Rockefeller Institute

THE TWORT-D'HÉRELLE PHENOMENON.

II. Lysis and Microbic Variation.

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Plates 7 and 8.

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In a preceding paper (1) the present knowledge of the phenomenon of transmissible microbic lysis discovered by Twort and by d'Hérelle was reviewed; there was described the method by means of which we dissociated a culture into two types of organisms, by merely allowing a culture of *Bacillus coli* to age. One, Type S, is very sensitive to destructive influences, such as desiccation and transmissible lysis. The other, on the contrary, is much more resistant. Besides these differences in vulnerability, the organisms are also distinguished by other properties; namely, motility and virulence.

Since that time we have had an opportunity to isolate a greater number of different types of *Bacillus coli*, all derived from the same original strain, as will be described in this article.

Characteristics of the Original Bacillus coli (coli O).

The culture employed was derived from a strain given by d'Hérelle to Bordet and Ciuca. Before beginning their experiment, Bordet and Ciuca made three successive isolations in order to start, as far as possible, with a culture arising from a single organism, and it was from this source that our culture was derived.

Besides the general properties of *Bacillus coli*, such as sugar fermentation and gas and indole production, this original strain of *Bacillus coli* possesses the following characteristics. The colonies on agar plates are large, flat, grape leaf-shaped, and very fluorescent. *Coli* O is non-motile and is very sensitive to the lytic agent, as is shown by the following tests.

Motility Test.—In order to test the motility of a strain, a stab culture is made into semisolid agar (0.5 per cent agar). If none of the organisms of a culture are motile, the growth is confined to the line of puncture. On the other hand, if they are all uniformly motile, they diffuse through the mass of agar and produce a uniform turbidity. If they have a variable motility the result is a combination of the two preceding pictures, that is to say, a dense growth appears close to the line of puncture and is surrounded by a diffuse zone of turbidity. This test is always controlled by a microscopic examination of broth cultures.

Resistance Test.—A suitable resistance test is found in the method of Bordet and Ciuca, slightly modified. These authors place a drop of lytic agent on a 3 hour culture of *B. coli* on agar slant. Resistant colonies grow on the path of clarification left by the drop. The number of these resistant colonies varies directly with the resistance of the strain.

In order to avoid the confusion that might result from the purely mechanical effect of the drop on the young growth, we prefer to place the drop on a sterile agar slant first, and then to seed the tube with *B. coli* 2 hours later. This method shows that *coli* O is very sensitive. Only very few or no resistant colonies appear in the zone of agar touched by the lytic agent.

Action of the Lytic Agent on coli O.—Bordet and Ciuca have shown that when a few drops of lytic agent are added to a broth culture of *coli* O, an almost complete dissolution occurs, with the exception of a very few resistant organisms. When transplanted on an agar slant these organisms produce a very scanty, irregular culture at the beginning, but after several passages the growth becomes more and more luxuriant and is distinguished from the original culture by certain characteristics. These organisms resist the lytic agent but have now acquired themselves lytic properties and become lysogenic, or capable of inducing dissolution in a culture of normal *Bacillus coli.* Moreover, when planted on slanted agar, a mucoid culture results. They are also less phagocytable and more virulent.

We found that all of the bacilli comprising a culture of this modified *coli* do not possess all of these characteristics, which are rather shared by different types of organisms in the culture. The following experiment shows that the modified *coli* is a very heterogeneous culture.

Experiment 1.—0.5 cc. of increasing dilutions of lytic agent (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, straight broth) were mixed respectively with 0.5 cc. of a 12 hour broth culture of *coli* O diluted to 10^{-5}. Immediately afterward, these mixtures were plated in Petri dishes with 10 cc. of plain agar and incubated at 37°C. After 12 hours the result shown in Fig. 1 was obtained; Plate A is the control, *e. g.* a normal

culture of *coli* O, and the following plates, cultures of *coli* O mixed with an increasing quantity of lytic agent.

As shown in a preceding paper (1), the number of resistant colonies left varies in inverse proportion to the amount of lytic agent. But aside from this fact, it is possible to distinguish three types of colonies: (1) mucoid (Fig. 2); (2) non-mucoid and regular (Fig. 1, E and F); and (3) non-mucoid and irregular (Fig. 1, C and D).

Mucoid Colonies, or coli O R 1.

Such colonies are found only occasionally and it may be necessary to repeat the experiment several times to find one colony of this type. They are regular, large, convex, translucent, and non-fluorescent.

If a trace of one of these colonies is streaked on an agar plate, a curious picture results. The mucoid and translucent growth is studded with numerous opaque spots (Fig. 3, B). Among the isolated colonies some are entirely opaque, some entirely translucent, and some mixed. Transplanted, an opaque colony gives only a homogeneous opaque growth. On the contrary, a translucent colony reproduces the same mixture of translucent and opaque colonies. Thus, the translucent growth is a very unstable form which in an ordinary culture is very quickly overgrown by the more stable opaque form. In order to avoid complications, only the latter form will be considered under the distinctive denomination of *coli* O R 1.

Coli O R 1, like the original *coli* O from which it is derived, is nonmotile but is completely resistant to the lytic agent. As is shown by the following test, it is not lysogenic or capable of inducing dissolution in a normal culture of sensitive *Bacillus coli*.

Lysogenic Test.—The technique is based on the fact that lysogenic organisms added, even in a very small number, to a young broth culture of sensitive *B. coli* produce lysis of this culture. The organisms to be tested are planted in 5 cc. of broth (pH 8.0). When the culture is 18 hours old, it is centrifuged and 0.5 cc. of the clear supernatant fluid, still containing a small number of organisms, is added to 5 cc. of a young broth culture (3 hours, pH 8.0) of normal *coli* O. When the growth of the mixture, compared with a normal culture of *coli* O as control, exhibits evidence of inhibition and dissolution, the test is positive.

This is not the case for *coli* O R 1.

To summarize, *coli* O R 1 is a non-motile, very resistant, and non-lysogenic organism producing large, round, mucoid, and non-fluorescent colonies.

Non-Mucoid Regular Colonies, or coli O R 2.

These colonies are found in the two last plates of Experiment 1; that is to say, in those plates in which the lytic agent is present in high concentration (Fig. 1, E and F). They are small, round, convex, hyaline, and non-fluorescent and retain these characteristics when transplanted on agar plates (Fig. 3, A). *Coli* O R 2 also is very resistant, non-lysogenic, and non-motile.

Non-Mucoid Irregular Colonies.

They exist only in the plates in which the lytic agent is present in moderate quantity (Fig. 1, Plates C and D). They are large, flat, non-fluorescent, and extremely irregular.

Experiment 2.—A trace of an irregular colony was streaked on an agar plate. In the first streaks the growth was scanty and irregular; but in the subsequent streaks the colonies became less irregular, showing sometimes only a slight indentation confined to a portion of the edge, and finally, perfectly regular large flat colonies could be found in the last streaks.

Irregular colonies can be transplanted in series many times with the same result. Nevertheless, after a certain number of generations, the proportion of regular colonies appearing in the last streaks increases.

Experiment 3.—An irregular colony, or the irregular edge of a partially irregular colony, was planted in broth. A very slowly growing culture resulted which was lysogenic.

Irregular colonies are thus lysogenic. On the other hand, the regular colonies found in the last streaks have lost this property, as is seen in the following experiment.

Experiment 4.—A regular colony, or the regular edge of a partially irregular colony, was streaked on an agar plate. Only regular colonies appeared. They were large, flat, and non-fluorescent. Transplanted in broth, they gave a normal rapidly growing culture of non-lysogenic and non-resistant organisms.

Thus it is evident that the lysogenic property is intimately related to this irregular growth first described by Bordet and Ciuca (2). This is not only the case for *Bacillus coli* but it is a general rule, since similar observations have already been made by Kuttner for typhoid bacilli (3), by Wollstein for Shiga bacilli (4), and by myself for staphylococci (5).

It is evident also that irregular colonies are diseased colonies and that indentations must be considered as lesions. Our experiments show that when the diseased material is streaked on agar plates, the lesions are very numerous in the first streaks but gradually disappear in the following ones and that in the last streaks only perfectly regular, healthy, and non-lysogenic colonies are found.

It results also from the preceding observations that all the organisms composing a culture of *coli* O do not respond in the same way to the lytic agent. (1) Some are so sensitive that they are dissolved even by very dilute filtrate (spots of clarification, Fig. 1, Plate B). (2) Some are just resistant enough to survive and grow in the presence of a moderate quantity of lytic agent, but they are still weak organisms, and, as they are more or less affected by the lytic agent, they produce diseased irregular colonies. They are the lysogenic organisms of Bordet and Ciuca. When streaked on agar plates, they can recover and produce in the last streaks regular, large, flat colonies made up of sensitive and non-lysogenic organisms. (3) Only a few individuals are sufficiently resistant to survive the strong action of concentrated lytic agent. They are absolutely invulnerable to the lysis and produce regular, small, convex, and hyaline colonies of very resistant and non-lysogenic organisms (*coli* O R 2). (4) Among these very resistant bacilli only a very few are mucoid.

The three principal characteristics of the modified *coli* of Bordet and Ciuca, *i.e.* resistance, lysogenic properties, and mucoid growth, are thus shared among different organisms.

B. coli O + lytic agent = { Irregular lysogenic colonies; Regular non-lysogenic colonies { *Coli* O R 1 = Resistant, mucoid; *Coli* O R 2 = Resistant, non-mucoid }

Action of the Lytic Agent on coli S and coli R.

As described in a preceding paper (1), two different types of organisms, *coli* S and *coli* R, have been obtained by allowing *coli* O to age. Both types have lost the property of producing mucoid growth when submitted to the lytic agent.

Experiment 5.—6 hour broth cultures of *coli* O and *coli* R were added respectively to 0.2 cc. of lytic agent, and incubated at 37°C. When a loopful of each partially dissolved culture was planted on agar slants 36 hours later, a scanty and irregular culture resulted which, after several passages, grew more readily, indeed, but never became mucoid.

This result is confirmed and explained by the following experiment.

Experiment 6.—The technique used in Experiment 1 for *coli* O was applied to *coli* S and *coli* R. Again irregular lysogenic colonies were found in the plates containing weak lytic agent, and regular resistant colonies in the plates containing concentrated lytic agent. But in any case, in spite of a large number of experiments, no mucoid colonies have been found.

In other words, neither *coli* S nor *coli* R contains any mucoid organism, or, at least, any organism capable of becoming mucoid.

Another striking observation can be made in this experiment. On account of its greater resistance, *coli* R gives a greater number of round resistant colonies and only a very few irregular lysogenic colonies. Moreover, when streaked on agar plates, the irregular growth cannot be preserved any length of time; hence, at the end of three or four passages, the growth is almost completely regular. Thus, *coli* R not only is less affected by the lytic agent than the more sensitive strains, *coli* O and *coli* S, but also recovers more quickly.

As the ultimate result of the lytic agent on *coli* S and *coli* R we thus obtain resistant, non-lysogenic, non-mucoid organisms. These organisms we shall term *coli* S R and *coli* R R respectively. *Coli* S R is non-motile, and, when transplanted, grows very slowly, producing only a slight turbidity in broth and very small round hyaline colonies on agar plates. *Coli* R R, on the other hand, is very motile and grows quickly.

$$\underset{\text{(non-motile)}}{\textit{B. coli}\ \text{O} + \text{age}} = \begin{cases} \underset{\text{(non-motile)}}{\textit{Coli}\ \text{S} + \text{lytic agent}} = \underset{\text{(non-motile)}}{\text{Resistant } \textit{coli}\ \text{S R}} \\ \underset{\text{(motile)}}{\textit{Coli}\ \text{R} + \text{lytic agent}} = \underset{\text{(motile)}}{\text{Resistant } \textit{coli}\ \text{R R}} \end{cases}$$

Further Variations of the Modified coli.

An agar slant of modified *coli*, obtained by Bordet and Ciuca, was kept in the ice box for several months before being used in the following experiments.

Experiment 7.—The old modified *coli* was transplanted each day on agar slants. The culture remained mucoid and lysogenic.

Experiment 8.—A trace of the modified *coli* was streaked on an agar plate and after 12 hours incubation two types of colonies were found: the more numerous were mucoid, the others—only a few—were non-mucoid. Both types of colonies were round and non-lysogenic.

Experiment 9.—Both types were streaked respectively on agar plates, and since this first isolation two pure cultures have been obtained which breed true—a mucoid one, or *coli* M 1 (Fig. 4, B), and a non-mucoid one, or *coli* M 2.

B. coli O + lytic agent = Modified *B. coli* + age = { *coli* M 1 (motile, mucoid); *coli* M 2 (motile, non-mucoid) }
(non-motile)

As a matter of fact, it will be seen later that these strains cannot be identified with any of the types above described.

Characteristics and Evolution of coli M 2.

When streaked on agar plates, *coli* M 2 produces large, flat, pale blue, translucent, and non-fluorescent colonies. Different individuals of that type vary greatly in their motility, as observed by microscopic examination and by planting in semisolid medium. Submitted to the resistance test, they show an average resistance; *i.e.*, they do not possess the absolute invulnerability of *coli* R R or *coli* S R, but the path of clarification left by the drop on an agar slant of *coli* M 2 can be completely recovered by a resistant growth as early as 12 hours after seeding. The lysogenic test is negative. All of these characteristics are maintained indefinitely. When freshly isolated, *coli* M 2 is spontaneously agglutinable in plain broth. But this character is transient and disappears after a few passages in broth or on slanted agar.

The most interesting property of *coli* M 2 is its remarkable ability to give mucoid growth when again submitted to the lytic agent.

Experiment 10.—0.2 cc. of lytic agent was added to a broth culture of *coli* M 2 (6 hour, pH 8.0). A rather easy and almost complete dissolution occurred. After 24 hours incubation, a loopful of the partially dissolved culture was streaked on agar plates and gave the result shown in Fig. 4, A. In the first streak, in which the living bacilli and the lytic broth had been spread in abundance, the growth was scanty and irregular, but became less and less irregular in the following streaks, and was finally completely regular in the last streaks. This, of course, was to be expected from our former observations, but the remarkable fact was that a certain number of mucoid colonies was present in the first streak, very often covering the debris of an almost completely dissolved irregular colony of *coli* M 2 (Figs. 5 and 6).

Experiment 11.—Same experiment as the preceding one, but the sample of the dissolved culture was taken and streaked only after 3 days incubation. In this case almost all of the colonies were mucoid.

B. coli M 2	+ lytic agent =	*B. coli* M 1
(non-mucoid)		(mucoid)

It may be concluded that different types of *coli* differ greatly in their ability to give a mucoid growth when submitted to the action of the lytic agent. Some, like *coli* S and *coli* R, lack this property entirely; others, like *coli* O, have it to a certain extent; and some, like *coli* M 2, have it to a very high degree.

Characteristics and Evolution of coli M 1.

Streaked on agar plates, *coli* M 1 gives very large colonies, extremely mucoid, opaque, and fluorescent. Like *coli* M 2, these organisms have a variable motility, an average resistance, and no lysogenic properties.

Experiment 12.—*Coli* M 1 was streaked every day on agar plates, and retained these characteristics even after a great number of passages. But occasionally a colony showed on a portion of its edge a kind of indentation where the growth was non-mucoid. This transformation of mucoid into non-mucoid growth has a very typical aspect which can be seen in Fig. 7.

Experiment 13.—A trace of the non-mucoid material of the indentation described in the foregoing experiment was streaked on agar plates and gave a pure culture of non-mucoid and non-motile organisms (*coli* M 1 a) which possesses absolutely all the characteristics of *coli* O, and which, therefore, can be considered as a reversion to the original type.

With the exception of this occasional reversion, *coli* M 1 is very stable on agar plates, since only mucoid colonies have been observed

in the course of more than 50 platings. *Coli* M 1 is very stable also when kept growing in a synthetic medium, as the following experiment shows.

Experiment 14.—*Coli* M 1 was seeded in a tube of synthetic medium, composed as follows:

Water	1,000 cc.
Glycerol	30 cc.
Sodium chloride	5 gm.
Calcium chloride	0.1 gm.
Magnesium sulfate	0.2 gm.
Dipotassium phosphate	2.0 gm.
Ammonium lactate	12.0 gm.

pH 7.4

Transplantations were renewed daily. At the same time a loopful of each 24 hour culture was streaked on agar plates, in order to control the behavior of *coli* M 1 in the synthetic medium. Nothing but mucoid colonies was found in the plates in the course of more than fifteen passages of *coli* M 1 in synthetic medium.

Quite different was the result when *coli* M 1 was planted in plain broth.

Experiment 15.—Same experiment as the preceding one, but plain broth was substituted for synthetic medium. Since the first passage in plain broth a certain number of non-mucoid bacilli has appeared, as shown by the corresponding plate in which, among mucoid colonies, a certain number of non-mucoid colonies was found. At the end of ten passages in plain broth, almost all the bacilli were non-mucoid.

Plain broth realizes, then, a condition which does not exist in agar plates or in synthetic medium, and which induces the transformation of the mucoid *coli* M 1 into a non-mucoid form, or *coli* M 1 b.

This new non-mucoid form derived from *coli* M 1 in broth is not at all similar to the non-mucoid reversion, or *coli* M 1 a, which appears occasionally on agar plates. The first is extremely motile, while the latter is not. Nor is it similar to the motile non-mucoid modified *coli* described above under the denomination of *coli* M 2, because *coli* M 2, as we have seen, becomes very easily mucoid again when submitted to the lytic agent, while *coli* M 1 b never reverts to the

mucoid form, even in the presence of lytic agent, as is shown in the following experiment.

Experiment 16.—5 drops of lytic agent were added respectively to 6 hour cultures of *coli* M 2 and *coli* M 1 b, and both mixtures were allowed to incubate at 37°C. for several days. A loopful of each mixture was streaked every day on agar plates in order to control the appearance of mucoid bacilli. While numerous mucoid bacilli have already appeared since the 1st day in the mixture of *coli* M 2 with lytic agent, not one could be detected in the mixture of *coli* M 1 b, even after 5 days of incubation.

B. coli M 1 (mucoid, motile)
- In synthetic medium = *Coli* M 1 (mucoid, motile)
- On agar plates =
  - *Coli* M 1 (mucoid, motile)
  - *Coli* M 1 a = *Coli* O (non-mucoid, non-motile)
- In plain broth = *Coli* M 1 b (non-mucoid, motile)

A striking characteristic of *coli* M 1 b is that of giving an extraordinary granular growth when freshly isolated and transplanted in broth. Soon after transplantation, heavy clumps appear, which sediment at the bottom of the tube, leaving a clear supernatant broth.

This property led us to make a rather unexpected parallel with the recent observations of De Kruif on the bacillus of rabbit septicemia (6). This author observed the dissociation of the bacillus into two types: one is extremely virulent, produces opaque and very fluorescent colonies on agar plates, and grows diffusely in broth (Type D); the other type is avirulent, produces translucent and weakly fluorescent colonies, and gives granular growth in broth (Type G).

The similarity with our last findings is evident. Like Type D, *coli* M 1 gives opaque, very fluorescent colonies, and grows diffusely in broth; on the other hand, *coli* M 1 b, like Type G, gives translucent and weakly fluorescent colonies and produces granular growth in broth. According to recent experiments of De Kruif, Type D is able to change into Type G when kept growing in broth; *coli* M 1 b also appears in broth cultures of *coli* M 1, as we have seen. Type G never reverts to Type D; similarly no reversion from *coli* M 1 b to *coli* M 1 has thus far been observed, even in the presence of lytic agent. Because of this parallelism, it seems that the facts pointed out by

De Kruif are not a special feature of the bacillus of rabbit septicemia, but have a more general bearing.

Besides, our attention has been called repeatedly to similar facts reported by Arkwright (7), who found that *Bacillus dysenteriæ*, *Bacillus typhosus*, *Bacillus paratyphosus*, and *Bacillus enteritides*, could be made to yield two forms, the rough ("R") form, which makes stable emulsions, and the smooth ("S") form, which is spontaneously agglutinable. This distinction is similar to that, for instance, in the case of *coli* M 1 and *coli* M 1 b, and also to that in the case of *coli* M 1 and *coli* M 2.

General Properties of the Different Types.

In the course of our studies on the transmissible autolysis of *Bacillus coli*, we have thus isolated ten different types of organisms, all

TABLE I.

Type.	Resistance.	Motility.	Mucoid growth.	Ability to yield mucoid growth.	Fluorescence.	Seroagglutination.
O	+	0	0	+	++++	0
O R 1	++++	0	++++		0	
O R 2	++++	0	0	0	0	
S	+	0	0	0	++	+++
S R	++++	0	0	0	0	+++
R	++	++++	0	0	++	++++
R R	++++	++++	0	0	0	++++
M 1	+++	+++	++++		++++	++++
M 1 a	+	0	0	+	++++	0
M 1 b	++	++++	0	0	++	++++
M 2	+++	+++	0	++++	0	++++

derived from the original *Bacillus coli* of Bordet and Ciuca. They are perfectly distinguished from each other by striking characteristics, such as size, shape, opacity and fluorescence of colonies, mucoid or non-mucoid growth, ability to yield mucoid growth in the presence of lytic agent, motility, resistance to desiccation and to lytic agent, etc. (Table I). The specific properties of the colon bacillus are preserved; namely, the fermentation of carbohydrates, with the exception of saccharose, and the production of indole. The following experiment deals with the agglutination reaction.

Experiment 17.—Rabbits were immunized respectively with *coli* O, *coli* S, and *coli* R. The agglutinating power of the three antisera obtained was tested on nine of our strains by the following technique advised by Bordet and Ciuca. Increasing dilutions of the three antisera were made with plain broth and 1 cc. of each dilution of each serum was seeded respectively with one of the nine strains and incubated at 37°C. When the bacilli grew in clumps, the result was considered as positive. When the growth was diffuse the result was considered negative. The results are shown in Table II.

TABLE II.

Antiserum.	Titer of antiserum.	Strain.								
		O	S	S R	R	R R	M 1	M 1 a	M 1 b	M 2
O	1:10	−	++	+	+++	+++	+++	−	+++	+++
	1:100	−	+	−	+++	+++	++	−	++	−
	1:500	−	−	−	++	++	+	−	+	−
	1:1,000	−	−	−	−	−	−	−	−	−
	1:5,000	−	−	−	−	−	−	−	−	−
S	1:10	−	+++	+++	++	++	+++	−	++	++
	1:100	−	+++	+++	++	++	+++	−	++	++
	1:500	−	++	++	+	++	+++	−	++	++
	1:1,000	−	+	−	+	+	+++	−	+	++
	1:5,000	−	−	−	−	−	+++	−	+	++
R	1:10	+	+++	+++	+++	+++	+++	+	+++	+++
	1:100	+	+++	+++	+++	+++	+++	+	+++	++
	1:500	−	+++	+++	+++	+++	+++	−	+++	++
	1:1,000	−	+++	++	+++	+++	+++	−	+++	++
	1:5,000	−	++	−	+++	+++	+++	−	++	++

Two interesting facts appeared in this experiment. (1) While all the other strains grew in clumps in the presence of any of the three antisera, only the original *coli* (*coli* O) and the reversion to the original type (*coli* M 1 a) were not agglutinable, even by their corresponding antiserum, *i.e.* the serum obtained from a rabbit immunized with *coli* O, which, however, agglutinated the other types. It is very interesting that Type M 1 a, which we already considered as a reversion on account of its similarity with the original *Bacillus coli*, shows the same lack of agglutinability as *coli* O. (2) The titer of the anti-*coli* S serum and of anti-*coli* R serum was greater than 1:5,000, while,

on the other hand, the power of anti-O serum was much lower and hardly reached a titer of 1:500. *Coli* O is not only non-agglutinable but possesses also weak antigenic power.

DISCUSSION.

What is the nature of the different variations above described?

The notion of contamination can be disregarded with certainty, not only in consideration of the care constantly taken but because it has been possible to reproduce the experiments at any time, always with the same expected result, and because a certain number of our types have been isolated simultaneously in Brussels by Bordet and Ciuca from the same original strain of *Bacillus coli.*

The range of the variation never goes beyond the limit of the species: all of the strains still possess the specific properties of *Bacillus coli.*

All of the types, once isolated and regularly transplanted, keep their individuality even after several months. They are thus stable. Nevertheless, they are not all uniformly stable, and certain types, like *coli* M 1, for instance, still readily undergo changes under certain definite influences.

It is out of the question also to say that the eleven types coexisted in the original strain, because in order to admit such a coexistence it would be necessary to imagine that at least eleven different organisms, each of them representing one of our variations, came together through the three successive isolations performed before the present studies were begun. We have thus been forced to accept the conclusion that the different types observed are the result of changes occurring in the original *Bacillus coli* in the course of these studies. The new types always appear under certain definite conditions of the environment, but it is still impossible to determine whether the external influence is the direct cause of the variation or only an occasional factor which makes apparent, by mere selection, a modified germ already present but in too small numbers to be detected, and produced independently of the environment itself.

It is a common observation that transmissible lysis of a bacterium, induced under certain influences such as stool filtrate, peritoneal exudate, tissue extracts, and vaccine, happens in a rather haphazard

and irregular fashion and seems a question of luck. This fact is easily explained by the hypothesis of a bacteriophage virus. But if the phenomenon is not due to a virus, but as suggested by Bordet and Ciuca, to some autolytic vitiation of the bacteria, the irregularity with which the phenomenon is promoted must find its cause in the bacteria themselves. It is possible that a given bacterium is not always capable of starting the lysis. Various forms of *Bacillus coli* appear to differ in their properties, for example in their behavior toward the lytic agent. Certain forms become mucoid in the presence of the lytic agent. Similarly, it may be possible for only a certain form to undergo autolysis. In order to start the dissolution it is necessary to employ the proper forms of bacteria.

SUMMARY.

1. When the few individuals still alive in a dissolved culture of *Bacillus coli* are transplanted on slanted agar, a culture results which possesses new characteristics. First observed by Bordet and Ciuca, this culture received the temporary name of modified *coli*.

In the study described above, we found that this modified *coli* is very heterogeneous and that its three principal characteristics, resistance to lysis, lysogenic properties, and mucoid growth, are shared among different types of organisms that can be isolated when the normal original *coli* (*coli* O) is plated together with increasing quantities of the lytic agent: (*a*) a certain number of bacilli are just resistant enough to survive and grow in the presence of a moderate quantity of lytic agent, but they are still more or less sensitive and produce diseased, irregular, and lysogenic colonies; (*b*) a few of the organisms are able to resist concentrated lytic agent; they are entirely resistant and give round, healthy, and non-lysogenic colonies (*coli* O R 2); and (*c*) among these resistant bacilli only a very few are mucoid (*coli* O R 1). All these types are not motile and not fluorescent.

2. The original *coli*, when allowed to age, can be dissociated, as we have shown in a preceding paper (1), into two types of organisms, the non-motile *coli* S and the very motile *coli* R. Submitted to lysis, *coli* S gives a very small number, *coli* R a much greater number of resistant organisms (*coli* S R and *coli* R R), but both types never yield any mucoid growth.

3. An old culture of the modified *coli* obtained by Bordet and Ciuca, when streaked on agar plate, gives two types of colonies: a mucoid and fluorescent type (*coli* M 1) and a non-mucoid and translucent type (*coli* M 2). Both types are motile.

Coli M 2, once isolated, keeps its individuality even after several passages in artificial media, but if again submitted to the lytic agent, a great number of mucoid bacilli are found among the organisms which are still alive.

Consequently, different types of *Bacillus coli* differ greatly in their ability to give a mucoid growth when submitted to the lytic agent. Some, like *coli* S and *coli* R, do not possess this property at all. Others, like *coli* O, possess it to a certain extent, and some, like *coli* M 2, have it to a very high degree.

4. The mucoid and motile *Bacillus coli* M 1, when streaked every day on agar plates, remains indefinitely mucoid and motile, but occasionally a mucoid colony shows an indentation made up of non-mucoid growth, which, transplanted, gives a pure culture of non-mucoid and non-motile organisms, *coli* M 1 a. This new type possesses all the characteristics of the original strain of *Bacillus coli*, and therefore must be considered as a reversion.

5. The mucoid and motile *Bacillus coli* M 1, kept growing in synthetic medium, remains perfectly stable; on the other hand, when it is transplanted in broth, *Bacillus coli* M 1 turns very quickly into a non-mucoid but still very motile organism, or *Bacillus coli* M 1 b. This last type, which produces translucent colonies on agar and grows granular in broth, never reverts to the mucoid form, even in the presence of lytic agent.

6. A single strain of *Bacillus coli* has thus been made to yield eleven different forms, all distinguished by striking characteristics, but still possessing the specific properties of *Bacillus coli*.

Nine of these forms have been submitted to antisera prepared with three different types (*Bacillus coli* O, *Bacillus coli* S, and *Bacillus coli* R). While seven out of these nine strains were agglutinated by any of the three antisera, only the original *Bacillus coli* (*Bacillus coli* O) and the reversion to the original type (*Bacillus coli* M 1 a) were not agglutinable, even by their corresponding antiserum; *i.e.*, the serum obtained from a rabbit immunized with *Bacillus coli* O, which, however, agglutinated the other types.

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EXPLANATION OF PLATES.

PLATE 7.

FIG. 1. Experiment 1. *Coli* O plated with increasing quantities of lytic agent (O, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}). Plate A. Control; normal culture of *coli* O. Plates B, C, and D. *Coli* O + moderate quantities of lytic agent; note the great number of irregular colonies. Plates E and F. *Coli* O + concentrated lytic agent; note the presence of only small regular colonies. No mucoid colonies are found in this experiment.

PLATE 8.

FIG. 2. When Experiment 1 is repeated several times, a mucoid colony can occasionally be found. Here is a plate of *coli* O mixed with concentrated lytic agent. Besides the small regular colonies, two large mucoid colonies are visible.

FIG. 3. Plate A. A small round colony of Plate F, Fig. 1, was planted and gave a pure culture of *coli* O R 2. Plate B. The lower mucoid colony seen in Fig. 2 was transplanted and gave a mucoid culture of *coli* O R 1; note the translucent growth studded with opaque spots.

FIG. 4. Plate A. Experiment 10. A loopful of a partially dissolved culture of *coli* M 2 was streaked on an agar plate. In the first streak the growth is very irregular and contains a certain number of mucoid colonies. In the second, the colonies have already become less irregular, and in the third they are normal. Plate B. Mucoid growth of *coli* M 1.

FIG. 5. Magnification of a portion of the first streak of Plate A, Fig. 4. Showing mucoid colonies among the very irregular colonies of *coli* M 2.

FIG. 6. The same. A mucoid colony recovering the debris of a colony of *coli* M 2.

FIG. 7. Magnification of the edge of a colony of *coli* M 1 where an indentation of non-mucoid growth (light) issues from the mucoid growth (dark).

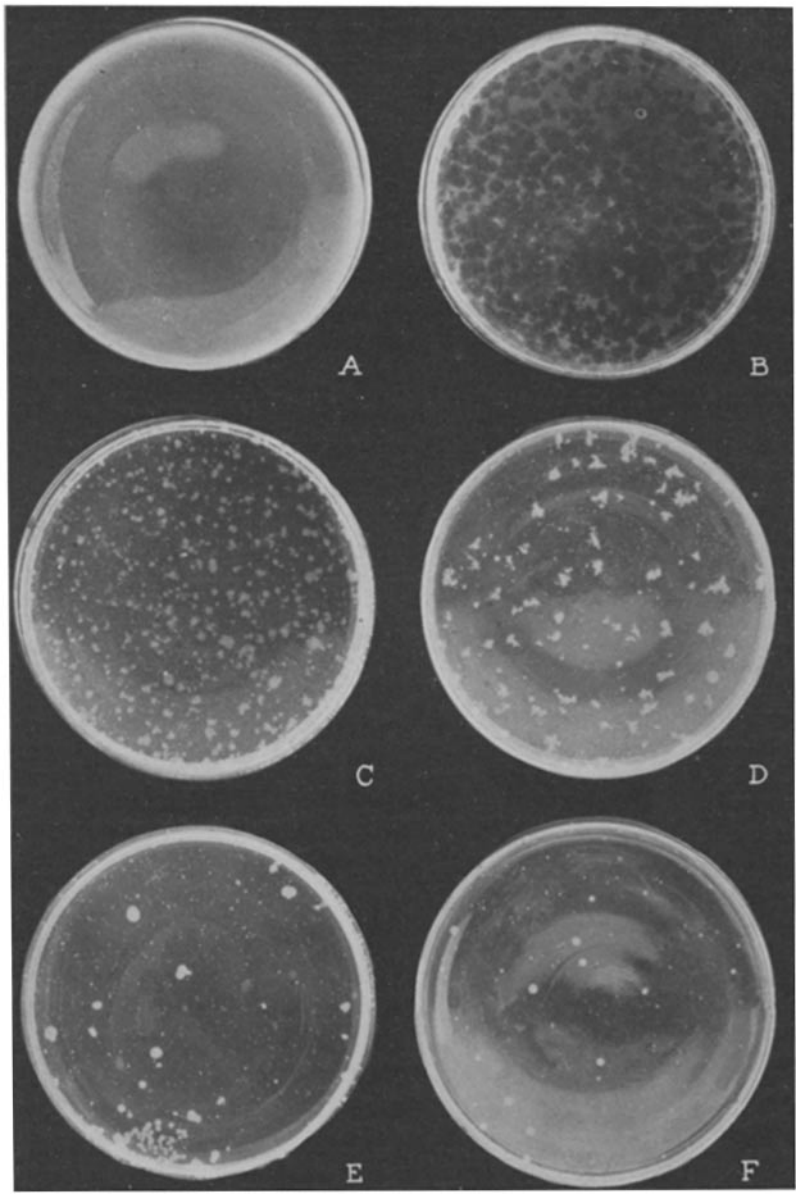

FIG. 1.

(Gratia: Twort-d'Hérelle phenomenon. II.)

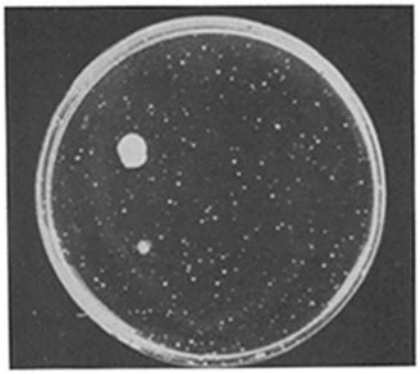

FIG. 2.

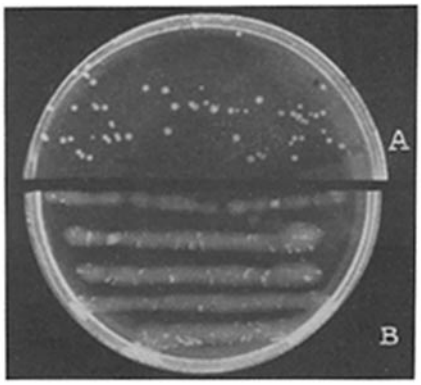

FIG. 3.

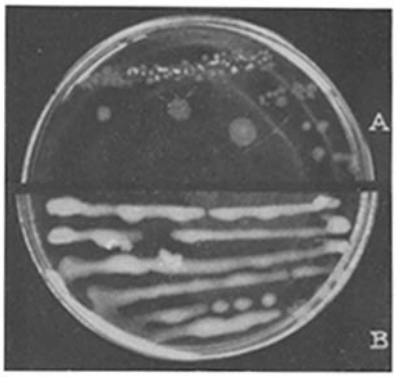

FIG. 4.

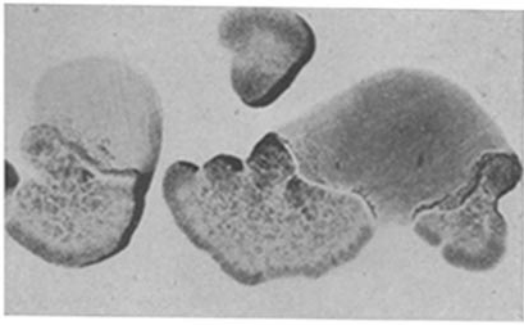

FIG. 5.

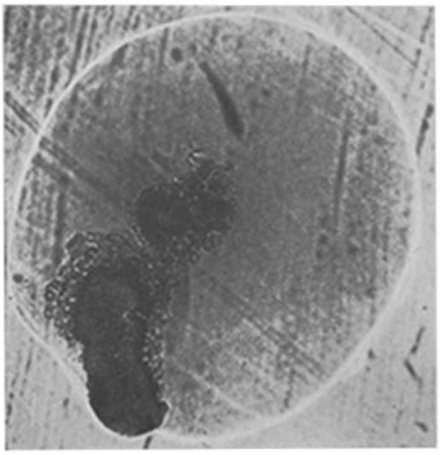

FIG. 6.

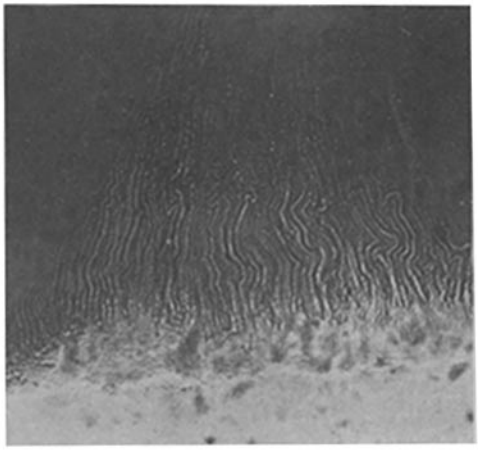

FIG. 7.

(Gratia: Twort-d'Hérelle phenomenon. II.)