STUDIES ON THE D'HÉRELLE PHENOMENON.*

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PLATES 5 AND 6.

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INTRODUCTION.

About 3 years ago d'Hérelle¹ found that stools of patients recovering from bacillary dysentery contain a filterable substance which is able to dissolve cultures of the Shiga bacillus and that a few drops of the dissolved culture reproduces the same phenomenon upon addition to another culture, and so on, indefinitely. Through these different passages the lytic property, instead of decreasing by dilution, is on the contrary increased and retains its activity even after several years.

Such a continuous transmission of the lytic property occurs only if the transfer is made into living cultures of the Shiga bacillus. Hence d'Hérelle concluded that the lytic agent is a filterable virus parasitic on the Shiga bacillus. To this supposed virus he has given the name of bacteriophage. He discovered similar bacteriophages for B. coli, B. typhosus, B. paratyphosus A and B, and some other bacilli.

Salimbeni² claimed to have isolated the bacteriophage, which he described as a myxameba possessing spores so minute that they are capable of passing porcelain filters.

Kabeshima,³ on the other hand, denies the living nature of the bacteriophage and considers it merely as a catalyzer secreted by the leucocytes of the infected intestine and capable of activating a lytic proferment present in the body of the microbes.

The observations of Bordet and Ciuc⁴ also make very doubtful the parasitic nature of the bacteriophage. These authors injected guinea pigs intraperitoneally

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* Most of the findings here reported have already been presented as preliminary notes in Compt. rend. Soc. biol., 1921, lxxxiv, 275, 750, 751, 753, 755.
¹ d'Hérelle, F., Compt. rend. Acad., 1917, clxv, 373; 1918, clxvii, 970; 1919, clxviii, 631; Compt. rend. Soc. biol., 1918, lxxx, 1160; 1920, lxxxiii, 52, 97, 247.
² Salimbeni, Compt. rend. Soc. biol., 1920, lxxxiii, 1545.
three times at 5 day intervals with cultures of \textit{B. coli}. 1 day after the last injection the peritoneal leukocytic exudate exhibited the properties of the bacteriophage of d'Hérelle; namely, a continuous transmission of lytic action.

Bordet and Ciuca also observed that a culture of \textit{B. coli}, once dissolved by an immunized exudate and then filtered, is able either to dissolve a second culture of \textit{B. coli} or to inhibit its growth in broth. But neither this dissolution nor the inhibition is absolute, since a few organisms always resist the dissolution and multiply, although slowly. The latter bacilli are distinguished from the original culture by certain characteristics: they resist the lytic agent but have now themselves acquired the lytic property and become lysogenic, or capable of inducing dissolution in a culture of normal \textit{B. coli}. Moreover, when planted on slanted agar a mucoid, sticky culture results; also they are less phagocyttable and more virulent for guinea pigs than the normal culture from which they were derived. All these properties are preserved even after passage through animals.

Thus there seems to arise under the influence of the lytic substance a race of bacilli which is adapted to this substance and is characterized by new and transmissible properties such as the increased virulence. Bordet and Ciuca call this race "modified \textit{B. coli}," and they infer that under the influence of the peritoneal exudate mentioned a variation of the colon bacilli occurs in the sense that they now secrete an autolysin which dissolves their own cells, with the exception of a few resistant organisms which survive and continue to produce the lytic secretion. Hence Bordet and Ciuca conceive the phenomenon to be that of a transmissible microbic autolytic property.

The facts as outlined are of fundamental importance, since they relate not only to the problem of the lysis itself but also to such disputed questions as the appearance of new races, the heredity of acquired characteristics, and the nature of virulence.

\textit{Influence of Hydrogen Ion Concentration.}

We first established the influence of the hydrogen ion concentration on the lytic action\footnote{We are indebted to Dr. Bordet for a strain of \textit{B. coli} with which he carried on his studies, together with a quantity of the corresponding lytic agent. The experiments were conducted with this material.} which, as is known, manifests itself in two ways, either in inhibiting the growth of \textit{Bacillus coli} in freshly planted broth cultures or in dissolving a culture already grown. As the former phenomenon is more easily observed than the latter, the inhibiting action of the lytic agent will be chosen as a criterion in the following experiments.

\textit{Experiment 1.}—Two tubes of plain broth were seeded with the same small quantity of \textit{B. coli}; i.e., with 1 drop of a 24 hour broth culture. Immediately
afterward 10 drops of the lytic agent were added to the second tube. In this condition, it is known that the first tube grows normally while the second remains perfectly clear for a certain period, which can be easily measured.

We have repeated this experiment with broths of varying hydrogen ion concentrations and observed that the inhibition is markedly influenced by the reaction of the medium; it is faint in a slightly acid (pH 6.8), neutral (pH 7), or even slightly alkaline broth (pH 7.4), but much stronger in a more alkaline broth (pH 8 or 8.5).

In a slightly acid broth (pH 6.8) containing 10 drops of lytic agent, *Bacillus coli* grows after 2 to 3 hours almost as well as in normal broth. But this early growth disappears very quickly by dissolution. After a few hours, however, a renewed growth begins and this time develops normally, reaching its maximum after 24 to 36 hours. Then another dissolution occurs, but this process is slow and incomplete. On the following day the culture becomes somewhat more opaque again.

Hence the impression arises of a succession of waves of growth and redissolution, at each wave of growth *Bacillus coli* becoming more resistant.

On the other hand, in a highly alkaline broth (pH 8.5) the inhibition is much more striking and it is only after 36 to 48 hours that growth appears.

Between these extremes we have observed all intermediate degrees, the inhibition increasing with the increase of the alkalinity.

*Lysis and Microbic Variation.*

Bordet and Ciucu have clearly demonstrated that the lytic agent spread on the surface of a young culture of *Bacillus coli* on slanted agar clarifies the culture by dissolution, but that after a certain period of incubation one can detect a few irregular colonies which have the distinctive characteristics of the resistant *Bacillus coli* (Fig. 1, *A*). We observed a very similar picture by merely allowing the normal culture of *Bacillus coli* to age. An old agar slant of *Bacillus coli* shows a uniformly dull film on which appear very distinctly, here and there, small vitreous colonies. The first impression is that of a contamination, but these colonies are undoubtedly *Bacillus coli* and their distribution reproduces that of the resistant colonies.
observed by Bordet and Ciua (Fig. 1, B). On transplanting the material of the dehydrated film between the vitreous colonies, no growth occurs. The organisms originally in this material are now dead. On the other hand, if one of the vitreous colonies is planted in broth, a growth results which possesses a great resistance to the lytic agent. Therefore, by merely allowing the normal culture of *Bacillus coli* to age, we have realized an artificial selection of more resistant organisms.

At the same time we have had the good fortune to isolate from a subculture of the original strain a colony of organisms which are, on the other hand, extremely sensitive to the lytic agent. Hence two types of *Bacillus coli* were isolated—one very resistant (Strain R) and the other very sensitive (Strain S)—both artificially selected from the original culture in which they coexisted. The difference in the susceptibility of the two strains is illustrated in the following experiments.

**Experiment 2.**—1 drop of the lytic agent was spread on the 3 hour slanted agar growth of Type S. With the exception of only two or three colonies, an almost complete clarification occurred (Figs. 2, A and 3, A and B).

With Type R, however, the clarification was transient and soon afterward the path left by the drop was covered with a multitude of minute colonies (Fig. 2, B) which, by confluence, overgrew again the surface previously clarified (Fig. 3, C and D).

**Experiment 3.**—Broth of varying hydrogen ion concentrations and containing a few drops of lytic agent was seeded respectively with Types S and R. Type R grew luxuriantly, especially in acid medium (pH 6.8), while Type S showed no growth, even in an acid broth.

In addition to the differences in their susceptibility to the lytic agent, both types are distinguished by other characteristics. Type S, multiplying more rapidly than Type R, produces promptly in broth a supernatant film with a whitish band, consisting of microorganisms, adhering to the wall of the tube at the level of fluid surface; Type R shows the same details but only at a much later period of growth. Both types produce indole and ferment carbohydrates, with the exception of saccharose. The fermentation tests were made by means of stab cultures in semisolid agar and gave us the oppor-

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*We have made similar observations with Shiga cultures.*
tunity to observe another striking distinction. The growth of Type S remains close to the line of puncture (Fig. 4, B); on the contrary, Type R diffuses uniformly throughout the whole mass of agar (Fig. 4, A). This distinction is due to the difference in the motility of the two types of organisms; Type S is non-motile; Type R possesses, on the other hand, an active motility very much like that of the typhoid bacillus. This affords a very useful means of separating the two types when they are mixed: a stab culture is made in one arm of a U-tube containing semisolid plain agar; only the motile Bacillus coli diffuses to the other arm of the tube, from which, after a few hours, we are able to recover it in pure culture.

### TABLE I.

<table>
<thead>
<tr>
<th>Guinea Pig No.</th>
<th>Type S</th>
<th>Dilution</th>
<th>Results</th>
<th>Guinea Pig No.</th>
<th>Type R</th>
<th>Dilution</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>Undiluted</td>
<td>Died after 26 hrs.</td>
<td>6</td>
<td>2</td>
<td>Undiluted</td>
<td>Died after 10 hours</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1:2</td>
<td>Survived.</td>
<td>7</td>
<td>2</td>
<td>1:2</td>
<td>&quot; &quot; 15 &quot;</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1:4</td>
<td>&quot;</td>
<td>8</td>
<td>2</td>
<td>1:4</td>
<td>&quot; &quot; 12 &quot;</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1:10</td>
<td>&quot;</td>
<td>9</td>
<td>2</td>
<td>1:10</td>
<td>Survived.</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>1:20</td>
<td>&quot;</td>
<td>10</td>
<td>2</td>
<td>1:20</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

In general, Bacillus coli is not very virulent, and guinea pigs can withstand intraperitoneal injections of large doses of this microorganism. Hence it is rather difficult to measure variation of virulence between different strains of Bacillus coli. However, in the following experiment, in which each type of this bacterium was employed, it will be noted that Type R is more virulent than Type S.

**Experiment 4.**—We injected into the peritoneal cavity of five guinea pigs respectively 2 cc. of a culture of Type S (grown in plain broth, pH 6.8, for 18 hours), using different dilutions, undiluted, 1:2, 1:4, 1:10, 1:20. Similarly five guinea pigs were injected with a culture of Type R. The results of this experiment are shown in Table I.

**Autopsies.**—Guinea Pig 1 (injected with coli S): The peritoneal cavity contained a purulent exudate with fibrinous clumps. Diffuse fibrinous membrane
covering the viscera, especially the liver. Microscopic examination of the exudate showed large numbers of leucocytes, a few containing phagocytosed bacilli. No free bacilli were visible (Fig. 5). The heart's blood yielded a sparse growth of Type S microorganisms.

Four other guinea pigs which died after injections of large doses of Type S have shown the same conditions, and in three of them cultures of the heart's blood remained sterile.

Guinea Pigs 6, 7, and 8 (injected with *coli* R): Only a faint fibrinous pellicle was noted on the surface of the liver. The peritoneal exudate was serosanguineous and on microscopic examination showed numerous bacteria and an occasional leucocyte (Fig. 6). The heart's blood yielded a profuse growth of Type R bacilli.

It appears from these observations that Type R is more virulent and less phagocytobable than Type S. The two types of *Bacillus coli* behave differently, therefore, not only on artificial media but also in the animal body, and, furthermore, retain their individuality even after one passage through a guinea pig.

In spite of the sensitiveness of Type S to the lytic agent, we have never observed a complete dissolution of all these organisms in a culture. Among the vast number of bacteria that are spread over an agar slant there are always a few—a dozen at least—that resist the lytic agent and form colonies. We have found that these resistant organisms were not a few individuals of Type R which had contaminated the culture of Type S; they were not motile. At least it proves that all the individuals which constitute a culture of Type S are not similar in so far as their susceptibility to the lytic agent is concerned. We assume that there are all degrees of resistance against the destructive agent, and if a few individuals can withstand the powerful action of the undiluted lytic agent there must be also a few so sensitive that they can be dissolved even by the lytic agent highly diluted, and that between these two extremes, intermediate degrees exist. This deduction is favored by the following experiment.

**Experiment 5.**—To a number of agar slants seeded with equal quantities of Type S bacilli and maintained for 3 hours at 37°C. was added 1 drop respectively of increasing dilutions of the lytic agent, as follows: undiluted, 10⁻¹, 10⁻², 10⁻³, etc. As the concentration of the active agent diminished, the number of the resistant colonies increased. At a certain point the clarification of the surface was reduced to small irregular zones and finally to small, perfectly circular areas (Fig. 7).
These small areas of clarification represent the few individuals of *Bacillus coli* which are sensitive enough to be dissolved even in a very dilute solution of the lytic agent. Each of them, in turn, becomes a center of regeneration of the active substance, which, diffusing out at the same distance in all directions, produces a small, circular zone of clarification surrounded by a sort of halo.

**Relation to the So Called “Colonies of Bacteriophage.”**

d'Hérelle, who first observed the localization of the dissolution when the lytic filtrate is very dilute, offers a different explanation for the formation of the small zones of clarification. He considers these areas as colonies of the invisible virus which he supposes to be responsible for the dissolution. When the filtrate is pure the individuals of the bacteriophage are so numerous that their colonies are confluent and produce a general lysis. But when the dilution is sufficiently high, each parasite is isolated and produces localized lysis.

He claims this peculiar aspect to be unquestionable proof of the living nature of the bacteriophage because he questions whether a diffusible substance can localize its action at certain points. As we have seen, we can explain this easily if we do not regard a culture of *Bacillus coli* as a homogeneous whole but as made up of organisms of varying resistance to the lytic agent. While d'Hérelle ascribed the localized dissolution to the dilution of the bacteriophage, we are inclined to look upon the dilution as accessory, and to search for the immediate source of the phenomenon in the relative resistance of the colon bacilli. If this is so, we should expect the similar production of small areas of clarification even with undiluted lytic agent on submitting to its action cultures of greater resistance. As will be shown later, this is precisely what we have often observed.

To sum up, the so called “colonies of bacteriophage” can be explained as well by the hypothesis of a lytic agent as by that of a parasite.

In the preceding experiment decreasing quantities of lytic agent were tested on a constant quantity of *Bacillus coli*. On the other hand, in the following experiment, increasing quantities of *Bacillus*
coli were submitted to a constant quantity of highly diluted lytic agent.

Experiment 6.—200 cc. of a 12 hour broth culture of Type S were centrifuged and to the sediment 2 to 3 cc. of broth were added. The mixture was then filtered through sterile cotton; in this way we obtained a perfectly homogeneous suspension with which the following dilutions were made: $10^{-5}$, $10^{-4}$, $10^{-3}$, $10^{-2}$. In large tubes was mixed respectively 1 cc. of each of these B. coli suspensions with 0.5 cc. of very dilute ($10^{-6}$) lytic agent. Immediately afterward 1 cc. of each of these mixtures with 10 cc. of plain agar was plated in Petri dishes and incubated at 37°C. After 12 hours incubation, the surface of the plates was covered with a uniform pellicle of B. coli in which appeared small areas of clarification which were evenly distributed and easy to count.

If the lytic agent is a living organism and these areas are colonies of bacteriophage, the number of areas should be approximately the same on all plates, since we have added to each the same amount of diluted filtrate. Moreover, since the size of a colony usually varies directly with the abundance of its food, and since Bacillus coli comprises the nutrient elements in this case, the size of the areas should increase with the quantity of Bacillus coli.

But the results of the preceding experiment show otherwise. The number of areas first increases, then reaches a maximum, and finally decreases, as the quantity of Bacillus coli is increased (Table II). The size of the areas instead of increasing, on the contrary, decreases.

These results favor the idea that the lytic agent is a diffusible substance. If the areas represent individuals of Bacillus coli so sensitive as to be dissolved even by a very weak lytic agent, the num-

<table>
<thead>
<tr>
<th>Dilution of</th>
<th>Dilution of</th>
<th>Experiment 6. No. of spots.</th>
<th>a.</th>
<th>b.</th>
<th>c.</th>
<th>d.</th>
</tr>
</thead>
<tbody>
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<td>lytic principle.</td>
<td>B. coli.</td>
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<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>$10^{-4}$</td>
<td>69</td>
<td></td>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>$10^{-3}$</td>
<td>249</td>
<td>56</td>
<td>70</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>$10^{-2}$</td>
<td>469</td>
<td>167</td>
<td>187</td>
<td>800</td>
<td></td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>$10^{-1}$</td>
<td>328</td>
<td>211</td>
<td>332</td>
<td>508</td>
<td></td>
</tr>
<tr>
<td>$10^{-1}$</td>
<td>Undiluted.</td>
<td>142</td>
<td>95</td>
<td>235</td>
<td>320</td>
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</table>
ber of these sensitive individuals should increase with the concentration of the *Bacillus coli* emulsion, and the number of spots should then increase proportionately. This is what is noted at the beginning of the curve. But unfortunately a second phenomenon of opposite tendency interferes with the preceding one: when the lytic agent is added to the *Bacillus coli* suspension, it combines partially with proteins which have nothing to do with the dissolution and so decreases the dissolving action in direct proportion to the increase in quantity of *Bacillus coli*. If, on one hand, the clarified areas increase with the increase of the *Bacillus coli* emulsion because the number of sensitive organisms is increasing, on the other hand, these areas tend to decrease because the activity of the lytic agent is decreasing. The combination of these two phenomena of opposite action should give us the curve that we have observed. The evidence at hand leads us to the hypothesis that bacteriophage is a diffusible, rather than a living substance.

*Non-Specificity of the Lysis.*

The lytic filtrate used in the preceding experiments was specific. While it is active on the *Bacillus coli* used in injecting the guinea pigs, it is without any action not only on other closely related species but also on other strains of *Bacillus coli*. But in the following experiments we have been able to extend the lytic action to other species.

*Experiment 7.*—With the aim of increasing our stock of lytic agent we added 1 cc. of the original filtrate to each of two flasks, one containing 25 cc. of a young broth culture (pH 8) of Type S and the other of Type R. Dissolution of the growth occurred. After 48 hours incubation, we filtered both cultures and thus obtained besides the original filtrate (Filtrate O), two new filtrates, Filtrate 1 (Type S) and Filtrate 2 (Type R).

With a sample of Filtrate 2 a very marked dissolution was observed of Shiga bacilli, Flexner bacilli, Hiss Y type of Flexner bacilli, and also a strain of *B. coli*.

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7 This phenomenon is similar to the one observed by Loeb (Loeb, J., Artificial parthenogenesis and fertilization, Chicago, 1913, 145) on the parthenogenetic fertilization of eggs with small quantities of acids. Very dilute solutions of acid are able to fertilize a small number of eggs, but not a larger number. The acid in combining with the jelly of the eggs loses a certain part of its activity; it still retains enough efficiency when the eggs are not numerous, because the total quantity of jelly is small, but loses it completely, on account of the abundance of jelly, when the number of eggs is great.

8 These observations were made by Dr. Martha Wollstein.
D'HÉRELLE PHENOMENON

which was unattacked by the original filtrate. This observation led us to control the action of all three filtrates on agar slants, seeded with the following bacteria: Types S and R, both isolated from the original culture of Bordet and Ciucu, B. coli communis, B. coli communior, B. dysenteria Shiga, B. dysenteria Flexner, Hiss Y type of Flexner bacilli, B. typhosus, and B. paratyphosus A and B.

Filtrate O, the original lytic agent, had only a weak lytic action, limited to Types S and R; Filtrate 1 was similarly weak, but produced a few small areas of clarification on the three dysenteric bacilli; Filtrate 2, on the other hand, was extremely active and produced a complete dissolution of Type S, an almost complete dissolution of Type R and of the three dysenteric bacilli, and a complete clarification of the strain of B. coli communis. Only the B. coli communior, the typhoid, and both paratyphoid strains were unaffected.

Our supposition was to attribute this increase of the power of the lytic agent to the fact that Filtrate 2 was prepared with a more re-

<table>
<thead>
<tr>
<th>Filtrate</th>
<th>Type S</th>
<th>Type R</th>
<th>Communis</th>
<th>Communior</th>
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<tbody>
<tr>
<td>0</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>++++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>3</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
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<td>4</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
</tbody>
</table>

TABLE III.

<table>
<thead>
<tr>
<th>Filtrate</th>
<th>Type S</th>
<th>Type R</th>
<th>Communis</th>
<th>Communior</th>
<th>Shiga</th>
<th>Flexner</th>
<th>Hiss Y</th>
<th>Typh.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>++++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td></td>
<td>+</td>
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<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
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</tr>
</tbody>
</table>

++ indicates complete clarification, no resistant colonies; +++, almost complete clarification, less than twelve resistant colonies; +, moderate degree of clarification, many resistant colonies; +, only a few small areas of clarification; —, negative result.

ristant strain, Type R. The following experiment demonstrates that this is the actual condition and the use of a resistant strain gives a method of increasing the efficacy of the lytic agent and extending its action to the other species as yet refractory.

Experiment 8.—By allowing 1 cc. of Filtrate 2 to act for 48 hours on 25 cc. of a young culture of Type R bacilli, we have obtained a still stronger filtrate (Filtrate 3) which, fortunately, produced four or five minute areas on a slant of typhoid bacillus. Following this last indication, we mixed 25 cc. of a young culture of typhoid bacillus with 3 cc. of Filtrate 3, and after 48 hours incubation, obtained a filtrate (Filtrate 4) extremely lytic for typhoid, as well as for para-
typhoid B bacilli. At the same time, the action of Filtrate 4 on \textit{B. coli} was diminished to a certain extent. Table III shows the activity of the different filtrates.

It is obvious that we can now multiply similar combinations by allowing one or another of our filtrates to act on a well selected strain as intermediary. It will be of interest to study the relation between different microbic species in this respect, and to note to what extent the lytic activity can be transmitted from one species to another.

\textbf{CONCLUSIONS.}

The inhibition produced by the lytic agent on the growth of \textit{Bacillus coli} is greatly influenced by the reaction of the medium; it is faint in a slightly acid (pH 6.8) or neutral (pH 7) or even slightly alkaline broth (pH 7.4), but is much stronger in a more alkaline medium (pH 8 or 8.5).

We have isolated from the original strain of \textit{Bacillus coli} two types of organisms; one (Type S) is sensitive to the lytic agent, the other (Type R) is much more resistant. These types are distinguished also by other characteristics: Type S grows quickly in artificial medium and is non-motile; Type R grows more slowly, is extremely motile, much less phagocyttable, and more virulent. Both types produce indole and ferment carbohydrates, with the exception of saccharose. Both types keep their individuality even after passage through a guinea pig.

We have also demonstrated that even a culture of a single type, Type S for instance, is not a homogeneous whole but is made up of organisms of varying resistance to the lytic agent; only a few are resistant enough to overcome the strong action of the undiluted lytic agent. On the other hand, only a few as well are sufficiently sensitive to be dissolved even by very dilute lytic agent.

This explains why dilute lytic agent spread on an agar plate seeded with \textit{Bacillus coli} confines its action only to certain places and produces the small round areas of dissolution that d'Hérelle considered as "colonies of bacteriophage." Moreover, we have observed the same localized action even with non-dilute lytic agent when submitting to its action cultures of greater resistance.
Our original lytic agent was found to be specific; it acted exclusively on the coli with which the guinea pigs were injected. By allowing this original lytic principle to act on broth cultures of our two types of Bacillus coli, we have obtained two new filtrates. The first, resulting from dissolution of the sensitive Strain S, is specific as is the original filtrate. But with the second, obtained from the resistant Strain R, Dr. Wollstein has found a marked action on Shiga, on Flexner, and on Hiss dysentery bacilli. In consequence of this observation, we have been able, by a method of successive passages through appropriate strains, to extend the lytic power to other species, as typhoid and paratyphoid bacilli, and have obtained by this somewhat different technique results similar to those recently published by Bordet and Ciucu.

EXPLANATION OF PLATES.

PLATE 5.

Fig. 1. Tube A: Experiment of Bordet and Ciucu. A young culture of B. coli on slanted agar covered with lytic agent. Only a few organisms resist dissolution and produce irregular colonies. Tube B: Agar slant culture of B. coli, 6 weeks old. Note on the uniformly dull film of the desiccated culture a few hyaline colonies which have resisted desiccation (coli R).

Fig. 2. Experiment 2. 1 drop of lytic agent placed on 3 hour cultures of B. coli (Types S and R). Results after 6 hours incubation at 37°C. Tube A: Type S (sensitive). The path of the drop is free from growth. Tube B: Type R (resistant). The path of the drop is already covered with a great number of minute resistant colonies.

Fig. 3. The same experiment. Results after 24 hours incubation. Tubes A and B: Coli S. Only a few resistant colonies may be seen. Tubes C and D: Coli R. Numerous resistant colonies have overgrown the area previously clarified.

PLATE 6.

Fig. 4. Stab cultures of Types S and R in semisolid agar. Tube A: Type R (motile). The growth diffuses evenly throughout the whole mass of agar. Tube B: Type S (non-motile). The growth remains close to the line of puncture.

Fig. 5. Peritoneal exudate of a guinea pig dead after injection of coli S. Numerous leucocytes. No free bacteria.

Fig. 6. Peritoneal exudate of a guinea pig dead after injection of coli R. No leucocytes. Numerous free bacteria.

Fig. 7. Action of increasing dilutions of lytic agent on B. coli S.
(Gratia: d‘Hérelle phenomenon.)
(Graizi: d’Hérelle phenomenon.)