Canadian Journal of Public Health
Vol. 40, Nov. 1949, pp. 473-478.

Progress in the Treatment of Typhoid Fever with Vi Bacteriophages

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IN two previous preliminary reports (1, 2) we described the results obtained from the treatment of 20 typhoid fever cases with Vi antityphoid bacteriophages I, II, III, IV of Craigie and Yen (3). These two reports indicated the modification which we effected in the original method of Knouf, Ward *et al.* (4), who treated 56 typhoid cases employing only specific Type II bacteriophage. We used a mixture of I, II, III and IV phages. The polyvalent phage was diluted with 500 ml. of 5 per cent glucose solution and administered by intravenous injection, with the drip method, in accordance with the technique prescribed by the above-mentioned authors. The polyvalent phage possesses the advantage of serving for the treatment of all typhoid cases, even of those in which the inciting strain of *Salmonella typhosa* is resistant to phage II. The favourable results obtained from these first 20 cases (no deaths) encouraged us to continue these experiments.

We have, however, further modified the polyphage and altered the method of administration. To the four Vi phages of Craigie and Yen, we added two new Vi phages which we isolated in the Province of Quebec and which we have designated as Type V and VI phages. These two phages, producing very small plaques, possess, however, a remarkable power of diffusion (attack), resulting in plaques of 5 mm. *in vitro* with homologous cultures. They are not neutralized by antisera I, II, III and IV and their antisera do not neutralize phages I, II, III and IV.

The drip method of injection requires a rather complicated equipment and is not readily adapted to treatment in the home. Upon modifying somewhat the concentration of the polyphage and on eliminating all bacteria not lysed in the course of the preparation of the phages, direct injection in the vein was tried, employing 3 or 4 injections at intervals of two days. By this direct method, 36 typhoid cases were treated in the Province of Quebec in 1948. Some twenty cases have also been treated with this polyphage outside the Province, but we have had no bacteriologic control of these outside cases.

From reports received from the physicians interested, it appears that, clinically, the results obtained with this direct treatment are similar to those secured with the drip method, as reported by Knouf, Ward and their collabo-

Presented before the Epidemiology Section at the thirty-seventh annual meeting of the Canadian Public Health Association, held in the Nova Scotian Hotel, Halifax, June 27-30, 1949.

rators and also by ourselves. A few hours after the injection the patient's temperature rises 3° to 4°F. and remains at this level for 3 to 5 hours. The majority of the patients experience, a few hours following the injection, a chill lasting 20 to 30 minutes. The temperature then falls rapidly to 94° to 97°F. After the subsequent injection the temperature rise and fall are less marked. Following the 3 or 4 injections the temperature is stabilized between 97° and 98°F. When the treatment is given early in the disease, during the septicemic stage, blood culture rapidly becomes negative and no positive faeces are obtained. Treated at this stage, some patients are completely cured within ten days. If treatment is begun when the faeces are already positive, the bacteriologic results vary considerably: the stools of some patients will be negative a week or two after treatment, whereas those of other patients may remain positive for several weeks and some patients may become germ carriers. Nevertheless, the efficacy of the treatment, as regards disappearance of symptoms and temperature, is similar in all cases.

Of the 36 patients treated in 1948, 21 were completely freed from typhoid organisms as determined by finding two samples of faeces, collected at an interval of two weeks, to be negative. Two patients remained carriers; one of these was later treated with aureomycin with no effect. The status of the other thirteen persons is not definitive because the required release specimens have not yet been submitted for examination. One patient died following meningeal complications. The mortality rate for the group is therefore 2.7 per cent, whereas that for the 56 patients treated in both 1947 and 1948 is 1.8 per cent. Knouf, Ward *et al.* reported a mortality rate of 5 per cent for the same number of cases.

In January, 1949, we prepared a new lot of polyvalent phage, the concentration of which varied in different ampuls from 10^{25} to 10^{30} particles per ml. The phages were propagated on sensitive cultures in a definite medium containing no meat infusion. The distribution in ampuls, the sterility tests and the tests on animals were done at the Institute of Microbiology of the University of Montreal. The final titration of each phage in the mixture was done at the Division of Laboratories of the Quebec Ministry of Health. Up to June 1, more than 550 ampuls have been gratuitously distributed, many to various foreign hospitals and laboratories.

Early in February two small outbreaks of typhoid fever occurred in the Province, with two different types of *S. typhosa* involved, Types C and E_4. The 13 cases from these outbreaks, aged from 16 months to 17 years, were hospitalized in the same ward at Ste. Justine Hospital, Montreal. All were treated with polyphage. Thanks to the collaboration of the physicians and authorities of this hospital, a number of experiments were made, the results of which may allay, in some measure, apprehensions such as those expressed, in a personal communication from Doctor James Craigie, of London, as follows:

"Your news about the treatment of typhoid cases with polyvalent Vi phage is most interesting and I look forward to hearing of the results of later developments in preparing and administering the phage. One thing, however, worries me about clinical trials. That is the risk of occasional untoward results—collapse or death—which might discourage more

extensive trials. I have in mind the danger of increasing the load of Vi and O endotoxins temporarily beyond the amount the patient can stand. A certain additional amount will be introduced with the phage preparation. If the phage produces prompt and massive lysis *in vivo*, further amounts of Vi and O endotoxins will be liberated. I think, therefore, that a detailed study of Vi and O antibody levels in the patients' blood, before, during and after phage administration would be helpful. The antibody titres might, with experience, guide decision as to whether the phage be given rapidly or by continuous intravenous drip.

"After the commencement of phage treatment, blood samples should be taken at 6 to 8 hours' intervals for the first two days to see if the changes in Vi and O antibody level are rapid or significant. Agglutination tests, provided that reference Vi and O sera are used in each set, will serve unless the results are of such a nature as to warrant mouse protection tests.

"The blood might also be titrated for phage and tested for phage antibodies. Phage antibodies develop rapidly in rabbits. If humans are as responsive, the continuation of phage injection beyond a week is unlikely to be of value since the injected phage will be promptly neutralized."

Twelve of the thirteen patients were tested before and after polyphage treatment as suggested by Doctor Craigie, except the very young from whom it was impossible to collect the required number of blood samples. One child died from pneumonia and pancarditis, some days after admission to the hospital. The following examinations were made:

Before every polyphage treatment, blood for sero-diagnosis and culture, and faeces were collected; the blood and faeces were examined for presence of *S. typhosa;* the type of *S. typhosa* was determined; the titres of Vi, O and H antibodies in the serum of the patient were determined; the power of the patient's serum to neutralize Vi phages was tested.

After injection of the polyphage, the titres of Vi, O and H antibodies in the patients' serum were determined at regular intervals; the power of the serum to neutralize Vi phages was tested; bacteriophages were sought in the blood and in the faeces; blood, faeces and urine were examined for the presense of *S. typhosa;* tests were made to determine whether the bacteria isolated were of the V or W form.

The results of these examinations may be summarized as follows:

Two of the twelve patients showed the presence of Vi antibodies in their sera before treatment.

The dilution titres of O and H agglutinins in the sera before administration of the polyphage varied between 1:500 and 1:5000.

No patient's serum neutralized the six Vi phages before treatment.

In every case, *S. typhosa* was isolated either from the blood or from the faeces. All cultures were of the V form and typable.

For administration of the polyphage, the physicians of the hospital, who had already treated some twelve patients previously, tried successfully a new procedure. At 9:00 a.m. 1 ml. of the polyphage was injected directly into the vein; this was followed by an injection of 250 ml. of 5 per cent glucose for hydration of the patient. The rise in the patient's temperature was noted every 15 minutes. If within 3 to 4 hours after the injection the temperature had not reached 106°-107°F., another 1 ml. of the polyphage was injected. On the 3 or 4 following days the same treatment was repeated. The physicians found no

unfavourable indication following this massive treatment. The typhoid aspect disappeared and the temperature dropped within a few days after the treatment.

We noted no increase in the Vi agglutinin titre in the serum of any of the patients, even in those which, before treatment, showed the presence of such agglutinins; the titre of the O and H agglutinins, however, increased rapidly within a few days to reach levels as high as 1:25,000.

Neutralizing antibodies appeared in the first week after polyphage injection. Phage I, II and III wer neutralized first, to be followed later by phages IV, V and VI.

The patients were freed from *S. typhosa* more or less rapidly depending upon whether the treatment was administered early or late in the course of the disease. None of the twelve patients became carriers.

Of the cultures isolated after the treatment, 99 per cent were of the V form and of the same type as before treatment.

Bacteriophages were found in the faeces a few hours after the treatment. We did not find them, however, in the blood; perhaps some question of the technique employed may be involved here.

In our previous reports we noted an early appearance of Vi antibodies in the sera of treated patients; to our surprise, we found no production of such antibodies in sera of the patients treated with the new polyphage. Naturally, we desired to know the reason for this difference.

The sera neutralizing phages I, II, III, IV, V and VI are prepared by injecting into rabbits the corresponding concentrated phages, each of which, in turn, is prepared by propagation on sensitive cultures. These cultures, being of the V form, must contain Vi antigen in order that propagation may take place, but they nearly always contain a certain proportion of bacteria of the W form—O and H. By agglutination tests of these sera with Vi, O and H antigens, we have found that, contrary to expectations, the six neutralizing sera agglutinate not at all the Vi antigen, but do agglutinate O and H antigens to titres from 1:10,000 to 1:15,000.

We repeated the experiments with rabbits, but injected phage II propagated exclusively on the strain Bahtnagar Vi I which is characterized by absence of O and H antigens (5). The phage was propagated on this V-form strain, after it was purified several times by isolation from a single plaque and by taking care that lysis was complete and that non-lysed bacteria were eliminated by centrifugation. A rabbit was injected with this Vi bacteriophage and the results observed follow:

March 8. Blood sample: Ty.O: — Ty.H: — Ty.Vi: —
Neutralization: O

March 8. 1.5 ml concentrated phage injected.

March 9. Blood sample: Ty.O: — Ty.H: — Ty.Vi: —
Neutralization: O

March 10. 1.5 ml phage injected.

March 11. Blood sample: Ty.O: + 1/50, Ty.H: + 1/50, Ty.Vi: —
Neutralization: O

March 14. 1.5 ml phage injected.

March 16. Blood sample: Ty.O: + 1/937, Ty.H: + 1/1750, Ty.Vi: —
Neutralization: 50% in dilution 1/10
25% in dilution 1/20
5% in dilution 1/40

March 22. 2.0 ml phage injected.

March 25. Blood sample: Ty.O: + 1/5625, Ty.H: + 1/7500, Ty.Vi: —
Neutralization: 99.99% in dilution 1/320
99. 9% in dilution 1/640
50.% in dilution 1/1280

These results indicate that the bacteriophage Vi does not produce Vi antibody but rather O and H antibodies. Using the same strain Vi I of *S. typhosa*, and killing it with formol, we find that if this is injected into the same animal, Vi antibody is produced in a few weeks without any increase in the O and H antibodies. Thus, the entire bacteria produce Vi antibody, whereas the same bacteria, disintegrated by Vi bacteriophage, produce only O and H antibodies, even when the strain is not supposed to contain the corresponding antigens. We have verified the suspicion that our first polyphages contained unlysed, dead bacteria of the V form, and it was for this reason, apparently, that the sera of our earlier patients showed the presence of Vi antibody.

The results of these experiments suggest the manner in which the bacteriophage acts in the phage treatment of typhoid fever and also suggests the reason for its failure to free carriers from harboured bacteria. Apparently, it is the antibodies or protecting endotoxins, rapidly assimilated, which cause the disappearance of the symptoms of the disease; the bacteriophage itself, in contact with the bacteria in the body of the patient, liberates new protecting endotoxins by destruction of the bacteria. This lysis thus appears to be the indirect cause of the cure as regards symptoms. In carriers this phenomenon does not occur because they are already immunized; and the bacteriophages cannot reach all the bacteria because some of the latter are distributed in difficulty accessible locations where few or no phage particles can reach them.

These experiments explain also another puzzling phenomenon: the absence of Vi antibody in the sera of the great majority of the patients and the presence always therein of O and H antibodies. However, it is almost always in the V form that bacteria are isolated from these patients and these V forms are not agglutinable by experimental O and H sera. The bacteria destroyed, whatever be the mechanism employed, produce O and H antibodies; whereas those bacteria not destroyed, as found in convalescents who have long harboured them and in carriers, apparently produce only Vi antibody with more or less O and H antibodies.

Summary

New methods of preparation of polyphages and their administration for treatment of typhoid fever are described. The results of a detailed study of the response, as regards appearance of various antibodies, bacteriophages and bacteria in the sera or faeces, of a group of 12 patients, before and after phage treatment, are reported. A theory of the action of bacteriophages on the bacteria in treated patients and in carriers is offered.

ACKNOWLEDGMENTS

Our thanks are due to the physicians and the hospitals who have kindly tested the polyphage treatment; to Mr. M. H. McCrady, Chief of Laboratories, for his continued encouragement and his aid in the preparation of this report; to Dr. M. Saint-Martin, Bacteriologist, and to Dr. R. Cyr, Associate Bacteriologist, for their collaboration in the experimental work; to Dr. J. Craigie for his advice; and to Miss J. Martin for her valuable assistance in the technical work required in this study.

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