antiendotoxins, an important step in the recovery from the disease.

With these ideas in view, I undertook the study of the effect of sensitized typhoid bacteria as a form of active immunity in the therapy of typhoid fever.

Preparation of Sensitized Vaccine.—Three strains of typhoid bacilli that had been isolated from the blood of patients at the hospital were used for preparing the vaccine. Agar cultures of each strain were grown for twenty hours; the profuse growths were washed off with sterile normal saline solution, about 2.5 c.c. for each agar slant. This mixed suspension was sealed in a sterile tube, which was then submerged in water at 60°C. for one hour. This generally sufficed to kill the bacteria. The sterility was tested in the usual manner. The exact total quantity of the emulsion was noted. The strength of the vaccine was standardized at this point of the preparation, that is, before rather than after the immune serum had been added, for I found it more difficult for purposes of standardization to obtain an even distribution of the bacteria (on the counting slide) after the bacteria had been sensitized (agglutinated). Wright's classic method of standardization was employed.

For sensitization I used the immune serum obtained from the same three (then convalescent) patients from whom the bacteria used for preparing the vaccine were isolated. (Their serums had a strong agglutination and complement fixation titer.) In this way it was certain that the antibodies would certainly fit the bacteria, as they both came from the same source; in other words, complete sensitization was attempted.

The same object can be accomplished by using the serum from an animal (goat or horse) that had been immunized with the strains of bacteria from which also the vaccine is to be made. The serum was inactivated at 56°C. for one-half hour before being added to the bacterial emulsion. Two c.c. of serum for each 1 c.c. of bacterial emulsion were used. The mixture under cover of toilet was left for twenty-four hours at incubator temperature not exceeding 37°C., and was frequently shaken. At the end of this time the agglutinated bacteria were gently stirred and then centrifuged for five minutes. The supernatant fluid was pipetted off, more sterile saline solution added, the emulsion again shaken and again centrifuged. Once more the supernatant fluid was removed and finally the same quantity of saline solution added as was employed before for washing off the bacterial growths from the agar slants. In this way the standardization estimated previously from a sample of unseparated vaccine held good for the sensitized preparation.

The concentrated emulsion after being stirred and shaken thoroughly to make an equal, fine suspension, was finally diluted with one-half per cent. phenol solution so that each cubic centimeter contained 2,500 million sensitized typhoid bacteria.

Dosage.—The dosage of typhoid vaccines for therapeutic purposes is far from definitely established. As a general rule, however, it may be said that bacteria can be given in much larger doses when sensitized than if non-sensitized. The author found that guinea-pigs inoculated intraperitoneally with double the lethal dose of non-sensitized dead typhoid bacilli would die within ten hours, while the same quantity of sensitized bacteria was well tolerated. Similar results were recently obtained by Cecil.18 No fixed therapeutic dose of either non-sensitized or sensitized typhoid vaccine has been adopted. In the present series of cases, 500 millions of sensitized bacteria were employed as the standard dose, both for the first and subsequent inoculations. Naturally, one had to be guided by (a) the severity of the illness, (b) the age of the patient and (c) the reaction from the inoculation. Thus, very sick individuals were given only 250 million bacteria. The dosage in children should be regulated according to the weight, 150 pounds being taken as the weight corresponding to 300 million bacteria.

Frequency of Inoculations.—Usually two to four inoculations were necessary until the temperature became normal; as a rule, one inoculation (250 millions) was administered during the convalescence with the idea of preventing a relapse. The interval between inoculations was five to seven days, or even longer. It must be remembered that with non-sensitized bacteria, the larger the dose of vaccine, the longer must the interval between the inoculations be. This rule does not apply as strictly to sensitized vaccine, nevertheless 300 million bacteria is a large dose, and more frequent repetition was not deemed advisable. Future experience should guide us in this direction.

Reactions from Inoculation.—Both local and systemic reactions were exceedingly slight. Sensitization in general tends to diminish local reactions.19 This has been demonstrated for the tubercle bacillus by Fritz Meyer, who uses a sensitized bacilli emulsion (S. B. E.), and also by Citron for the bacilli of mouse typhoid and swine pest, in which instances marked infiltration following the inoculations was avoided by sensitization of the micro-organisms. Besredka noted only very slight local reactions following the prophylactic inoculation of sensitized typhoid bacilli. In my cases a small, red, infiltrated area about the size of a quarter usually appeared around the points of injection within twenty-four hours after the inoculation and persisted for one to two days. In some cases the local reactions were larger, about 3 inches in diameter, but never were they as severe as is observed after the prophylactic administration of the ordinary vaccine. As for systemic reactions, no definite manifestations of a negative phase, that is, an increase in the severity of clinical symptoms, were evident.

Results.—In a consecutive series, seventeen cases of typhoid fever were treated with the sensitized vaccine as soon as the diagnosis was definitely established. In the majority of instances this was the second week of the disease. The usual other indications in the treatment of typhoid fever, such as sponge baths or tub baths, rectal irrigations, etc., were not neglected. Of this series, only one patient died. The latter had developed a large, deep, spreading abscess of the thigh.

19. It is interesting to note in this connection the recent finding of Jobling that addition of homologous serum to bacteria lessens the occurrence of anaphylaxis, an observation already noted by me in 1910.
and scrotum requiring operation. Four patients had relapses. One patient had several very severe intestinal hemorrhages, the first following about thirty-six hours after the first vaccine inoculation. The subsequent vaccine inoculations, however, were well borne, so that it would be unfair to assume the principle of "post hoc ergo propter hoc." With this possible exception, no manifestations of a negative phase were observed and no other complications noted. Only in two out of the seventeen cases did the temperatures subside acutely, reaching normal forty-eight to seventy-two hours after the vaccine inoculation; in the great majority, the temperature did not go any higher, but began to show remissions, coming down to normal gradually. It would be futile to tabulate the results of the treatment in reference to any other individual symptoms, as such detailed differences should only be based on a very much greater material. On the whole, the impression was gained that the inoculated patients ran what might be termed mild courses with comparatively few complications. I am fully aware how guarded one should be about the results of any form of specific therapy in a disease like typhoid fever. Normally, all types of illness may exist. Clinicians have frequently observed that during some typhoid seasons the patients will present mild infections without any special treatment whatever. One could attribute beneficial effects to vaccine treatment with certainty only if rapid improvement or crisis in the course of the disease would set in soon after the inoculation. Such acute changes can be expected more often by treatment with a specific serum than with a vaccine, for by the former (passive immunization) antibodies are injected ready for neutralizing the poison; while in the latter instance, the antibodies must first be manufactured by the tissue cells. This ever-existing factor in vaccine therapy makes it absolutely necessary to start the treatment in typhoid fever as early in the course of the disease as possible, at a time when the reactive power of the individual is still responsive and unimpaired by the infection.

**SUMMARY**

In summary it may be said:

1. The treatment of typhoid fever by sensitized typhoid bacteria offers a more rational therapy from an immunologic point of view than by the ordinary non-sensitized vaccine.

2. The repeated inoculation of large doses of sensitized vaccine even in very sick patients, was not attended by any harmful effects or a distinct negative phase; the general course of the disease seemed milder and the complications less; the occurrence of a crisis in the infection took place in a small percentage of cases, the improvement usually being gradual. Perhaps more striking results will be attained by a larger number of smaller doses more often repeated.

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20. The patients referred to were treated in 1911, but publication was deferred until a series could be treated by smaller and more frequently repeated doses. This was not, however, possible.

**Geographic Distribution of Diseases.**—In certain diseases, of which the cause or means of spread is unknown, morbidity reports show their geographic distribution and varying prevalence and the conditions under which cases occur. This information has great potential value in attempts to ascertain their causes and means of spread.—John W. Trask, Supplement No. 12, Public Health Reports, April 3, 1914.

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**GUIDES IN VACCINE THERAPY**

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Since the opsonic index variation method proved to be practically unsuited as a guide in the general application of vaccine therapy, most users of vaccines have not attempted to discover individual indications as to dosage, but have established a general routine initial dose, to be increased by a certain amount at a certain interval, and have persistently carried out their program as long as the treatment was adhered to. For this reason one object of the present article is to gather and describe such symptoms and manifestations as there is reason to believe give clue to that which takes place in the body following vaccine administration, and which may reliably guide to individual case variation of both dose and interval.

In general there has been a tendency to overdo and abuse this form of treatment which, in many instances, when properly handled, proves of immense value; and through frequent blundering and total disregard of the principles involved there is great danger that it will be brought into disrepute with the profession at large. To aid in counteracting this I am offering the record of my experience and interpretation of results obtained by other physicians for whom 1 have prepared vaccines, and I feel certain that the facts noted will be found so much in line with observations which all who have used these agents must have made—although, perhaps, they may not have paused to analyze them—that their truth and value will be readily understood, and may help in guiding aright others with less experience in this line.

It has been truly said that it is as wrong as it is useless to attempt to convince through argument, but that one's duty is to offer evidence and let the prejudiced convince themselves. This principle applies very strongly to those who, despite what one would think are insuperable objections, make use of stock vaccines. No one for argument would imagine that an attack of measles, although known to confer great specific immunity, could aid in preventing or in overcoming a typhoid infection; and yet if we employ a stock vaccine, in at least ninety-nine instances out of a hundred we follow a similar procedure. Let any one who has fixed on a variety of stock vaccine for use in a given case look up the possible bacteriology of the disease process which he proposes to treat, and then calculate his chances of striking, even by name, the guilty organism, let alone the particular strain active— which may even be one as yet unrecognized by science—the last consideration a factor which so often confuses exact bacteriologic diagnosis and serum therapy, but which is entirely avoided in vaccine treatment when autogenous products are used.

The fact that a given stock is composed of a number of strains cannot reassure, since at least all but one would be given needlessly and could not but burden, if they did not seriously injure, the patient's reactive mechanism—as in the case of the rabbit dosed with colon bacilli in order to render him susceptible to organisms to which he is normally immune, an effect which could be produced by the use of any stock vaccine on the market. Besides the polyvalence of most vaccines relies solely on difference in source and not on actual known biologic differences.