VACCINE TREATMENT IN THE PREVENTION OF DYSENTERY IN INFANTS.*

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This work was undertaken during the summer of 1910 in order to find out, if possible, whether vaccine treatment would have any effect on either the morbidity or the mortality of the dysentery of infants. The months chosen for this work were July, August, and the early part of September. During these months, by far the greatest number of cases of infantile dysentery occur in this country. The difficulties of controlling such cases in giving the vaccine treatment and in getting the specimens for bacteriological examinations can easily be understood.

All the cases vaccinated were under two years of age and clinically showed no signs of dysentery or of any very acute gastro-intestinal disorders. The vaccine was not administered to any child having a temperature above 38° C. In as many cases as possible specimens of the stools were obtained from the treated cases for bacteriological examination and an equal number of control cases were followed bacteriologically and clinically. In this way it was hoped that some conclusion could be reached which would represent the value of this work carried out under the prevailing conditions.

Historical.—The history of preventive vaccination against dysentery dates back to 1898, when Shiga (1) tried the subcutaneous injection of small amounts of killed cultures of the *B. dysenteriæ* (Shiga). This method produced such marked general and local reactions that he abandoned it in favor of a combination of killed cultures and a specific serum injected simultaneously. From 1898 to 1900 (2) he vaccinated 10,000 Japanese in this way. These first injections were followed three or four days later by a dose of vaccine twice as large, but with no specific serum. His results at this time showed no diminu-

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tion in the morbidity. From 30 to 40 per cent. the mortality diminished to almost nothing. The immunity secured was of very short duration, lasting only three or four weeks.

Lüdke (3), in 1905, published an experimental study on the production of agglutinins and antibodies in rabbits and on the power of the serum so produced to kill the dysentery bacillus. Both Shiga and Lüdke showed that after using the Shiga dysentery bacillus the general and local reactions were very marked and at times quite dangerous. In 1909, Ch. Dopter (4) published a complete study of the preventive vaccination by the dysentery bacillus. In conjunction with Vaillard (5), in 1903, he had shown that adult mice or white rats were the best laboratory animals for experimentation with this organism. He therefore worked with adult mice weighing twenty grams, giving them .00001 of a gram of a killed dried culture of dysentery bacilli. His results showed that with mice such vaccination in 40 to 50 per cent. of cases can confer an immunity against a fatal dose and that such an immunity appears about twelve days after the first injection and lasts from four to six weeks. During the negative phase, i. e. while the animal is acquiring its immunity, it is more susceptible to a fatal dose than the control. The local and general reactions of such vaccines were quite marked. It is of great importance to note that there is a negative phase, for this fact makes it impractical to use this method when there is an epidemic of dysentery, or when the disease is very prevalent, as it is during the summer months. Dopter cites the use of this method in several human cases. For instance, Kruse gave himself a subcutaneous injection of one cubic centimeter of a killed culture. At the site of inoculation there was marked swelling, edema, and pain. After about four days, during which time there was slight abatement of the local reaction, the general symptoms of fever and prostration appeared. Shiga injected into his forearm a small dose of a killed culture. In a few hours his temperature rose to 38.6°, and there was marked swelling, edema, and pain at the site of inoculation. His axillary glands also became swollen. After about three days, during which time the symptoms abated slightly, the swelling and temperature recurred. Rosenthal vaccinated himself and his laboratory boy, inoculating one cubic centimeter of a killed culture subcutaneously. There was a marked local reaction, as well as general constitutional effect, headache, and arthritis. These personal experiences show the discomfort of such a method of vaccination. The same results were obtained by Dopter by using the autolytic products of the dysentery bacillus (Shiga). The immunity did not last longer than six weeks and took from ten to twelve days to appear. The negative phase was also present.

Shiga used the antidysentery serum to produce a passive immunity. The duration of such an immunity is very short, not exceeding ten days. This was shown by Kruse in 1904 when he vaccinated ten people, each with two cubic centimeters of antiserum. Only one of this number took the disease, the first symptoms in this case occurring three days after the injection of serum. This amount, he concluded, was too small. In 1907, Lallemant used ten cubic centimeter doses of antidysenteric serum in treating sixty cases at the insane asylum at Quatre-Mares. None of this number became sick during the epidemic. Rosculet (6) tried this method in quite a large epidemic of dysentery by giving five cubic centimeters of an antidysenteric serum to each of eighteen people.

In none of this number did the disease develop, although all were exposed; while of eighteen other people who were not vaccinated, fourteen contracted the disease. Michiels (7), during an epidemic at Chauvigny, gave an injection of ten cubic centimeters to each of fifteen people. Only one of this number contracted the disease, the first symptoms appearing in this instance nine days after the injection. It would appear from this case, and from Michiels's own personal experience, that the duration of this passive immunity does not exceed ten days.

Mixed Vaccination with Antiserum and Bacterial Vaccine.—In August, 1900, dysentery broke out in a Japanese village. During one month there were twenty-eight cases. Shiga (8) inoculated all the inhabitants over four years of age that were not already infected. The first injection consisted of antiserum and bacterial vaccine in equal amounts, and four or five days later he gave a mixture of eighty parts of bacterial vaccine to twenty of antiserum. All these injections were followed by mild general and local reactions. In this village epidemic, only two cases appeared after these inoculations.

Dopter tried these same experiments on mice. He concludes from this study that vaccination with antiserum and bacterial vaccine gives a speedy immunity which is practically immediate in the great majority of cases. Immunity, however, lasts only about four weeks. This method dispenses with the negative phase and therefore prevents the subject from being more susceptible to an infection with the dysentery bacillus. The local and general reactions from this method are also markedly less than when the bacterial vaccine is used alone. Dopter concurred with Beinarowitsch in believing that the quantity of serum given affects the duration of the immunity in an inverse ratio, the smaller the quantity of antiserum given in combination with the bacterial vaccine, the longer the immunity.

Dopter tried a new plan for producing immunity, using sensitized dysentery bacilli for vaccination. The vaccine was made up as follows: Shiga dysentery bacilli (killed by being heated at 60° C. for an hour and dried in a vacuum) were made into an emulsion with physiological salt solution. To this emulsion was added antidysenteric serum of a very high agglutinating power. This was allowed to stand at room temperature for about twelve hours. By this time the bacilli were strongly agglutinated, had become sensitized, and had fallen to the bottom of the tube. The supernatant fluid which was clear was decanted. The precipitate was washed and centrifugalized twice in physiological salt solution and the last sediment was made into an emulsion with physiological salt solution.

Dopter carried out a long series of experiments with mice, using this sensitized vaccine, and he concludes (1) that mice vaccinated by sensitized bacilli acquire an immunity in about four days; and (2) that there is no negative phase —the vaccinated animal being no more susceptible than the control. The immunity persists at least four and one half months. There are scarcely any local or general reactions from this sensitized vaccine.

These are practically the same conclusions as those arrived at by Besredka (9) who used sensitized cultures in studying the cholera vibrio and the plague bacillus. The only possible objection to this means of producing immunity is that the immunity does not appear for four days, although there is no negative

phase during this period. The sensitized vaccine method just described and the method of administering antiserum in combination with bacterial vaccine, seem to be the two methods of choice.

For this special investigation the latter method was chosen for the following reasons: (1) It had been tried on human cases, apparently with good results, whereas the first method, which, however, seems more likely to produce the best results, had not been tried on human cases. However, an experimental study of this method is being undertaken and we hope to be able to say something further concerning it as a means of preventive vaccination. (2) A previous study (10) had shown that the main cause of infantile dysentery is the organism of Flexner and not that of Shiga, which bacillus has been used in all the previously noted experiments. During this study we found, as have also other investigators, that the Flexner dysentery bacillus is far less toxic to the ordinary laboratory animal than is the Shiga type of *B. dysenteriæ*. This we learned in trying to produce an antiserum of high potency in rabbits. Although we were fairly successful in producing an antiserum to the Flexner type of the *B. dysenteriæ,* it was extremely difficult to do so with the Shiga type. Further, we found, as other investigators have done, that the Flexner organism may be present in the intestinal tract without causing the characteristic clinical symptoms of acute dysentery, thus showing that even in the human system this type of dysentery bacillus can exist without producing marked toxic symptoms. It seemed fair, then, to conclude that a vaccine made from an organism isolated from a case of infantile dysentery (Flexner), would be the most appropriate one to use as a preventive. The following plan was therefore adopted. A standard vaccine was made from a twenty-four hour agar growth of *B. dysenteriæ* (Flexner) of such strength that one cubic centimeter of the emulsion contained one hundred million bacilli. At the first injection, one cubic centimeter of an antidysenteric serum was used in combination with fifty million bacilli, or one half cubic centimeter of the standard emulsion.

The plan was to make three injections, the second to be given five days after the first. This was not always possible, however, from the character of the clinic. The majority of the patients

received only one or two injections and the interval separating the first from the second inoculation varied from five days to three weeks. Fifty-one patients received one injection, and forty-four received two or three. In all, ninety-five patients were vaccinated.

The manifestations following the vaccinations varied from a slight local reaction and a temperature of 99° F., with slight fussiness, to a very marked local swelling, edema, and considerable tenderness. In no case was there any abscess formation, and usually the most marked reactions disappeared within twenty-four hours. All the vaccinations were given in the morning, and each patient was visited from four to six hours later, when the local condition and temperature were noted. The highest temperature noted was 103° When the temperature was over 101° the patient was always visited the following morning, and in no instance was the temperature above 99° on this second visit.

From thirty-three of these vaccinated patients, one to four fecal cultures were taken by the method used by Kendall (11).

In this is employed a small glass tube with rounded ends, one of which is plugged with cotton. The tube is placed within an ordinary thick-walled culture tube and the whole is sterilized. The cultures were obtained by passing the sterile tubes into the rectum, and at the end of the clinic these tubes were expressed to the laboratory where the contents of the tube were discharged into a tube of plain broth. From this emulsion, the isolation of the *B. dysenteriæ* was carried out by a modification of the method used by Kendall and Walker. Using varying amounts of the emulsion according to the character of the stool and the strength of the emulsion, two large plates were made on Endo's medium and incubated for eighteen hours. On these plates *B. dysenteriæ* appear as slightly elevated, clear, colorless colonies measuring in diameter from 5 to 1.5 millimeters, according to the total number of colonies on the plate.

All suspicious colonies were fished into litmus mannite semisolid media and incubated for eighteen to twenty-four hours. If at this time there was growth characteristic of the Flexner or Shiga type of *B. dysenteriæ,* a tube of plain broth (reaction —.5) and a tube of litmus milk were inoculated with the culture and incubated for a day. If at the end of this time the litmus milk showed the characteristic lilac color, microscopic agglutination of the broth culture at a dilution of 1 to 200 was tried.

Later in these investigations, in order to make a diagnosis more quickly, since a large number of specimens were being studied, suspicious colonies from the plates were fished directly into plain broth (made from meat extract, reaction —.5). This was incubated for about eighteen hours and then agglutinating horse serum was added to the tube. A drop of Flexner serum was used first, and then, if in three or four hours there was no agglutination, a drop of Shiga

antidysenteric serum was added. If, after either, agglutination was positive, the supernatant broth was drawn off and sterile bouillon was added to the tube. The tube was shaken, a loopful transferred to another tube of broth, and plates made from this. After incubation, the colonies were fished and carried through mannite semisolid litmus milk and the agglutination test made a second time. The strains were finally transferred to agar slants for preservation. By this method a tentative diagnosis was reached in about thirty-six hours. It was found that a positive microscopic agglutination could be allowed to stand for several hours and the agglutinated organisms would grow when transferred to fresh broth.

Of the thirty-six cases studied culturally, six proved already to have the dysentery organism. Five of them had the Flexner type of the dysentery organism, and one the Shiga.

Two of these patients received three vaccinations, as follows: the first vaccination consisted of one cubic centimeter of antiserum and one half cubic centimeter of the standard emulsion (fifty million bacilli); on the second inoculation, they received one half cubic centimeter of the standard emulsion and no antiserum; and on the third inoculation, one cubic centimeter of the standard emulsion. Three of the patients received only two injections, the first with antiserum and the second without. One patient received only one injection of antiserum and vaccine.

The immediate reaction in all these cases was very mild, the highest temperature being 101.5°. All the local reactions were of the mildest type and there was no general reaction noted beyond slight fussiness. In no case was the immediate intestinal condition aggravated, and in all but one case no further intestinal trouble has appeared up to the present.

One patient receiving two doses of vaccine, the last being given on August 7, did well until an acute intestinal infection developed on September 8. This child was entered in one of the hospitals as having an acute infectious diarrhea. This attack was only of moderate severity, and the child when seen last, October 24, was in the best condition.

In none of these six patients was the dysentery organism suspected before the vaccine was given, as it was the intention at first to avoid giving vaccine treatment to any patient having dysentery infection.

The results, however, in these six cases show very clearly that vaccine made from the *B. dysenteriæ* (Flexner) is not contra-

indicated in those cases which may have this organism in a clinically unrecognizable form.

Out of the ninety-five patients vaccinated, there were two deaths. In the two cases that ended fatally, the original cultures were negative for the *B. dysenteriæ*. Each patient received two vaccinations. One child contracted acute infectious diarrhea seventeen days after its last inoculation and died ten days later. The outcome is interesting from the standpoint of hygiene. The family lived under conditions of the worst kind, and with flies in superabundance. Undoubtedly the features which, more than any others, contributed to the fatal result in this case, were the utter lack of intelligence on the part of the family and their indifference to the sickness of the child. With these difficulties it was impossible to cope.

The second case was that of a very delicate child who lived under the worst sanitary conditions of filthy people. Here also the flies were numerous. This child did well for one month after its last inoculation, when it contracted acute infectious diarrhea and died two weeks later.

The other ninety-three cases did perfectly well throughout the whole summer and when last seen, sometime in the latter part of October, were in the best condition.

It is interesting to compare these cases with ninety-seven which did not receive any vaccination and which proved culturally to be negative as far as the presence of the *B. dysenteriæ* was concerned. Of this number, three died, apparently of acute infectious diarrhea, after illnesses of from three to five days. No bacteriological examinations were made on these cases and autopsies could not be obtained.

In twenty-five cases studied culturally but not vaccinated, *B. dysenteriæ* were found. In twenty-four of these the Flexner type of the organism was present, and in only one, that of Shiga. There has been one death from this group of twenty-five cases. In only one or two of these cases was the presence of the *B. dysenteriæ* suspected at the time the culture was taken, and in the case that died, the final acute attack was very short and severe. This is of interest when compared with the subacute form of attack in the three cases that developed acute infectious diarrhea later in the summer.

Agglutination.—The agglutination test was applied in thirty-six of the ninety-five cases vaccinated. Agglutination was negative at 1 to 50 in some, and positive at 1 to 400 in several of the vaccinated cases. No difference was noted in the agglutinating power of the six cases in which the dysentery bacillus was isolated. A few control agglutinations were obtained from some of the cases in which the dysentery bacillus had been isolated by culture, but which were not given the vaccine. One of these cases agglutinated very strongly up to 1 to 100, another only mildly at 1 to 100, and a third only mildly at 1 to 50. Several control cases which were culturally negative gave agglutination reactions. One patient who was not vaccinated, from whom the organisms were not cultivated, and who clinically was not suffering from infectious diarrhea, gave a strong agglutination at 1 to 100.

The home conditions of all the vaccinated patients were studied and recorded. There can be no question that whatever the treatment outlined at a clinic may be, or whatever preventive measures are instituted, these are of little or no avail in the unsanitary conditions under which a fair majority of these children were compelled to exist.

The question of the presence of flies appeared to be a most important one, and toward the end of the summer an attempt was made to discover how far the fly entered into the carrying of the dysentery organism. To this end sterilized fly-traps were set in the homes of several patients from whom the dysentery bacilli had been recovered. In two out of three such attempts, the dysentery organism was recovered from an emulsion made from these flies. The manner of procedure was as follows:—

An ordinary wire fly-trap, sterilized by heat, was left at the house for two or three days. The traps were brought to the laboratory and were set in a cold room (4° C.) for fifteen minutes. The cold numbed the flies to such an extent that they could be picked out of the cage with sterile forceps and placed in broth. The drowned flies were ground in a sterile mortar and from the emulsion plates were made, some of them being on Endo's medium. Any suspicious colonies were run through the ordinary media for isolating the dysentery organism, and finally treated for agglutination. In the two cases in which the Flexner organism had been isolated, this

same organism was also recovered from the flies. It is interesting to note that the *B. dysenteriæ* are carried by the ordinary house-fly and that these flies may be one of the causes of spreading this infection during the months when they are so abundant.

CONCLUSIONS.

This investigation is very limited and the number of patients treated is few, yet the following conclusions may not be amiss:—

1. The vaccine in no instance did harm, and the reactions in the majority were very mild and of short duration.

2. Cases in which bacteriologically *B. dysenteriæ* were proved to be present, but in a clinically unrecognizable form, were not affected differently from those which were culturally negative. This may in part be due to the fact that this mode of vaccination produces an immediate passive immunity, while the bacterial vaccine is producing an active immunity.

3. The work is of interest and holds out a possible means of preventing the great mortality from infantile dysentery during the summer months.

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