THE BACTERIOPHAGE

by

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In the last few years attention has been focused on the works of d'Herelle, Twort, Bordet, Gratia and others on the so-called "Bacteriophage," a phenomenon which consists of the breaking down and solution of bacteria by a filtrable agent which may be associated with pure cultures.

This bacteriolytic action was early spoken of by Hanken (1) while working with the waters of India. However, the first real investigation of it was made in 1915 by Twort at the Brown Institute. Twort, (2) after experimenting with varied materials obtained results with vaccinia. He innoculated agar slants with the vaccinia before the glycerine had completely sterilized it and found that the micrococci showed at times a translucent or transparent change which started as clear spots at the margins. He found that some of these colonies could not be sub-cultured, also that if a colony of the white micrococcus that had started to become transparent was plated out, then the micrococcus grew and a pure streak culture from certain of these colonies could be obtained. But if plate cultures, (made by innoculating the water of condensation of a series of tubes and floating this over the surface of the medium) were left, the colonies turned transparent, this action starting from the edge. Other experiments

showed that if a normal colony of the micrococcus were touched with some of this glassy colony, the normal colony would become "watery." It was found that this trans - parent material was still active in high dilutions and all attempts to sub-culture the filtrate proved negative for it would not grow by itself on any medium. By further experiment, Twort found that this filter passing material

1. Resisted heat to 60 for one hour.
2. It could be separated from the organism from which it was derived by filtration. (Berkfeld).
3. It had no action on dead organisms and was most active against young actively growing organisms.
4. It increased in quantity when allowed to act on a culture.
5. It acted to a less degree on closely related organisms and did not act upon unrelated forms such as the colon-typhoid group.
6. It could be transmitted indefinitely from one culture to another.

Twort also obtained similar results with a micrococcus, and a member of the colon-typhoid group of bacilli obtained from the mucous membrane of a dog suffering from distemper; also with a bacillus from the intestinal tract.

He claims in the case of vaccinia, the transparent material contains an enzyme. If it is part

of the micrococcus it may be either a stage in its life history which will not grow on artificial media but stimulates fresh cultures of the micrococcus to pass into the same stage, or an enzyme secreted by the micrococcus which leads to its own destruction and the production of more enzyme. He does not however, insist that his experiments have definitely disproved the possibility of its being an ultra microscopic virus.

Bordet and Ciuca (3), who have also done a considerable amount of work on the bacteriophage, entirely disagree with d'Herelle in thinking that lysis is due to a living filterable virus. They believe that the microbes, when exposed to external influences, such as a leucocytic exudate, undergo a modification by which they are then capable of producing an autolytic principle. This property is transmitted to the following generations by the germs which were sufficiently resistant and thus could multiply.

Bordet was the first to declare that a lytic principle could be obtained without starting from a stool filtrate but could be isolated by filtering an exudate. This method however is not considered satisfactory by the majority of those who have tried its duplication. He and his co-workers were also the first to produce an antilytic serum by innoculating rabbits with

increasing amounts of dissolved culture filtrate. This serum was able to neutralize completely the action of their bacteriophage.

Since then Kuttner (4) has obtained this lytic principle from normal tissue extracts of guinea pigs and from normal rabbit sera. She does not agree with d'Herelle but favors the idea that the bacteriophage is a secretion of the bacteria of the nature of an autolysin. This autolysin usually liberated in old bacterial cultures, as a result of cell disintegration acts as a catalyst which destroys the equilibrium occurring in actively growing cells between the constructive and destructive forces in favor of the latter. Solution of the bacterial cell consequently results and thus more of the autolysin is liberated.

D'Herelle's work has undoubtedly aroused the greatest interest in the Bacteriophage. He has recently published a book on this subject, the result of four years of careful study. He made daily examinations of the stools of a patient suffering from a severe dysentery (Shiga). He innoculated broth with feces, incubated it overnight, filtered through a Berkfeld filter and added about twelve drops of the filtrate to young active broth cultures of Shiga bacillus, using one tube innoculated with Shiga only as a control. During the heighth of the disease such cultures yielded normal

growths. However, when the patient presented symptoms of improvement, the bouillon innoculated with culture and filtrate appeared sterile. When he added a drop of this dissolved culture to another young broth culture it also was dissolved. After repeating this process several times it was found that the broth cultures were dissolved even more quickly than at first. From this he concluded that the Bacteriophage was increasing in potency and that it was capable of cultivation in series.

D'Herelle also examined the above cultures after incubation periods of one, two and three hours by innoculating the material on to agar slants. If examined immediately after planting on agar, the agar was covered by a normal film of Shiga Bacilli, but with two areas about 2 mm. in diameter entirely free of growth. In the one hour tube six of these clear areas were found. In the two hour tube there were about one hundred and in four hours no growth of Shiga bacillus could be seen.

After many experiments of this type he came to the conclusion that this dissolving principle may be present in the intestinal contents of all living beings. It is usually parasitic for Bact. coli but in the course of intestinal disease it becomes a parasite on the invading organism by the process of adaptation. D'Herelle

holds steadfastly to the idea that he is dealing with an ultramicroscopic filtrable virus which is parasitic on bacteria and as proof he states the following facts:

I. "The dissolution of bacteria by the Bacteriophagic principle takes place in series." He found this to be true after more than 1000 passages. If it were an enzyme action it would soon cease to show because of the greater and greater dilution of the enzyme solution in the course of the successive passages.

II. "The lytic enzymes come from material corpuscles which will pass through filters; these corpuscles multiply in the course of the bacteriolysis."

III. "All bacteriophagic ultramicroscopic corpuscles grown at the expense of any bacterial species constitute one and the same antigen." By experiment he found that the serum of an animal prepared by a culture of any bacterial species dissolved by bacteriophage contains an amboceptor which fixes itself on any other bacteriophaged culture. Hence the amboceptor is specially anti-bacteriophagic and not anti-bacterial."

IV. "The lytic activity of the corpuscles varies." This variation in activity is due to a difference in the multiplication of the corpuscles innoculated. To explain this, the active particle must be a microbe, as a microbe acts by its virulence and toxicity while an enzyme acts only by its quantity.

V. "The virulence of a lytic agent may be increased by successive passages."

VI. "Bacteria when attacked by bacteriophage defend themselves and are capable under certain condition of acquiring an immunity against the parasite. This could happen only if the bacteriophage were parasites of a living organism."

VII. "The resistance of bacteriophage to the action of phyeical and chemical reagents is that of a living being and not that of an enzyme. Bacteriophage is killed by eight days contact with glycerin, this being the liquid used to preserve indefinitely an enzyme in soluble form."

VIII. "It is possible to extract the lytic enzymes free from the living bacteriophage micro-organisms."

IX. "Bacteriophage is capable of adaptation."

X. "The properties of bacteriophage are variable, this being also a characteristic of living beings.

Although d'Herelle does not believe that the lytic material isolated by himself and the transparent material of Twort to be the same, most of the workers now consider that the same phenomena are involved in both cases.

Up to this time there has been comparatively little work done on the stools of typhoid carriers. Therefore it was decided to study in what proportion

of carrier cases a bacteriophage is found. It was thought possible that the isolation of an anti-typhoid bacteriophage from a suspected carrier might serve as another means for their positive identification.

A stool was obtained from an individual suspected of being a typhoid-carrier on account of the presence of a positive Widal test. This man had typhoid fever four or five years previously and two years ago had given a negative Widal. The present positive result was confirmed on three examinations at weekly intervals.

B. coli but no B. typhosus was isolated from the stool. About 5 gms. of the feces was carefully suspended in 50 cc. of bouillon and incubated at 37 for fourteen hours. It was then filtered through an ordinary filter and paper mash and finally through a Mandler filter previously sterilized. The day before the test was made agar slants were innoculated with B. dysenteriae Shiga, B. dip. Flexner, B. typhosus, B. Para typhosus A 13 para typhosus B. and the homologous strain of B. coli. From all of these young cultures seven groups of four tubes of peptone broth each were innoculated. To the first tube was added one drop of the filtrate, to the second ten drops and to the third two cubic centimeters. One tube was innoculated with the organism to serve as a control. All were incubated at 37 C. After twelve,

eighteen and twenty-four hours they were examined. All tubes showed a normal growth. In order to be sure that there was no bacteriophage present, active against these organisms, a platinum loop full of each tube was spread over the surface of slanted agar. After incubation these tubes presented a normal growth and no lytic colonies occurred. It would appear from this isolated trial that in this "carrier's" stool at the time of examination, there was no bacteriophage active for the stock strains of B. Shiga, B. Flexner, B. typhosus, B. Para typhosus A., B. Para typhosus B. or B. Coli.

Other experiments with a normal stool were carried out in the same manner as the previous attempt. The results however, were in this case also negative. The number of typhoid carrier cases in routine examinations has been disappointingly limited to the single case quoted above. This work is to be continued on future cases as they appear, and results appended later.

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