

GELATIN SURFACE COLONIES OF BACILLUS COLI COMMUNIS.

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(PLATE XXXI.)

THE surface and deep colonies of *Bacillus coli communis* in gelatin plates are always recorded as an important diagnostic feature of the organism. The surface colonies are particularly important; and, indeed, the colonies in the depth can, from the point of view of practical diagnosis, be neglected. They are not characteristic, and vary with depth, etc.

The importance of the surface colonies, from the practical aspect of the subject, is that this characteristic is largely made use of in the various methods employed for isolating this organism from water, soil, etc. Surface gelatin colonies, unless the plate is overcrowded, are usually most typical, and quite sufficiently developed for diagnosis and sub-culture after about forty to forty-eight hours' incubation at 37° C., so that throughout this paper, unless otherwise specially indicated, two days' surface plate gelatin plate colonies are those described and photographed.

I have not observed an alteration of type from the use of different percentages of gelatin, but my experiments in this direction have been but few. It was, however, thought desirable that standard and uniform gelatin media should be used, so that in every case 12 per cent. gelatin of uniform reaction, *i.e.* +1 per cent. to phenolphthalein was used. In every case the brushings were performed by adding one loop of broth to 10 c.c. or so of sterile water. A sterile rubber brusher was dipped into this, and, after mixture, withdrawn and brushed over one to two plates containing solidified gelatin. In this way perfectly discrete colonies, and not too numerous, can practically invariably be obtained.

In the course of work (extending over two years) bearing upon this organism and its intimate allies, and during which over two hundred *B. coli* from different sources, but mainly from water supplies, have been isolated, it has been frequently noticeable that though the surface gelatin colonies are as a rule typical, yet considerable

variations are met with. For some of my later work I have carefully recorded this character, making sketches, and in many cases photographing, the colonies.

In a few instances the variability of this character for the same organism was studied.

In dealing with this microbe, it is of great importance to be certain that the organisms described as *B. coli* are really this organism, and not merely allied. A summary of the culture characters of the organisms specially studied for their gelatin surface colonies is therefore given.

All these organisms (except one, No. 79, isolated about a year previously) were consecutively isolated, and with but few exceptions were obtained from different sources. In a few cases several distinct *B. coli* were found in plates from the same source, while in a few cases organisms were from the same source, but were isolated on re-examination many months later. With these exceptions they were all from different sources.

It will be seen that, except Class II., all are undoubted *B. coli*. In Class II. a few organisms not, or doubtful, *B. coli* are included, but their colony characters are considered by themselves.

CLASS I. Includes seventy-two organisms. It can be divided into the following sub-groups.

Group 1. Includes organisms with the following characters:—Short bacilli with rounded ends. No spores. Stain by ordinary stains. True motility present. Uniform turbidity in broth in twenty-four hours, with or without surface film. Bluish translucent growth on gelatin slope. No liquefaction

TABLE I.—CLASS II. ORGANISMS.

	171.	209.	199.	167.	200.	191.	186.	188.
Glucose gas .	+	+	— ¹	+	—	+	+	+
Lactose gas .	—	(little).	+	—	+	—	—	—
Neutral red re- action.	+	Partial	(slow). — ¹	+	(slow). —	+	+	+
Acid production (litmus).	+	+	+	+	+	+	Acid first Alkaline after a week.	+
Coagulation (milk).	—	—	—	—	+	+	—	+
Motility . .	—	—	+	+	(3 days). +	+	+	(6 days). +
Potato . .	Colour- less growth.	Pale yellow growth. Traces.	Yellow growth.	(very active). Pale yellow growth.	Yellow growth.	Yellow growth.
Indol production (one week).	+	+	+	+	—	+	+	+

¹ After growth in laboratory for about a week, fresh preparations fermented glucose and gave a complete neutral red reaction.

of the gelatin within four weeks. A positive neutral red reaction in glucose agar. Production of acid (in milk). Coagulation of milk within ten days. Fermentation of glucose, with gas production (tested in agar shake preparations). Fermentation of lactose, with gas production (tested in Durham's tubes in 1 per cent. lactose and 1 per cent. peptone medium). Production of indol in broth (1 week) or peptone water (ten days). Potato cultures examined for about two-thirds of the organisms isolated, generally yellow to brown growth.

Group 2. Exactly similar to Group 1, but no true motility observed in twenty-four hours' broth.

Group 3. Exactly similar to Group 1, but no milk coagulation (one month).

Group 4. Exactly similar to Group 1, but no indol production.

Group 5. Exactly similar to Group 1, but no glucose gas production, and no neutral red reaction.

CLASS II. Organisms doubtful, or closely allied to *B. coli*.

In Class II. the surface colonies of eight organisms were recorded.

All eight organisms were short bacilli, with rounded ends and no spores, producing uniform turbidity in broth, and, except No. 199, which produced a white opaque growth, growing as a bluish semi-translucent growth on gelatin slope, without subsequent liquefaction. In other characters, however, they differed from typical *B. coli*, as is seen in Table I. :—

The number of organisms in the different groups is as follows :—

CLASS I. Group 1 = 40, Group 2 = 25, Group 3 = 3, Group 4 = 3, Group 5 = 1. 72 in all.

CLASS II. Eight organisms.

Source of these eighty organisms :—

	Class I.	Class II.
Drinking water	45	3
Typhoid stool	3	0
Sewage	1	0
Sea-water	9	0
Oysters and cockles	7	3
Soil	7	2
	<hr/> 72	<hr/> 8

In investigating the 40–48 hours-gelatin surface colonies several types are met with, and some classification can be made.

TYPE A.—This is undoubtedly the common type, at anyrate as met with in water supplies.

It is well seen in the photographs, and may be described somewhat as follows :—

To the naked eye a bluish translucent flat colony, with an irregular margin.

Under a low power of the microscope it appears as a flat, colourless, or pale brown growth, with an irregular outline and wavy indented margin. It has either a central circular area or not. Over the surface and extending in from the margin, and particularly observable away from the centre, are a number of wavy lines (marmoration). Photographs of No. 165 (Plate XXXI. Fig. 8) and No. 181 (Plate XXXI. Fig. 27) illustrate this type.

Subtype A. Very similar to Type A, but the colonies are uniformly granular, and pale brown all over, with neither central area nor wavy lines.

TYPE B.—To the naked eye a somewhat raised circular colony, whiter and more opaque than Type A, and generally somewhat smaller. Under a low power it appears as a well-defined colony, nearly circular, with well-defined slightly wavy border. These colonies are brown, uniformly and finely granular all over, showing no wavy lines. With or without a central area. Photograph of *B. coli* 196 (Plate XXXI. Fig. 7) illustrates this type.

TYPE C.—Small colonies, usually much smaller than either A or B, quite circular, with perfectly smooth, well-defined margins. Brown and uniformly granular, with no central area and with no surface markings. The photograph of *B. coli* 204 (Plate XXXI. Fig. 25) illustrates this type.

The rest of the organisms may be grouped under one heading, *Group D*. Group D includes all the irregular varieties and all the colonies not corresponding to Groups A, B, or C. The Group D organisms (Class I.) comprise seven bacteria, *i.e.* Nos. 160, 147, 184, 185, 189, 79, and 217.

Photographs are shown of 160 (Plate XXXI. Fig. 6), 184 (quite opaque and structureless) (Plate XXXI. Fig. 23), 185 (Plate XXXI. Fig. 24) (showing a distinct tendency to be fissured), 217 (Plate XXXI. Fig. 13), and 79*a* (Plate XXXI. Fig. 28) (*i.e.* not the original 79, but after keeping in the laboratory). 147 was chiefly characterised by marked lines radiating from the centre. 189. Small, nearly circular, colonies, with slightly wavy border. Nearly circular dark brown area in or near centre of all the colonies. 217. Colonies very similar to those of 185, the chief difference being that they were rather smaller.

The *coli* organisms were derived from five different sources. In the table given below (Table II.) the incidence of the different types is given, and also according to the origin of the *B. coli* isolated :—

TABLE II.

Source of the Organisms.	CLASS I.					CLASS II.				
	Type A (incl. A ₁)	Type B.	Type C.	Group D.	Total.	Type A.	Type B.	Type C.	Group D.	Total.
Drinking water . . .	33	3	6	3	45	2	1	0	0	3
Typhoid stool and sewage.	3	1	0	0	4	0
Sea-water	6	1	0	2	9	0
Oysters and cockles . . .	1	1	4	1	7	1	1	0	1	3
Soil	1	0	5	1	7	0	0	1	1	2
Total	44	6	15	7	72	3	2	1	2	8

The totals are too small for fine numerical calculations, but some broad deductions may be made. Type A may be considered the typical form of *B. coli* gelatin surface colonies. Type B does not differ so very markedly, and may be considered subtypical; while Type C and Group D are quite atypical as compared with the ordinary type.

Considering Class I. alone, we find :—

<i>B. coli</i> from all sources,	61 per cent.	quite typical,	70 per cent.	nearly typical,	30 per cent.	quite atypical.
„ drinking water 73·3	„	„	80	„	„	20
„ all other sources 34·8	„	„	43·4	„	„	56·5

Or claiming Types A and B as normal, and C and D as abnormal, we obtain the following table— :

TABLE III.

Source of the Organisms.	CLASS I.				CLASS II.			
	Normal.	Abnormal.	Percentage Normal.	Percentage Abnormal.	Normal.	Abnormal.	Percentage Normal.	Percentage Abnormal.
Drinking water	36	9	80·0	20·0	3	0
Typhoid stool and sewage .	4	0	100·0	0·0	0	0
Sea-water	7	2	77·8	22·2	0	0
Oysters and cockles . . .	2	5	28·6	71·4	2	1
Soil	1	6	14·3	85·7	0	2
Total	50	22	69·5	30·5	5	3	62·5	37·5

This table clearly shows that the source of the organism has a very considerable influence on the type of colony produced.

The comparison of the shell-fish *B. coli* with the sea-water *B. coli* is particularly striking, since they are both from a common source. Three-fourths of the sea-water colonies were typical, but only one-fourth of the shell-fish colonies. The difference must be ascribed to the modifying influence caused by the passage through the cockle, and in part to the fact that the method of isolation had some influence.

It is also noteworthy that the Class II. organisms, as far as their limited number will allow a comparison, followed the lines of the Class I. organisms, and this fact helps to add a link to the chain of evidence which would declare these organisms as true *B. coli* organisms modified by environment. The only other factor which would be likely to influence the type of colony is variation in the method used for the isolation of these organisms.

Only two methods were used—one the neutral red glucose broth method, and the other the addition of the material to tubes of media composed of 1 per cent. lactose and 1 per cent. peptone. They were all (that is, Class I.) isolated by the first method, except six from soil and three from shell-fish. One soil organism and four shell-fish organisms were isolated from neutral red broth.

Of the six soil organisms isolated from lactose peptone, one was Type A and five Type C. The one soil organism isolated from broth was a Group D organism. Of the seven shell-fish organisms, two belonged to Type C and one to Group D, when lactose peptone was used to isolate; while of the four isolated from neutral red broth, one belonged to Group A, one to Group B, and 2 to Group C.

It is probable, therefore, that the method of isolation is also of importance in determining the type of colony.

All the Class II. organisms, except No. 191 (from "made soil"), were isolated from neutral red broth. No. 191, isolated by lactose peptone, was a Type C organism.

The variation of colony characters under cultivation was also studied.

A question of scientific and, to a less extent, of practical interest is the study of gelatin surface colonies from the point of view of the variability of this character with varying environment. Bacteria have been but little studied from the point of view of broader biological problems and laws. We really know very little concerning the variation of cultural characters under environment, and it is very doubtful how far any definite environment induces a definite cultural variation. Different observers have recorded alteration of type of colonies of *B. typhosus* and *B. coli* kept in sewage, but the experiments recorded below show that marked deviations from the "typical" take place under ordinary laboratory conditions.

In these experiments the bacteria were kept under very ordinary conditions, *e.g.* in broth or on agar slope at 37° C., or room temperature, etc.

A number of points of interest arise, of which the following appear to be the most interesting:—

1. Will atypical gelatin surface colonies of *B. coli* become typical under cultivation?
2. Will typical gelatin surface colonies of *B. coli* remain typical?
3. Has the culture medium any definite and constant action on the colony character?
4. Do the variations, if such are met with, proceed in a definite way; are there, for instance, definite types of variation.

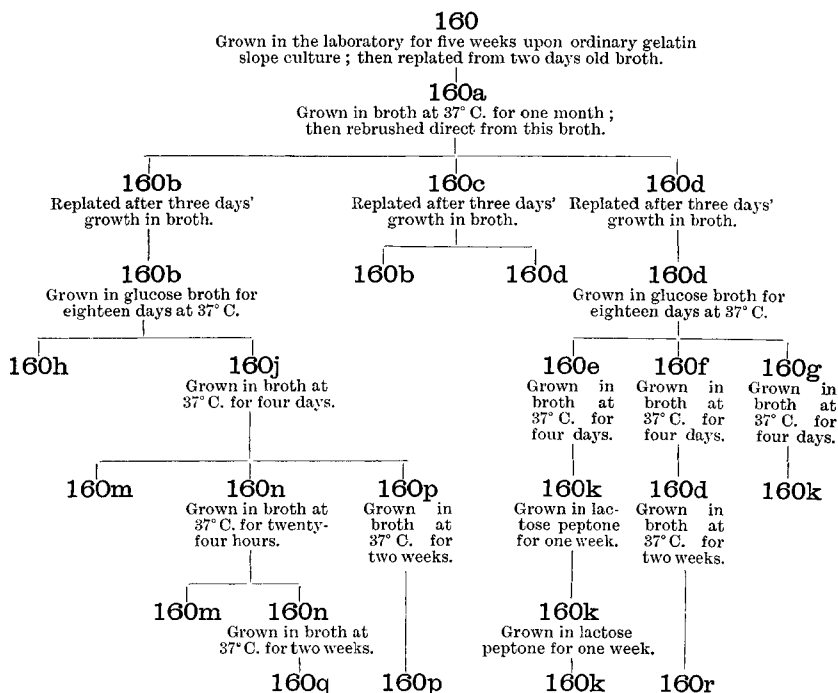
A number of atypical colonies are included in Group D. Several of these were studied in detail, and two at considerable length. These two will be first considered.

No. 160. A quite typical organism when isolated, except for three points, *i.e.* the gelatin surface colonies were atypical (see Plate XXXI. Fig. 6), no indol was formed, and the bacilli in broth showed very active motility. It was isolated from an upland surface water. Its gelatin surface colonies were so atypical that its characters were not worked out until after it had been replated, to be certain that it was a pure growth. It was then kept under the conditions described in Chart I., being replated after the periods mentioned, and each of the different kinds of colonies noted, and in many cases photographed.

Photographs¹ of 160 (Plate XXXI. Fig. 6), 160*b* (Plate XXXI. Fig. 10), *c* (Plate XXXI. Fig. 4), *d* (Plate XXXI. Fig. 3), *e* (Plate XXXI. Fig. 12), *m* (Plate XXXI. Fig. 1), *p* (Plate XXXI. Fig. 2), *k* (Plate XXXI. Fig. 11),

¹ The photographs were made directly from the Petri dishes. They were nearly all taken with the same degree of magnification, *i.e.* about 50 diameters.

q (Plate XXXI. Fig. 9), and *r* (Plate XXXI. Fig. 5) shown, while a brief description of the other colonies is appended.

CHART I.¹

No. 160a. Perfectly typical *B. coli*, Type A.

No. 160f. Colonies rather resembling Type C, *B. coli* colonies described above.

No. 160g. Irregular rather opaque colonies, with wavy markings all over.

No. 160j. Very similar to 160g.

No. 160n. Very similar to 160m, except that the colonies are smaller and with finer lines radiating out into the gelatin.

No. 160h. Type B colonies, except that there is a small central area. They were very few in number.

With colonies so peculiar as 160 b, e, etc., a suspicion of contamination naturally arises. Repeated subcultivations on the different media were, however, made from time to time, and particularly with the very abnormal colonies. In every case typical *B. coli* reactions were obtained. Also, 29th January 1903, the four final varieties, *p*, *q*, *k*, and *r*, were subcultivated side by side, to see how far cultural variation might also have taken place. The results are shown in Table IV.

¹ General Explanation of the Charts.—The note below the figures gives the conditions under which the organism was cultivated. The figures indicate the different kinds of colonies produced by this procedure, when plated out on surface gelatin. Thus 160a, when grown in ordinary peptone broth at 37° C. for one month, gave three kinds of colonies, 160b, 160c, and 160d.

TABLE IV.

<i>Coli.</i> 160.	<i>p.</i>	<i>q.</i>	<i>k.</i>	<i>r.</i>
Broth (24 hours)	Uniform turbidity; deposit and scum.	Clear broth, with suspended flakes and thick scum.	Clear broth, with suspended flakes and thick scum.	Turbid, but not uniform; thick scum.
Motility (from 24 hours' broth)	Active motility.	Moderately active motility.	Very active motility.	Fairly active motility.
Litmus milk	5 days; partial coagulation. 6 days; complete coagulation.	5 days; complete coagulation.	5 days; complete coagulation.	5 days; no coagulation. 6 days; complete coagulation.
Acid production.	4 days; well-marked acid production. + well marked.	4 days; well-marked acid production. + fairly marked.	4 days; well-marked acid production. + well marked.	4 days; no acid. 6 days; distinct acid production. + well marked.
Indol (9 days' broth culture). Glucose neutral red agar shake.	2 days; very marked gas and complete reaction.	2 days; very marked gas and complete reaction.	2 days; very marked gas and complete reaction.	Complete reaction only in 3 days; marked gas.
Lactose gas	+ gas.	+ gas.	+ gas.	+ gas.

It will be noticed that they all show very similar characters, and that the differences are very slight. *p* is similar to the original 160, even to the date of milk coagulation, except that the original organism, as isolated, produced no indol. The varieties are now even more typical and produce indol.

We have here an organism which, at first very abnormal in its gelatin colonies, became quite typical under cultivation. When, however, kept in broth at 37° C. for a month, it shows quite abnormal colonies, which in this case never became normal again. Its cultural characters were nevertheless maintained. It is to be noted that 160*b* and 160*d* were not ephemeral phases, but were stable, at any rate for a few days. The variations met with were of a very extraordinary character, particularly 160 *b*, *e*, and *k*, and were not in the slightest degree like ordinary *B. coli* colonies. 160*k* also seemed to be a fairly stable variety.

No. 147. Isolated from a shallow well. A typical *B. coli* except the colonies. These were abnormal, and showed marked linear arrangement radiating from the centre; near margin quite clear and non-granular.

It subsequently became perfectly typical, *i.e.* 147*d* and 147*e*.

After replating after further growth in broth, on one of the plates two quite similar but abnormal colonies were found. One of these was subcultivated and worked out. They seem to have been a kind of "sport," and when further plated gave rise to a number of abnormal varieties, with, however, a return in some cases to typical *B. coli*.

The persistence of the radiation characters in some of the types is very noticeable, *e.g.* 147 *n* and *l* (Plate XXXI. Figs. 16 and 19). The cultural characters of 147 *j* and *k* were fully worked out, and were identical with those of the original 147.

This organism illustrates the fact that an abnormal colony will become normal again, but that abnormal varieties may again develop.

Photographs of 147 *f* (Plate XXXI. Fig. 17), *g* (Plate XXXI. Fig. 18), *j* (Plate XXXI. Fig. 15), *k* (Plate XXXI. Fig. 20), *n* (Plate XXXI. Fig. 16), *l* (Plate XXXI. Fig. 19), *p* (Plate XXXI. Fig. 21), shown, and a sketch of 147*h* (Plate XXXI. Fig. 14).

A brief note of some of the other colonies is appended.

147*a*. Similar to 147, but lines less marked.

147*b*. *B. coli*, Type B.

147c. Small circular well-defined margin; pale yellow brown; uniformly granular, with faint surface markings.

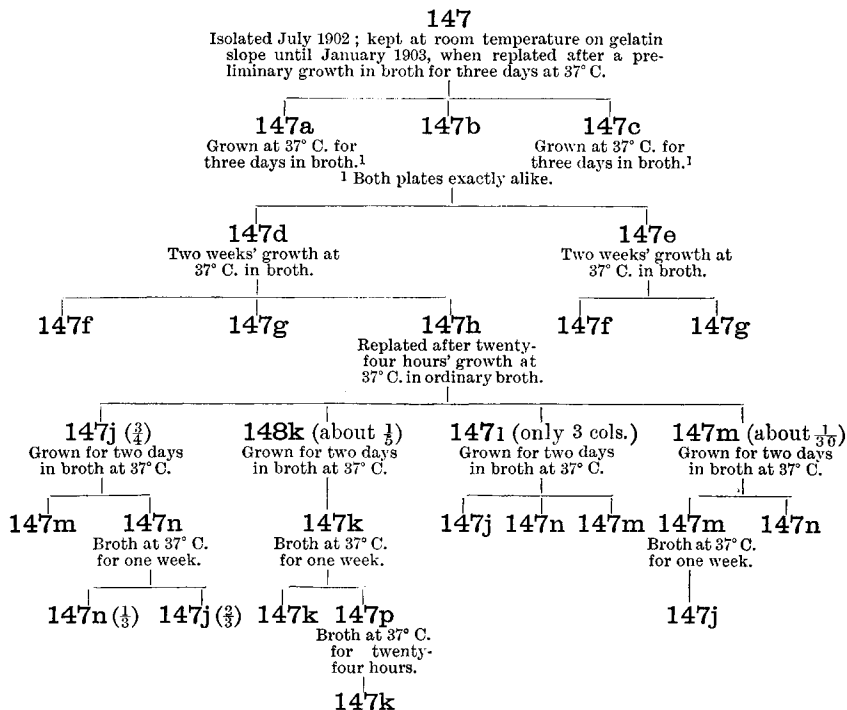
147d. Quite typical *B. coli*, Type A.

147e. Quite typical *B. coli*, Type B.

147m. Very similar to 147n, but surface lines less marked.

147k. Plate XXXI. Fig. 20, shows a considerable resemblance to 160b (Plate XXXI. Fig. 10) and also 160k (Plate XXXI. Fig. 11).

CHART II.



Coli 79. This organism when isolated was not typical. The gelatin surface colonies showed a very marked radiation, which was still more marked in the agar surface colonies; only traces of indol were produced, the organism did not ferment glucose, while no neutral red reaction could be produced. Lactose was, however, fermented. On being kept in the laboratory it became typical as regards these and all other characters, except in gelatin surface colonies. It was kept under varying conditions, but all attempts to cause it to become typical in regard to its gelatin colonies were unsuccessful. Photographs 79a (Plate XXXI. Fig. 28) and 79b (Plate XXXI. Fig. 26) show the well-marked radiation. 79a was grown for five weeks in sterile tap water plus a trace of broth at outside temperature, but when replated, colonies were unchanged. A further four weeks' growth gave 79b. This specimen was isolated from a deep well water.

Coli 184 (Plate XXXI. Fig. 23). Dense opaque colonies. Isolated from sea-water contaminated with sewage. On cultivation in broth, and also after inoculation of a guinea-pig and recovery from the spleen, typical *B. coli*, Types A and B, were obtained.

Coli 185 (Plate XXXI. Fig. 24) and 217 (Plate XXXI. Fig. 13). Colonies were abnormal and fissured when isolated, but it was not investigated to see whether it would become normal.

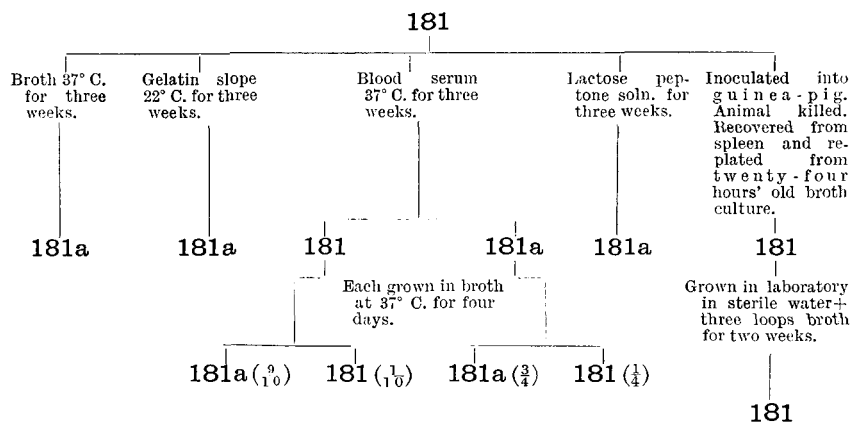
Coli 189. Became typical, Type B and some Type C, after being kept in the laboratory.

Out of these six atypical colonies four were further investigated, and three out of the four became typical wholly or in part, while one remained atypical.

Variation among some of the other groups was also studied. Group A includes forty-four organisms; of these, Nos. 165 and 181, among others, were further investigated.

No. 165 (Plate XXXI. Fig. 8). A quite typical *B. coli* isolated from a well water.

CHART III.



It was inoculated into a flask of 100 c.c. sterile water and kept outside for five weeks. This, brushed over gelatin plates, gave colonies all perfectly typical and unchanged. The cultural characters of the organism also remained quite unchanged. Here a typical *B. coli* remained typical under the conditions of the experiment.

Coli 181 (Plate XXXI. Fig. 27). Isolated from a pure water, mixed spring and upland surface. This organism was grown on a number of different media and under different conditions. It was possible to produce a certain amount of variation from the typical (181a) (Plate XXXI. Fig. 22), but no marked abnormalities were met with.

See Chart III. and Photographs 181 and 181a.

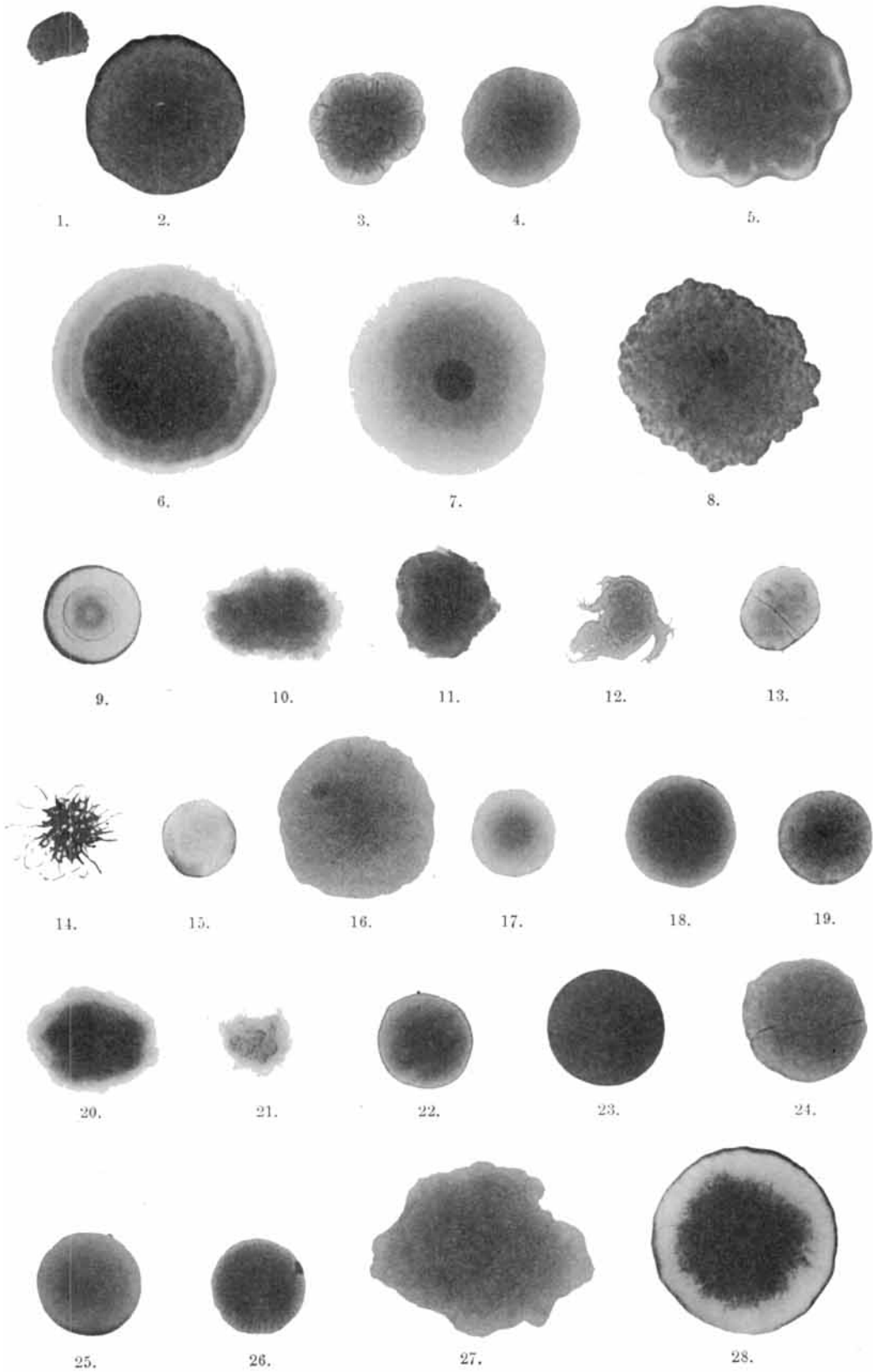
Several other organisms of this class showed very little variation. Several Type C organisms were also studied, *i.e.* 178, 208, 177, 173.

Coli 178. Isolated from same water as *Coli* 79. Quite typical except for gelatin surface colonies. These were Type C, but were very small. Grown in the laboratory on agar slope for six weeks, and then plated from twenty-four hours' old broth, it gave larger colonies, but still of the Type C type. Grown then for a further month in broth at 37° C. and then replated, perfectly typical *B. coli* colonies resulted, although a certain number of the colonies were very granular and not quite typical.

Coli 208. Colonies just like No. 204 (Plate XXXI. Fig. 25). Isolated from dustbin refuse deposited on land one month old. Under cultivation became typical Types A and B.

Coli 177. Isolated from a shallow well. Quite typical except for gelatin colonies. These were Type C. Grown for one week in broth at 37° C. and then plated, 90 per cent. of the colonies were perfectly typical *B. coli*, Type A (177a), while the remaining 10 per cent. were unaltered Type C.

177a. Grown in broth at 37° C. for three weeks and then plated. All the colonies remained typical colonies (Type A). The unaltered 177 colonies were grown under three different conditions, and yielded either 177 or 177a



colonies. This is an example of an organism whose not quite typical colonies rapidly became, for the most part, typical under cultivation. Very trifling variation met with.

Coli 173. A type C colony when isolated. Grown in broth at 37° C. for nine days and plated. All the colonies perfectly typical (Type A) *B. coli* colonies. One of these typical colonies was inoculated into sterile water and grown outside for two weeks: colonies remain quite typical (Type A).

Coli 206. A Type C colony when isolated from "made soil." Grown in lactose peptone for two weeks at 37° C. and then replated. Colonies very similar to the original. When grown for two days further in broth and replated, fairly typical (Type B) colonies were obtained.

All these five organisms with Type C colonies gave normal typical colonies, wholly, or for the most part so, after a short artificial cultivation.

These experiments show, and they were confirmed by others which it does not seem necessary to publish, that extreme variations from anything at all like ordinary *B. coli* colonies may be met with among certain *B. coli*, and this by the use of very simple means.

From the broader biological standpoint it is difficult to know what value to attach to these results. A question which may have an obvious explanation, but if so I am not aware of it, is, Why do colonies tend to grow uniformly and typically at all? A colony in the bacteriological sense is not a colony in the true biological sense. It is rather an aggregation of bacilli which rapidly change their appearance. Such aggregations one would naturally expect to differ, and the surprising thing is that, on the whole, such uniform results are met with. That they take this form must be because the multiplication and growth takes place along the lines of least resistance. How far are these determined by the toxic products excreted by the bacilli into the surrounding medium? The products excreted into the broth must certainly have some action, and account in part for the differences met with in a plating from a two-days' and a two-weeks' broth culture, but it does not explain why two or more kinds of colonies may be met with on plates from a single broth tube. Where we have two or three kinds of colonies on one plate, such differences cannot be accounted for by slight and local variations in the nutrient medium, amount of air received, etc. There is no evidence of anything of the kind, while such diverse colonies often maintain the same differences on replating, and then all the colonies on each separate plate are alike.

It looks as if in the broth, before plating, two or more varieties of *B. coli* had developed.

There also seems to be some inherent difference between an organism giving normal and one giving abnormal *coli* colonies when isolated.

The former under cultivation never deviated widely, and readily returned to the typical; while the latter may become quite typical, but nevertheless tends to revert, and to revert to varieties very atypical indeed.

The following conclusions were arrived at:—

1. That as met with there is a common type of *B. coli* colonies on surface gelatin plates, but that considerable variations from this may be met with.

2. That these variations are markedly influenced by the material from which isolated, and to a less extent by the method used to isolate.

3. That apparently typical *B. coli* gelatin colonies may be met with which, when culturally investigated, are not true *B. coli*.

It is therefore not possible to diagnose this organism from its surface gelatin colonies alone, and numerical estimations based upon the number of such colonies are fallacious.

4. That the colony characters of *B. coli* may undergo considerable variation, even when kept under such comparatively ordinary conditions as in broth at 37° C., and in certain cases markedly abnormal colonies may result.

5. The greatest variation is shown by colonies atypical when isolated, and on the whole a typical *B. coli* colony tends to keep typical.

6. No conclusions could be drawn as to type of colony in relation to the medium from which it was immediately plated.