THE APPLICATION OF BACTERIOPHAGE TO PUBLIC HEALTH

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Some years ago Hankin observed that the water of the Ganges and Jumna Rivers in India have marked bactericidal action for bacteria in general and for cholera vibrio in particular. The water of Jumna river contains, at the city of Agra, more than 1000 bacteria per cc. Five kilometers below this it contains only 90 to 100 bacteria per cc. When this water is passed through a Berkefeld filter it shows a marked bactericidal action for the cholera organisms, in four hours destroying all organisms when a little of the water is placed in a broth culture of cholera vibrio. When this water is boiled it loses its bactericidal action and cholera vibrio grow well, as they also do in water taken from wells in the vicinity of the rivers. Hankin had no adequate explanation of the peculiar phenomenon.

In 1916, Twort was attempting to isolate filterable viruses from nature which might be grown on ordinary culture media. In this he was unsuccessful. In his work on calf vacinnia (smallpox vaccine) he noted that cultures of micrococci growing from the glycerinated vaccine became glassy and transparent and could not be subcultured. By transferring a little of the glassy material to new tubes of micrococci, these also became dissolved. Action was strongest with young cultures and could not be demonstrated in very old cultures. The lytic principle passes through the finest clay filters, but would not grow of itself on any culture media. Twort was not certain whether the lytic action was due to a minute parasitic organism, an amoeba or an anzyme secreted by the micrococcus.

The investigator who has taken up the work of these two pioneers and made a most careful study of it is d'Herelle. It is his theory that there is one parasite for all bacteria which adapts itself to the various kinds of organisms. These parasites pass through the finest clay filters, but can be cultivated only in the presence of the bacteria. Bordet states the phenomena is a defensive process due to the leucocytes which give to the organisms an hereditary property of producing a lytic ferment. The only difference between these two theories is whether the lytic ferment is produced by an ultra-microscopic virus or whether it is produced by the bacteria themselves. Kabeshino claims that lysis is due to a prodiastase present in the bacterial cell which is activated by a catalysor contained in filtrates of intestinal contents; in other words, that lysis is due to a ferment from the tissues. Salibeni has found a myxamoeba in lytic filtrates. Elivia and Pozerski described the destruction of Shiga bacillus by salts of quinine. Whatever the nature of the principle, however, it is certain that in persons convalescing from disease, notably typhoid fever, bacilliary dysentery and cholera, a substance is present in the stools that will dissolve the bacteria from which the patient is suffering when these two are placed together.

As was stated before, it is impossible to grow this lytic principle, whatever it may be, except in the presence of young cultures of bacteria, but bacteria free cultures will remain active for as long as six months. Anaerobiosis does not favor the lytic action. The material can be passed from tube to tube of bacteria an indefinite number of times and will remain active in clear filtrates for six months. In centrifuged specimens of filtrates, nothing can be demonstrated by staining the sediment. However, when a little of the sediment is examined with the ultra microscope, very fine points of light are seen dancing in the medium. d'Herrelle claims these are the organisms in question. The principle reacts to physical and chemical agents very much as do bacteria, being destroyed by antiseptics and heat and being quite susceptible to acids and alkalies, preferring a slightly alkaline medium for growth.

The lytic action of this ultra microscopic organism, if such it is, can well be demonstrated in a test tube experiment. If to young cultures of Shiga bacillus on slant agar or in broth, some of the lytic principle is added, all of the bacteria disappear and the medium becomes perfectly clear. After a short time, however, a growth reappears, but this is not typical of the organism in question. Grattia designates these organisms by the letter R for resistant, and those dissolved by S for susceptible organisms. The resistant organisms can be transferred from generation to generation but are not typical either in morphological or cultural characteristics. Old and dry cultures of bacteria contain more resistant organisms than young cultures.

The lytic principle has been found so far only in intestinal contents with the exception of the experiments of Twort where he found it in vaccine virus. d'Herrelle is of the opinion that all normal persons harbor the principle, although in some persons it may be weak and it must be activated for demonstration. A person does not become ill upon infection if he has sufficient bacteriophage in his system. If the bacteriophage is weak he may develop signs of the disease but recovers after the lytic principle becomes activated. If the lytic principle does not become activated or if the resistant strain of organism develops, the patient may not recover. Possibly the production of organisms resistant to the bacteriophage is responsible for relapses in typhoid fever. d'Herrelle claims that the only persons from whom he could not obtain bacteriophage filtrates were those destined to die. In the lower animals, as in men, the bacteriophage is easy to demonstrate. It has been found in horses, chickens, calves, buffaloes, rabbits and other animals. Very probably it is widely distributed in nature.

The bacteria susceptible to lysis by bacteriophage are mostly those from the intestinal tract, though others have been described. The dysentery bacilli,—Shiga, Hiss and Flexner,—all are very susceptible. The typhoid group including the paratyphoid and the colon group are likewise easily lysed. Bacillus proteus, Staphlococcus Bacillus subtilus, the cholera organisms, and Bacillus pestis have been made the subject of study. The diphtheria bacillus has not received very much attention, although d'Herrelle found in the manure of diphtheria antitoxin horses a substance which would dissolve the diphtheria bacillus. Among the lower animals, for the organisms of fowl typhoid, hemorrhagic septicemia and plague of the rat, a lytic substance was present in the intestines. As d'Herrelle says, probably the parasite is the same for all organisms, but becomes adapted to specific organisms according to the conditions.

The most interesting results obtained with bacteriophage from a public health standpoint are the inoculation experiments of d'Herrelle. In an epidemic of avian typhoid which was scattered over 25 poultry farms, from 20 to 50 percent of the fowls had died from the disease. He gave to 600 of the fowls by ingestion 1 cc. of the bacteriophage culture activated against B. gallinarium, and to 1500 more 0.5 cc. of the same culture subcutaneously. Not one of the fowls on the 25 farms which had received the bacteriophage died, although the epidemic continued to rage on other poultry farms in the vicinity. In Indo-China hemorrhagic septicema among the buffaloes is an exceedingly fatal disease. By injecting into 100 buffaloes 0.25 cc. of bacteriophage principle, d'Herrelle immunized the animals to such an extent that they received without harm 1000 fatal doses of the pathogenic bacteria necessary to kill buffaloes under ordinary conditions. The blood of these immunized animals conferred immunity upon other animals when infected. Rabbits, which are very susceptible to the toxin of bacilliary dysentery, were rendered immune by the subcutaneous injection of a small amount of bacteriophage. In seven cases of dysentery in man, the injection of 1 cc. of bacteriophage was followed in from 24 to 36 hours by the disappearance of blood and bacillus from the stools.

From these results it would seem that it would be a simple matter to immunize our population against all intestinal diseases, by simply injecting a small amount of bacteriophage. In case a person contracted a disease, he could be cured by injection of bacteriophage. Probably the problem will not be solved so easily. d'Herrelle warns against repeated doses of bacteriophage since these make animals hyper-susceptible and a greatly reduced amount of infection is required to produce death.

Bacteriophage is interesting from a public health standpoint. While it is not permissible to theorize on a subject which can be settled only by scientific research, yet there are some matters of particular interest to which I wish to call attention. Do persons become ill because of lack of bacteriophage and recover when this principle is activated or when it is supplied artificially, as by ingestion? Is the higher mortality among the primitive races accounted for, in tuberculosis for instance, because of the lack of bacteriophage against tuberculosis? And is the greater resistance of the so-called civilized races to tuberculosis due to the fact that the population in general has imbibed more or less freely from each other of bacteriophage principles? In the self purification of streams polluted by sewage, does bacteriophage have any part in destroying the intestinal organisms? From the observations of Hankin in India, it would appear that this is so. These are only a few of the problems of public health which are before us to be worked out.