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DR. DOUGLAS SYMMERS, *President*

THE BACTERIOPHAGE REACTION OF D'HÉRELLE

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The interesting studies published during the past three years by d'Hérelle,[1] Kabeshima,[2] Salimbeni,[3] Bordet and Ciuca,[4] Gratia,[5] and Maisin[6] have drawn our attention to an agent that seems to have an important function in the destruction of the intestinal bacteria and in the recovery from the diseases included in the typhoid-dysentery group. Such a bacteriophagic agent was originally described by Twort[7] in the *Lancet*, 1915, in connection with observations on *staphylococcus* cultures derived from glycerinated vaccine virus, and on *coli* cultures from the intestinal tract of dogs suffering from distemper. Similar observations were also made by him on a large bacillus obtained from the intestinal tract of children suffering from diarrhœa.

The agent is described by d'Hérelle as a filterable virus which grows upon and destroys the pathogenic bacteria with which it is associated. It is found toward convalescence in the intestinal diseases, is not absolutely specific, and can be cultured indefinitely in series by transplanting a fraction of a loopful of the lytic fluid into fresh suspensions of the bacteria in bouillon.

These observations were first carried out by d'Hérelle in connection with the Shiga dysentery and then extended to the other types of dysentery, to typhoid, paratyphoid, and fowl typhoid.

Kabeshima does not consider this agent as a living virus, but as a ferment derived originally through the action of a leucocytic catalyzer upon the bacteria, causing the liberation of a ferment which can continue to act indefinitely in series of fresh suspensions of the organism.

Salimbeni's observations indicate that we are dealing with a myxameba-like organism which has two stages—a filterable spore stage and a vegetative fungus stage. These observations are probably erroneous. The appearances, which he describes, are probably artefacts developing during different stages of lysis of the bacteria.

More important, however, in explaining the nature of this agent is the work of Bordet and Ciuca, who consider that this lytic power is an hereditary lytic property produced by the action of leucocytic ferments upon bacteria, some of which subsequently acquire a property of being lytic for the original strain from which they were derived. Bordet and Ciuca obtained the lytic agent by the injection of a *B. coli* strain into the peritoneal cavity of guinea pigs. The action of this lytic peritoneal exudate upon the original strain showed up colonies of the *B. coli* which were resistant to lysis and very mucoid and translucent in appearance. The bacteria of this "modified" strain of *B. coli* were actively motile, resistant to the action of the lytic agent, and much more pathogenic for guinea pigs than the original strain. These "modified" bacteria have acquired the property of producing inhibition of growth and lysis of the original strain. This property is preserved through later generations and represents a new biological function of the bacteria.

One of Bordet's co-workers, Gratia, has shown more recently that in an old and evidently dead culture of the Bordet strain of *B. coli* small colonies were found that were markedly resistant to ageing. Upon transplantation these colonies proved to have the same resistant properties to the lytic agent as those of the resistant strain obtained directly by the action of the lytic fluid upon the original strain of *B. coli*. These studies, according to Gratia, seem to have an important bearing upon the questions of virulence, the heredity of acquired characteristics, and the formation of new races.

Through the kindness of Dr. Hardé, who brought specimens of lytic fluid from d'Hérelle to this country and placed them at my disposal, I was able to make some studies on the nature of this agent. The pressure of other work has prevented me from continuing these most interesting studies, and I can only submit the following limited observations:

One specimen marked "Bateriophage anti-Shiga," dated May 19, 1919, produced inhibition of growth in a fresh suspension of the Shiga bacillus and caused lysis of bouillon cultures grown

24 to 48 hours. Subcultures were sterile. A trace of this new lytic fluid showed the same property toward fresh bouillon suspensions, which in turn exerted the same powerful lytic action, which could be continued on indefinitely in series.

By great dilution the action of this lytic agent was diminished. In place of the complete inhibition of growth on the agar subcultures which were made immediately after the addition of the diluted lytic fluid to a fresh suspension of *B. Shiga*, a surface growth developed which showed here and there circular depressions with no growth surrounded by a halo leading off into the growth. These circular depressions were considered by d'Hérelle as representing colonies of the bacteriophage, by Gratia as the evidence of the lytic action of products of the resistant bacteria upon the non-resistant bacteria.

By making agar plates of the Shiga bacillus and streaking the surface crosswise with a loopful of the lytic fluid I could observe the following day a complete area of clearing along the path of the streaked lytic fluid which was surrounded by a regular surface culture of the Shiga bacillus. Studying the margin of the clear area, I noticed that the culture of the Shiga bacillus had at this point a shelving edge where it was quite translucent, and which upon microscopical examination showed the presence of most interesting structures. There was first a fine granular background, representing probably detritus derived from the bacteria; second, long, slender, refractive crystals, singly or in small groups of two and three; third, numerous irregular structures, not motile, pentagonal or hexagonal in shape, or of an irregular round form, which stood out clearly against the granular background. These bodies resemble closely in size and appearance crenated red blood cells. They could be floated in a hanging drop and seemed to have a somewhat spherical shape. They could be stained with Giemsa and less clearly with Gram's stain in ordinary preparations and in preparations made by impression from the agar plate. In the stained preparations the structures appeared as amorphous non-nucleated masses. It is probable that these structures represent *hyaline masses of protoplasm* resulting

from the action of the lytic agent upon the bacteria. Here and there along the margin of the streak small circular indentations could be seen in the culture proper which corresponded closely in appearance to the colonies of bacteriophage described by d'Hérelle. The microscopical examination of the margins of the circular areas showed numerous structures similar to those described above—granular detritus, refractive, slender crystals, and irregular protoplasmic hyaline bodies. The more central part of the clear streaked area showed in places upon microscopical examination similar structures.

After two to three days there appeared in the clear area small colonies which upon transplantation grew with difficulty. On the agar plates the subcultures of these resistant colonies did not show the above-described structures which indicate the action of the lytic agent upon non-resistant bacteria.

The specificity of the lytic fluid was also studied. It was found to completely inhibit a second strain and only partly inhibit a third strain of *B. Shiga*, and to produce complete inhibition of a Flexner-Harris and a Mt. Desert strain of dysentery bacilli. It had no action upon a typhoid strain (Pfeiffer), *B. coli*, *B. sanguinarium*, and paratyphoid A and B.

Microscopical studies of hanging drops made according to the method described by Salimbeni gave no evidence that we are dealing with a myxameba. The irregular hyaline structures described previously showed no motility. The lytic agent was found to produce very active and almost instantaneous agglutination of bacteria.

The lytic agent resists the temperature of 70° C. for one half hour, but is partly destroyed by a temperature of 75° C. for one half hour.

Studies were also made with the anti-typhoid bacteriophage sent over by d'Hérelle. On the agar plates inoculated with the *B. typhosus* (Pfeiffer) and streaked crosswise with a loopful of the lytic fluid, structures similar to those described above were found along the margin of the culture and within the cleared area.

The anti-typhoid lytic fluid produced complete inhibition and