has been further extended by the isolation of "special forms" of bacteria and by the possibility of demonstrating the production of bacterial antitoxins for the neutralization of toxins produced by other varieties of bacteria. Moreover, it must be remembered that all the vital processes of a bacterium have some relation to each other, and it is therefore necessary to study these problems together, or the true significance of any one may not be fully appreciated.

III.—CONCERNING THE THEORIES OF THE SO-CALLED "BACTERIOPHAGE."

By J. BORDET, M.D.,
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[THE following explanatory statement was read by Dr. Gratia for Professor Bordet.]

Through Dr. Gratia I obtained access to the paper Dr. d'Herelle intends to present on the lytic phenomenon due to the so-called "bacteriophage." I was not a little surprised to find that Dr. d'Herelle in this paper attributes to my co-worker, Dr. Ciuca, and to myself, as regards the intimate nature of this phenomenon, an opinion which is wholly different from what we felt entitled to uphold from the very beginning of our studies on the subject.[1] Dr. d'Herelle quotes our names next to Kabeshima's, and enlists us among the authors who assume that the lytic principle is a leucocytic secretion. In fact, this view seems to us altogether untenable, and is almost the reverse of the opinion we have constantly emphasized.

I think we were first to advocate the view that the lytic principle is produced by the microbe itself which shows the lysis—in other words, that the transmissible lysis is in reality an autolysis betraying a nutritive vitiation primarily started by external influences, an example of which may be the contact with a leucocytic exudate. No doubt it would be quite unnecessary to translate literally the many passages of our papers where this assumption is advocated. Some lines, however, may be quoted:

"External influences such as that of a leucocytic exudate modify the bacterium, inducing the latter to elaborate a lytic substance capable of diffusing itself and bringing about the same autolytic phenomenon through successive cultures. When the autolytic process occurs a large number of the microbes present may perish, but some of them, being more resistant, are, during a certain length of time, still capable of reproduction in spite of their producing the active principle, thus imparting to new cultures of the same microbe the same autolytic tendency."

In another paper we add:

"According to d'Herelle, the lysis is due to a living being, to a filtering virus. We, on the contrary, believe that the lytic principle originates from the bacteria themselves, which, when touched by this active substance, are capable of regenerating it, the factor responsible for the phenomenon being thus unceasingly reproduced—on the condition, however, that the bacteria be still living and provided with the alimentary substances necessary to their growth."

I wonder how Dr. d'Herelle could possibly give such an erroneous account of our work as in his paper. The many authors who have written on the subject did not, like Dr. d'Herelle, misinterpret a theory which we have so often and so distinctly outlined and explained. I shall allow myself to quote, for instance, the paper recently published by Dr. Bruynoghe,[2] who writes:

"According to Bordet and Ciuca, the microbes undergo—through the agency of a leucocytic exudate—a modification by which they are henceforth capable of elaborating an autolytic principle, this property being further transmitted to the following generations by the germs which were sufficiently resistant, and thus could multiply. This interesting view permits the understanding of the fact that the lytic principle is only regenerated when the bacteria are living, since the theory asserts that this principle is produced by the bacteria themselves."

I think there is no need to dwell longer on the subject. But one must agree that I could not refrain from correcting d'Herelle as regards our views, nor from presenting them again as they are expressed in all of our papers. The mere titles of these are clear enough; we always designate the phenomenon under the name of "the microbian transmissible autolysis."

REFERENCES.
[1] *C.R. Soc. Biologie*, October, 1920. [2] *Le Scalpel*, March, 1922.

IV.—ANDRÉ GRATIA, M.D.,

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1. The Twort phenomenon and the d'Herelle phenomenon are identical. They are two different aspects of one and the same phenomenon: the transmissible lysis of bacteria.

When the "dissolving material" of Twort found in diseased agar cultures of micrococci obtained from vaccinia lymph is transplanted into a young broth culture of staphylococci a dissolution of the latter occurs, and the filtrate of the dissolved culture exhibits all the characteristics of a typical staphylococcus bacteriophage according to the definition of d'Herelle.

On the other hand, typical staphylococcus bacteriophage could be obtained also by other means—namely, by the leucocytic exudate technique of Bordet and Ciuca, or by the puncture of a subcutaneous abscess. When small amounts of this staphylococcus lytic agent are introduced in melted agar which is afterwards slanted and seeded with sensitive staphylococci a culture results, apparently normal at the beginning, but which, a little later, turns into the typical glassy transparent material of Twort. In other words, the Twort phenomenon leads to the d'Herelle phenomenon, and, inversely, the d'Herelle phenomenon leads to the Twort phenomenon.

2. There are no unquestionable proofs that the bacteriophage is a living organism.

The assumption of the bacteriophage being a filtrable virus for bacteria was suggested by two main facts: (*a*) The power of reproduction possessed by the lytic agent, and (*b*) the localization of the lysis to certain round spots of clarification when a very diluted lytic agent is poured over the surface of an agar culture of sensitive bacteria. Although easily explained by the virus theory, yet both facts are not unquestionable proofs of the living nature of the bacteriophage, because they are by no means exclusive features of living beings.

Fire is not living, and yet fire is endowed with power of reproduction. When once lighted, thanks to an initial impulsion such as an electric spark or the mere striking of a match, it can be indefinitely reproduced if fuel is provided. A still more striking, because more biological, example is found in blood coagulation. Suppose a series of test tubes containing a stable plasma—bird's plasma, for instance—which will remain indefinitely fluid. To the first tube we add just a few cubic centimetres of distilled water. As a result of that initial thromboplastic action, which does not need to be repeated in the future, thrombin suddenly appears in the first tube and the plasma clots. If a few drops of the exudate serum in the first tube are pipetted off and poured in the next tube, this second tube clots, in its turn, with a new regeneration of thrombin, which, transferred in the third tube, brings about the coagulation of that tube with again a new production of thrombin, and so on indefinitely. In this way we realize the transmissible coagulation of blood in series, with the continuous regeneration of thrombin, and thrombin is not a living being.

The localization of the lytic action of diluted bacteriophage can be explained by the hypothesis of a chemical substance as well. It must be kept in mind that a culture is not a homogeneous whole, but made up of organisms showing all kinds of qualitative and quantitative individual differences—that is, as far as their susceptibility to the lytic agent is concerned. When a very concentrated lytic agent is poured over the surface of an agar culture an almost complete dissolution occurs, with the exception of just a few organisms resistant enough to overwhelm the strong action of the concentrated lytic agent. On the other hand, when a diluted lytic agent is used only the few extremely sensitive bacteria will be influenced, and each of them becomes a centre of regeneration of the lytic agent, which, diffusing evenly in every direction, produces perfectly round spots of clarification very often surrounded by a kind of halo of diffusion. Between these two extreme conditions all kinds of intermediate degrees exist. Further, any substance, living or not, is composed of particles, molecules, atoms, or ions. When we pour out a glass of soda water, there appear on the wall of the glass small round bubbles of gas, the size of which increases exactly as the so-called colonies of bacteriophage, and yet gas is not a virus.

3. The idea of the bacteriophage being a product of bacterial activity is suggested by the close parallelism existing between the regeneration of the lytic agent on the one hand, and the activity of growth of the bacteria on the other hand.

No regeneration ever occurs in dead cultures, nor in living cultures when put in such conditions that they cannot grow—in saline emulsions of bacteria, for instance, or at low temperature. A slight lysis, with but a small regeneration of lytic agent, is induced in the slow-growing culture of *B. coli* in a synthetic medium. On the contrary, an abundant regeneration occurs in a fast-growing culture in broth. A recently seeded broth culture to which is added just a trace of lytic agent will not be inhibited; but a few hours later, at the very moment the culture reaches its acme of growth, a rapid dissolution occurs with an abundant regeneration of lytic agent.

4. The conception of the bacteriophage being a chemical substance is favoured by the chemical-like affinity existing between a given lytic agent and the corresponding susceptible strain.

I first observed that small amounts of lytic agents lose a certain part of their activity when put together with too thick emulsions of sensitive bacteria. Bordet, with a different technique, could even obtain the complete disappearance of traces of lytic agent in the same condition. Still more convincing are the results of Yaumain and of Da Costa, who observed the absorption of relatively important amount of lytic agent by dead emulsions of the corresponding sensitive bacteria. This specific affinity which is the necessary condition for a lytic agent for inducing the dissolution of a given bacterium is not favourable to the virus theory, because we question how a virus could be definitely fixed by dead bacilli, which, however, it is unable to attack.

5. The bacteriophage is not one and the same antigen. Several lytic agents showing antigenic specificity must be considered.

The *coli* lytic agent can be completely neutralized by proper amounts of corresponding *coli* antilytic serum, but is not at all affected by staphylococcus antilytic serum, which, on the other hand, is only able to neutralize staphylococcus lytic agent and not *coli* lytic agent. This neutralization reaction is thus specific, and demonstrates the plurality of the bacteriophage.

The non-specific results obtained with the alexin fixation reaction and advocated by d'Herelle in favour of the unicity of the bacteriophage, are of no value, because they are vitiated, as can be easily demonstrated, by the presence in the bacteriophage of bacterial dissolution products which have lost their specificity and play therefore the rôle of common antigen between different lytic agents.

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The problem which has exercised the minds of all who have personally investigated the subject has been to elaborate some theory which will adequately explain the facts. Dr. d'Herelle maintains unaltered his originally expressed view that the phenomenon of transmissible lytic effect is fully explained by assuming that a living ultramicroscopic virus parasitizes and brings to solution the particular organisms with which it has most affinity, and that the apparent increase of lytic effect in passage is due simply to growth of the alleged live virus. Even if, as d'Herelle claims, his premisses warrant the conclusion to which he arrives, I should still hesitate in accepting it. I should desire to be satisfied that the alleged cause was a *vera causa* in the sense of the old logicians—that, in fact, it was a cause which did not tax too severely one's sense of the naturalness or likelihood of things. But are the premisses so indubitably established? Is no other general explanation possible? The investigations so far undertaken display an extraordinary variety of experiment, sometimes yielding uniform, sometimes highly conflicting, results, and I feel strongly that, throughout, there has been little attempt to study the phenomena on quantitative lines, with a view to eliminating or evaluating the various known possible factors and agencies that may, either in conjunction or separately, give rise to the end-result. It is possible, however, from the sheer mass of data recorded to take the following facts as presumably established.

1. That certain organisms, particularly those of the intestinal group, submit readily to lysis under the influence of a variety of primary stimuli. These may be filtered faecal extracts from healthy or diseased persons, or they may be extracts of normal tissues or body secretions. Filtrates of these organisms so dissolved are able to carry on the process.

2. That some strains of these same organisms are naturally resistant to these same stimuli.

3. That some strains of these same organisms show lytic changes normally. I attach great importance to this fact. Dr. Lepper, at the Lister Institute, recovered from the urine of a case of pyelitis a coliform strain which behaves in culture as if it were a mixture of bacilli and "bacteriophage." Its growth curve in broth showed repeated depressions, which can be explained only by autolytic effects. On agar the characteristic patchy growth of irregular nibbled colonies appears.

4. That the stimulus if not too strong causes a dissociation of the strain acted upon, into resistant and non-resistant types.

5. That potent lytic extracts can be prepared from the organisms themselves during artificial growth, the process being apparently facilitated by repeated filtration of the growth and reinoculation of the filtrate with the organism concerned.

6. That potent lytic extracts can be obtained from the filtered growth of organisms growing symbiotically.

In whatever way the initial stimulus is obtained the transmission in series can, in my view, be explained only on the assumption that potent autolytic ferments discharged from the lysed organisms are able to initiate similar effects in passage. My own work has been concerned with filtrates of *B. pyocyaneus*, which are known to dissolve readily organisms like *B. anthracis* and *V. cholerae*, but which may fail entirely with certain other species. I have not yet been able to show definite carry-over by ferments from organisms so dissolved, but it is probable that the proper conditions have not been secured. Malfitano, twenty years ago, showed that *B. anthracis* suspended in distilled water liberated autolytic ferments, and he pointed out what is of extreme importance in securing the carrying over—namely, that the organism must be so killed that the ferments discharged are not injured. It is important, therefore, if one wishes to eliminate superfluous factors, to seize on the simplest exposition of the experiment. The simplest exposition so far is, I take it, that in which the organism's own extract can initiate the "bacteriophagic" action. That extracts of organisms may exhibit lytic powers is, of course, no new observation, and it is helpful now to bring these older observations into line with new experiment. The second simple procedure which yields, apparently in a remarkably short time (in a matter of hours, in fact), a potent lytic stimulus is the inoculation of organisms into the circulation of an animal and the demonstration of "bacteriophagic" powers in the serum drawn a few hours later. If the results on this point are confirmed, we seem to have the phenomenon reduced to the simplest form, and it is remarkable that it fits in with certain phenomena the explanation of which has long puzzled us. I may refer, for example, to the extraordinarily rapid development of immunity to plague after inoculation of highly purified plague extracts in rats (Rowland), and to the remarkable though temporary increase of normal antibody which rapidly follows the injection into animals of autolysed bacterial bodies or refined substances like nuclein. I feel that here we have a promising link between "bacteriophage" work and certain previously recorded phenomena.

It is difficult at this stage to evaluate the alleged therapeutic results of these "bacteriophages." There is no doubt, however, that just as *pyocyaneus* filtrates can abort an anthrax infection in rabbits when injected simultaneously with the organism, so a similar local action may well be expected with the filtrates of d'Herelle. It seems to me unlikely, however, that much is to be expected from their therapeutic use in virulent infections in highly susceptible animals. I am not greatly impressed with the evidence of specificity of these "bacteriophages" based on complement fixation tests with immune serum. I take it that, from the work of Otto, Munter, and Winkler, the immune serums obtained by immunization with bacteriophage filtrates is essentially of the same kind, if not of the same order, as those obtained by immunization with the raw organism or its autolysate. Any alleged differences would seem to be due simply to the fact that the bacteriophage filtrates are in a highly refined colloidal state. To this high refinement, which is facilitated by repeated filtration and reinoculations, Otto, Munter, and Winkler attribute the potency of their products as compared with supernatants obtained by the centrifuge only. Just as the anaphylatoxin effect was whittled down to an interaction between two colloids, so I would urge for the investigation by quantitative