ON THE SPECIFICITY OF BACTERIAL MUTATION,

WITH A RESUMÉ OF THE RESULTS OF AN EXAMINATION OF BACTERIA FOUND IN FAECES AND URINE, WHICH UNDERGO MUTATION WHEN GROWN ON LACTOSE MEDIA.

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(*From the Lister Institute, London.*)

(Plates I to IV.)

Neisser in 1906 claimed to have discovered an organism which, when grown on lactose-agar, threw off suddenly a new strain capable of fermenting lactose. The identity of the new strain was established by Massini (1907) by many cultural and serological tests, and from that time onwards it has been generally accepted that new bacterial strains of great permanence may arise so suddenly as to justify this type of variation being named "Mutation."

The new strain arose when the original strain was grown on lactose-agar plates. The colonies on these plates showed papillary projections, and if litmus was present the papillae showed themselves to be strongly acid in reaction while the rest of the colony showed no sign of acidity. Replating of the papillae showed them to contain a new lactose-fermenting strain. This acquired power of fermenting lactose was held with great tenacity. The new strain was unable any longer to produce papillae on its colonies when grown on lactose-agar.

Arnold Burk (1908) described a similar organism but the evidence of identification in this case was almost entirely cultural, as the strains did not produce good agglutinating sera.

Reiner Müller (1908 and 1909) showed that various other organisms produced papillary projections on their colonies on agar plates, provided that certain definite carbohydrates were added to the agar. The bacilli contained in these papillae had, in a proportion of the strains examined

acquired the power to ferment the carbohydrate. The new strains had, however, in all cases lost the power to produce papillae on the same medium, and this fact constituted the essence of the mutation processes he described.

Various interpretations have been put forward accounting for these mutation processes. The first one suggested was that of contamination. The fermenting papillae on the non-fermenting colony suggested the presence of two organisms in intimate symbiosis difficult to separate by ordinary plating. The demonstration of similar agglutination properties in the case of the original and variant strains, the similarity of all cultural tests, with the exception of the one or two affected in the variation process, the fact that all the members of a species without exception may show the mutation when grown on a particular carbohydrate, *e.g.* all typhoid strains on iso-dulcite agar, and the demonstration of the process in cases in which the culture was started from a single bacillus, disposed of this objection and established on a firm basis this mutation process as occurring in bacteria.

These facts being demonstrated, certain observers and thinkers have maintained that our methods of bacterial classification have been undermined thereby; they suggest that if these sudden changes affect one character of an organism it is not unlikely that they may affect several simultaneously, and the *B. typhosus* of to-day may have been some other organism yesterday and may be yet another to-morrow.

In this rather depressing chaos R. Müller (1909) has suggested that these mutations are in a very high degree specific. If it can be proved that each and every member of a particular species, in a particular environment, alters in the same way and in approximately the same time, and that no other species so vary, then the process which was supposed to undermine current methods of classification would rather associate itself with them and tend to amplify them. The evidence R. Müller has brought forward in support of the specific character of mutations is the following:

(1) All the typhoid strains he examined (120) when plated on iso-dulcite agar, produced papillated colonies. These papillae always appeared within five days.

(2) Of 200 strains belonging to the coli-group, only three or four were able to produce these papillae when grown on the same agar. These few exceptions he compares to the organisms other than *B. typhosus* which agglutinate with a typhoid serum, of which a number have been described.

In view of the above facts he likens this iso-dulcite mutation test to the agglutination test in point of delicacy for *B. typhosus*. These papillae produced by *B. typhosus* are found to contain a new typhoid strain which is unable, when grown again on iso-dulcite media, to produce papillae.

R. Müller has shown that a similar raffinose mutation has a differentiating value as between *B. Aertryck* and *B. paratyphosus* B.

This proposition of R. Müller's seemed well worthy of investigation, and I therefore determined to select, from a series of organisms isolated from faeces and urine, those strains which had the power of "mutating" when grown on lactose-agar, and to examine the organisms so isolated to see if they formed one or more compact and well-defined species. For the sake of brevity I shall call such strains lactose-mutators, and similarly in the case of organisms showing mutation processes on raffinose- or saccharose-agar, I shall speak of them as raffinose- or saccharose-mutators respectively.

The specificity of the lactose mutation in the case of coli-typhoid organisms.

The strains hereinafter to be described have been obtained mainly from the excretions of suspected typhoid carriers. The technique practised has been the following: the material was plated out on MacConkey's medium and, after 24 hours, a fair sample of the non-fermenting colonies was inoculated into mannite broth tubes. Those showing gas formation were not further examined in the search for *B. typhosus*. These mannite cultures formed the main source of my material and for them I am indebted to Dr Ledingham.

Relative frequency of lactose-mutators in faeces and urine.

Out of 50 successive samples of material which were plated on MacConkey's medium and incubated for 24 hours 16 showed lactose-fermenting colonies only. The other 34 samples produced colonies which at this interval of time appeared to be exclusively non-fermenting. Mannite cultures of 70 such colonies were examined by me. They were replated on MacConkey's medium a second time to exclude further the possibility of contamination, and care was taken to have scattered colonies so that fermentation could easily be detected. After 48 hours' growth 36 of these strains were found to be typical lactose fermenters, so that many of the original colonies from which they had been taken had been too small to show any acid reaction after the short space of

24 hours. The remaining 34 plates consisting of non-fermenting colonies only were incubated for seven days. It was found that during this period no less than 21 of them developed definite papillae on their colonies on these lactose plates. We must, therefore, look upon lactose-mutating organisms as really comparatively common in the gut, though Massini found only one strain in the material he examined. These 21 strains were now plated out again on lactose further to ensure purity in the material worked from; no contaminations were found.

Sub-cultures from papillated plate colonies must be carefully distinguished according to the portion of the colony from which they are taken. If they come from the flat portion of a colony they perpetuate the original race, if from the papillae they contain as a rule mixtures in varying proportions of the new and old strains. In addition to the 21 strains so examined, I am indebted to Dr Ledingham for two other strains isolated from faeces, and to Prof. McWeeney for one strain which was, I understand, obtained from faeces and which was agglutinated by a $\frac{1}{50}$ dilution of the serum of the patient from whom it was obtained. For appearances of these strains when plated out on MacConkey's medium, see Table I (pp. 200–2).

To sum up Table I, we find that there were 19 strains giving papillae with acid reaction within one week when grown at 37° C., three strains giving papillae in the same time, none of which were acid in reaction, and two strains giving no papillae until after one week's incubation.

This description depends on a naked-eye examination of the plates and is summarised as follows:

Papillae in seven days		Papillae later than seven days at 37° C.
Acid in reaction	Non-acid in reaction	
Strain 12	Strain 8	Strain 23
13	24	17
6	16	
18		
10		
19		
3		
2		
4		
21		
5		
14		
7		
15		
11		
20		
22		
9		
1		

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Number of papillae per colony.

This varies greatly in different strains. It depends also on the length of time the plates have been incubated, and the size which the colony has attained. After four days the papillae may be non-existent in plates of some strains or as many as 40 on similar sized colonies of another strain. Strains 1, 9, 3, 2, 4 and 21 belong to a group showing 20 to 40 papillae at this stage, while the others show, excepting 24 where they are innumerable, diminishing numbers. Types are shown in the accompanying drawings (Plate I) and photographs (Plate II, figs. 1–4).

Size of papillae.

The papillae vary greatly in size in different strains. The absolute number of papillae has very little relationship to size. They may be few and small as *e.g.* in the case of strain 14, or very numerous and small as *e.g.* strain 24. The strains which show 20 to 40 papillae by the fourth day have papillae of moderate size. The largest papillae are got in the case of some of the strains showing few papillae on the colonies. These points are best illustrated by accompanying photographs (Plate II, figs. 1–4).

The strains were all plated out again on MacConkey's medium and incubated seven days at 37° C. and again they showed very similar appearances to those described in the foregoing Table I in respect of number of papillae, their reaction and time of their appearance. Plating was undertaken a third time from fresh single colonies of these strains. In this case the plates were incubated at 37° C. for five days and subsequently left at room temperature for a further 11 days. The appearances got in this series differed from the former only in the following particulars, viz. the acid reaction of the papillae was not present in the case of strains 10 and 19, while strain 17 which gave no papillae during seven days' incubation at 37° C. produced papillae on the plates grown 11 days at room temperature after five days' preliminary incubation.

On the nature of these strains.

There can be no doubt that the 19 strains which gave papillae of acid reaction on lactose-agar (see Table I) belong to the *coli mutabile* class, of which class Massini's organism was the first described member; two of the three strains, which produced in seven days naked-eye

TABLE I.

Cultures grown at 37° C. All observations naked eye unless otherwise stated.

The appearance of colonies of the following strains when grown on lactose-agar plates with neutral-red is summarized from one experiment as follows. None of these strains produced fermenting colonies, though the papillae they produced were in the case of the majority of strains of acid reaction.

Number and sources of strain	After 24 hours	After 48 hours	After 72 hours	After 96 hours	After 7 days
1 Faeces	Smooth non-fermenting colonies only	As many as forty papillae on the largest colonies, many of the papillae are of acid reaction	... ...	The central papillae of the colonies are more acid than those further from the centre. The extreme periphery shows no papillae in the case of this strain	—
2 ,,	Ditto. (Plate II, fig. 2)	As many as twenty papillae were present on one colony. None acid in reaction	Up to forty papillae on some colonies	Papillae faintly acid in reaction	These three strains are and have been throughout very similar in appearance
3 ,,	Ditto.		Ditto.	Papillae faintly acid in reaction	
4 ,,	Ditto. (Plates I, and II, fig. 3)		The papillae reach as many as forty on some colonies	Papillae faintly acid in reaction	
5 Urine	Ditto.	A few non-fermenting papillae present	As many as six non-fermenting papillae present on largest colonies	As many as eight papillae present on largest colonies. A few acid in reaction	—
6 Faeces	Ditto. (Plate II, fig. 1)	... ...	Well-marked naked-eye papillae present, none of acid reaction	As many as six papillae on one colony	As many as ten papillae present of slight acid reaction
7 ,,	No papillae present. No fermenting colonies present	A few papillae present	As many as two papillae on one colony. The papillae have marked acid reaction	... ...	—

8	Faeces	Ditto.	No papillae present	Small papillae present	As many as six papillae present on single colonies, no acid reaction of papillae present	Papillae still show no acid reaction
9	,,	Ditto.	As many as five papillae present on largest colonies. Some of the papillae are acid in reaction	... ...	As many as thirty-one papillae present on one colony	All signs of fermentation in papillae gone. They are now a dirty yellow colour
10	,,	Ditto.	... ...	As many as four papillae on one colony. All non-fermenting	... ...	As many as ten papillae on colonies. A few of the papillae with faint acid reaction
11	Urine	Ditto.	... ...	As many as three non-fermenting papillae present	Non-fermenting papillae only	A few of the papillae very acid in reaction
12	Faeces	Smooth non-fermenting colonies only	All non-fermenting colonies with no papillae	... ...	Up to two large papillae on single colonies. The papillae are of acid reaction	Up to three intensely acid papillae on the largest single colonies
13	Urine	Ditto.	... ...	... ...	Up to three large papillae of acid reaction, on single colonies. Colonies all alkaline themselves	—
14	Faeces	Ditto.	... ...	... ...	No papillae present	A few small papillae present on colonies, one or two of the papillae are of acid reaction
15	,,	Ditto.	No papillae present	As many as four colourless papillae of good size on the colonies	Non-fermenting papillae only	As many as six papillae on one colony, a few of the papillae are acid in reaction
16	Urine	Ditto.	... ...	No papillae present	A few non-fermenting papillae present	Many non-fermenting papillae present
17	Faeces	Ditto.	... ...	... ...	No papillae present	No papillae present after seven days' incubation. A few non-fermenting papillae appeared on the colonies after the plates had stood on the bench for some time later

TABLE I (*Continued*).

Number and sources of strain	After 24 hours	After 48 hours	After 72 hours	After 96 hours	After 7 days
18 Faeces	Smooth non-fermenting colonies only	... ...	No naked-eye papillae yet, few visible with a hand lens	As many as six papillae on one colony	As many as seven papillae on one large colony, three of which papillae on one colony have definite acid reaction
19 Urine	Ditto. (Plate I, fig. 2)	... ...	A few single papillae present on some of the colonies	... ...	As many as four papillae on one colony. Some papillae intensely acid in reaction
20 ,,	Ditto.	... ...	Similar to strain 19 throughout	... ...	Similar to strain 19
21 Faeces	Ditto.	No papillae present yet	Papillae present, small, as many as six on large colonies	As many as twenty papillae present on largest colonies. The colonies look to-day very much like those of "2," "3" and "4" strains	—
22 ,,	Ditto.	No papillae present	... ...	As many as seven papillae present on largest colonies. A few of the papillae are acid in reaction	—
23 ,,	Ditto.	... ...	... ...	... ...	Many small papillae present on examination with a hand-lens. No papillae present with naked eye. They appeared after being allowed to stand on the bench after seven days' incubation
24 ,,	Ditto. (Plate II, fig. 4)	... ...	... ...	No papillae present ...	Papillae very small and numerous. The colonies look like morocco leather on the surface. The papillae are not of acid reaction

papillae with no acid reaction, are, as will be subsequently shown, almost certainly of the same class.

Strain 23 which showed papillae of such small size that they required a hand lens for their observation, and strain 17 which only produced small neutral papillae when grown at room temperature for 11 days after previous incubation, connect this group with the non-lactose fermenters which do not show any tendency to throw off lactose-fermenting mutants. Strain "24" which formed tiny papillae but failed to ferment lactose after two months' growth on media containing it seems only a doubtful relative of this group.

Massini (1907) showed that if a papilla of his strain from a colony on a lactose plate be inoculated into ordinary broth and the latter plated on lactose-agar, a mixture of fermenting and non-fermenting colonies results. The non-fermenting colonies again produce papillae as in the case of the original strain, while the fermenting colonies give rise to a pure race of fermenting progeny which show no tendency to revert to the non-fermenting condition of the original strain. In my series of strains, replating on lactose-agar from papillae gave red and white colonies usually. The only negative strains were 15 and 24, where the replating gave only non-fermenting colonies; in the case of these two strains the replating from papillae was thrice repeated.

It must be remarked, however, that the proportion of red and white colonies on the plates is very variable when obtained in this way. The plates may sometimes consist of all fermenting or all non-fermenting colonies, in which case replating from a papilla has to be undertaken a second or even a third time, to get the typical mixed plates. The *permanency* of the fermenting character of these strains has not yet been tested, but our knowledge of other variants which arise suddenly in this fashion leads one to suppose that they will retain their fermenting character for many months, at least in those cases where the papillae appear early, *i.e.* from the second to the fourth day. Probably those arising later will have a slight tendency to reversion similar to dulcite-fermenting variants of *B. typhosus* (Penfold, 1910).

The next question to consider was the behaviour of these strains when grown on litmus-lactose-peptone-water. The findings are recorded in Table II.

To sum up the results of Table II we may say that the strains take up the position of slow lactose fermenters except strain 24 which was entirely unable to ferment lactose. The seven strains showing partial acidity on the first day could only be called slow fermenters when

TABLE II.

To show the time of appearance of acid reaction when the following strains were grown on litmus-lactose-peptone-water.

The strains were plated and after 24 hours growth at 37° C. two separate colonies were inoculated into the above medium and incubated at 37° C.

Time of observation	Col.	1	2	3	4	5	6	7	8	9	10	11	12
After 24 hrs.		As	As	As	As	As	As	As	As	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 48 ,,		Af	Af	Af	Af	Af	Af	Af	Af	Af	Af	As	As	—	—	—	—	A	—	—	—	—	—
,, 3 days		—	—	—	—	—	—	—	—	—	—	—	—	As	As	—	A	As	As	—	—	—	—
,, 4 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 5 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 7 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 8 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 9 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A	—
,, 10 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A
,, 11 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 12 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 14 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 22 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 23 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 25 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 35 ,,																							

	Col.	13	14	15	16	17	18	19	20	21	22	23	24
After 24 hrs.		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 48 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 3 days		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 4 ,,		A	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 5 ,,		—	—	A	A	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 7 ,,		—	—	—	—	—	—	A	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 8 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 9 ,,		—	—	—	A	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 10 ,,		—	—	—	—	A	—	—	—	—	—	—	—	A	—	A	—	A	A	A	A	—	—
,, 11 ,,		—	A	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 12 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 14 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 22 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 23 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 25 ,,		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,, 35 ,,																							

As = slight acid reaction. Af = full acid reaction.

compared with the lactose-fermenting variants the same strain threw off, or when compared with normal strains of *B. coli*. There is not the slightest doubt that just as selection was seen to occur in the plates, so here it occurred in the broths, and the varying times taken to produce acidity doubtless depended on the number and the nature of the variants produced. It is interesting to note that all the strains showing a large number of papillae of acid reaction, excepting strain 21, gave in lactose-peptone-water relatively early acidity. Strain 6 gave only up to 10 papillae on its largest colonies and strain 7 only two in 72 hours, so that their early acidity in peptone water depended rather on the nature than the number of the variants. The variants were fewer but obviously better fermenters and could, therefore, take advantage of the lactose food supply very fully as soon as they arose.

Strain 24 after being grown 35 days on lactose-peptone-water was sub-cultured again with the same medium and again failed to produce acidity within 30 days. This strain as before stated seems to be a very doubtful relative of this group.

Sub-culture of the lactose broths of the slowly fermenting strains was practised, when it was found that the length of time taken to produce acidity in the second lactose-peptone-water culture was reduced to one to five days except in the cases of 23 which still took 14 days, and 24 which, as before stated, did not ferment the lactose-peptone-water at all. This subject will be dealt with when the variant strains are described.

The general biological properties of the strains are described in the Table III annexed. The carbohydrates were added to peptone water in a Durham's tube and the fermentation properties tested in the usual way.

By the normal strain is meant the strain as isolated from the body. The variant strain was in the majority of cases obtained by replating on lactose-agar a papilla from a neutral-red-lactose plate of the normal strain and selecting for sub-culture a red colony—the variant strain.

Where these plates did not show red colonies as in the case of strain 15, the lactose broth which had fermented was plated on lactose and readily yielded a fermenting colony. In the case of strain 24, however, even this method was not possible since as before stated it failed to ferment the lactose even in the peptone water tubes.

In Table III the differences between the normal and variant strains are alone noted, not the points of agreement.

The Ehrlich test for indol was used throughout.

The following abbreviations are used in this table to permit of concise statement:

Af = full acid reaction.

A followed by a fraction indicates varying degrees of partial acidity.

G = gas. Gn = no gas.

The fraction following the letter G indicates the volume of the gas tube filled by gas. Amounts of gas less than $\frac{1}{12}$ of the gas tube are indicated by p and p′; the former letter indicates a pinshead bubble, the latter a larger amount though less than $\frac{1}{12}$ of the tube. The gas tubes used were 2 inches long by $\frac{1}{4}$ inch internal diameter.

Further signs in the table are in common use and do not require explanation.

The following are the main facts to be culled from Table III:

A. The essential differences in cultural characters between the variant and original strains are, first, the original strain ferments lactose broth slowly; second, it does not clot milk or does so only slowly, while as a rule in the case of the new strains lactose broth is fermented and milk is clotted much more quickly.

B. Glucose, laevulose, mannose, galactose, arabinose, inulin, sorbite, and mannite are either fermented or not fermented as the case may be by all the members of the group examined, *i.e.* they afford no help in differentiating the members of the group from each other.

C. Strains 22 and 9 are the only two non-gas producers. This gives however no idea of the relative frequency in the *coli mutabile* class of gas-producing and non-gas-producing forms from faeces and urine, as 21 of my strains were selected from gas-producing organisms exclusively.

D. *Xylose* is fermented by all the members of the group. The variant strains of 1 and 9 do it more quickly than the normal strains, whereas in the case of 4 the opposite is the case.

E. *Iso-dulcite.* "21" is the only strain not fermenting iso-dulcite. Strains 12, 13, 6, 5, 18 and 14 all require 48 hours to ferment it, and strain 16 seven days. The other strains all ferment it in 24 hours. The gas yield from this sugar, in the case of the gas-forming strains, is always small. In this respect they agree with intestinal bacteria in general.

F. *Maltose.* All strains fermented maltose quickly, excepting "24," which did not attack it at all.

G. *Lactose.* The variant strains fermented this more quickly than the normal. Strain "24" did not attack it at all.

H. *Raffinose.* Nine of the strains fermented this sugar, viz. 16, 9, 12, 13, 5, 14, 21, 24 and 15.

I. *Saccharose.* Twelve strains were positive on this sugar.

J. *Dextrin.* All strains fermented this except "24."

K. *Salicin.* Nine strains fermented this glucoside, three in 48 hours, and the rest within seven days.

L. *Glycerine.* All strains fermented this; the time varied from one day to one week.

M. *Adonite.* Strains 7 and 10 were the only ones to ferment this.

N. *Dulcite.* Eight strains were negative, the rest positive.

O. *Sodium formate.* All strains produced gas from this salt except "22" and "9" which failed to produce gas from any of the carbohydrates.

P. None liquefied *gelatine.*

Q. All acidified *milk*, the variant strains more intensely than the normal.

R. *Indol* production occurred in the case of 20 strains.

TABLE IV.

No. of strains	Iso-dulcite	Saccharose	Raffinose	Salicin	Adonite	Dulcite	Motility in 24 hours broth	Gas formation	Indol
6	+	−	−	−	−	+	+	+	+
18	,,	,,	,,	,,	,,	,,	,,	,,	,,
4	+	−	−	+	−	+	−	+	+
3	,,	,,	,,	,,	,,	,,	,,	,,	,,
2	,,	,,	,,	,,	,,	,,	,,	,,	,,
14	+	+	+	−	−	+	−	+	+
12	,,	,,	,,	,,	,,	,,	,,	,,	,,
5	,,	,,	,,	,,	,,	,,	,,	,,	,,
13	,,	,,	,,	,,	,,	,,	,,	,,	,,
15	,,	,,	,,	,,	,,	,,	,,	,,	,,
1	+	−	−	+	−	−	+	+	+
23	,,	,,	,,	,,	,,	,,	,,	,,	,,
10	+	−	−	−	+	−	−	+	+
21	−	+	+	+	−	−	−	+	−
7	+	−	−	+	+	−	+	+	+
11	+	−	−	−	−	−	−	+	−
19	+	+	−	−	−	+	+	+	+
8	+	−	−	+	−	+	+	+	−
24	+	+	+	−	−	+	+	+	+
17	+	+	−	−	−	−	+	+	+
16	+	+	+	−	−	+	−	+	+
22	+	+	−	−	−	+	−	−	+
9	+	−	+	−	−	−	+	−	+
20	+	+	−	+	−	+	+	+	−

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Staining and Morphology. All were gram-negative rods. The majority (13) were non-motile in 24 hours broth culture. Voges and Proskauer's test was in all cases negative when carried out on 48-hour cultures.

Many of the tests used are seen to have no differentiating value in the series of organisms examined. The preceding table gives briefly those that have value (Table IV).

This table shows that the 24 strains comprise no less than 16 varieties. Amygdalin which was fully fermented by strain "16" produced in other instances various shades of green. I have omitted it from the table of classification, as strain "16" was already differentiated.

The scheme of classification of these forms mutating on lactose is a little different from that used by MacConkey (1909) for ordinary forms of *B. coli.* His recommendation was followed in not using the reduction of nitrates or litmus-whey tests. These he found of little differentiating value. Further I did not use inosite. On the other hand, iso-dulcite, raffinose, and salicin were found of distinct differentiating value and they are therefore included in the synoptic table.

The motility tests were made with 24-hour broth cultures and this may account for the relative frequency of non-motile organisms. MacConkey's tests for motility were done in 6-hour broth cultures which gives a greater proportion of positive results. Leaving aside the tests wherein Table VI differs from MacConkey's scheme and comparing his table with mine in respect of the identical tests we find:

MacConkey's examination of 178 strains of lactose-fermenters from human faeces showed three large groups, viz.

1st { *B. Grünthal* and its non-motile form.
B. vesiculosus.

2nd { *B. coli communis* and its non-motile form.
B. Schäfferi.

3rd { *B.* 71 and its non-motile form.
B. neapolitanus.

Group 1 had 41 out of the 178 strains.
Group 2 had 48 out of the 178 strains.
Group 3 had 57 out of the 178 strains.

Out of the 24 strains of my series

2 belonged to Group 1.
6 belonged to Group 2.
8 belonged to Group 3.

Therefore my largest groups correspond fairly to those of MacConkey's table.

The three groups in question constitute 82 % of MacConkey's series and 66 % in my series, and if the strains of urinary origin be excluded from my series a still closer approximation to MacConkey's series is obtained. That is to say, these organisms of the mutabile group, unable to ferment lactose without variation and selection, show a very similar constitution judged by cultural characters to the lactose-fermenters of the faeces. It appears not at all improbable from this consideration that the *coli mutabile* organisms probably give rise to coliform organisms by variation and selection within the gut, and that the ultimate flora of the gut depends largely on the nature of the non-lactose fermenting strains which get into it and are able to throw off lactose-fermenting variants.

It is noteworthy that strain 6 after the process of variation and selection produced a typical *B. coli* Escherich strain in the strictest use of the term. The *B. coli mutabile* of Massini did not produce indol. I believe no genuine *B. coli* Escherich in the strict use of the term has been previously experimentally selected.

Little has been done with these strains in respect of agglutination tests, as they have been found to give little help in classifying the coliform organisms of the gut. I made, however, a serum from a typical *B. coli* Escherich and tested it against strains 4, 3 and 2 with the following result:

	1/50	1/250	1/1000	1/5000
4	...	–	–	–
3	–	–	–	–
2	?	–	–	
Normal *B. coli*	+++	+++	+++	++

Further strains were not tested.

The specificity of the lactose mutation.

It would appear therefore that a large number of organisms exist in the gut which are able to throw off lactose-fermenting mutants. These strains appear to belong to many species which are closely related to the lactose-fermenting species found in the same situation. The mutation process as it occurs on lactose media is therefore not specific in any strict sense.

Of course it might be maintained that the different varieties of lactose-fermenters found in the intestine are not separate species. There seems no inherent reason for believing that fermentation tests

have one value when applied to the non-lactose fermenters and another when applied to the lactose-fermenters. If *B. typhosus* is a distinct species as opposed, for example, to the dysentery organisms, then one must admit that *B. lactis aerogenes* and *B. coli* Escherich are equally good species though they may not produce such characteristic pathological conditions.

The specificity of the iso-dulcite mutation.

Reiner Müller showed that all natural strains of *B. typhosus* which he examined threw off mutants on iso-dulcite media. I examined a large number of strains and confirmed this. He further showed that a few pseudo-dysentery strains do this, but out of about 200 coliform organisms examined, only three or four possessed this property.

If this latter assertion is supported by further work, then the mutation test must be admitted to have a high degree of specificity in this case.

R. Müller has further stated that the same strain will only produce papillae as a rule on one carbohydrate. He states that *B. typhosus* did not show this mutation on any other of 18 carbohydrates tested, one of these being dulcite. This latter statement is, however, not correct as I have elsewhere shown. It seems probable that the same organisms may vary suddenly in several directions.

The raffinose mutation.

R. Müller found that *B. paratyphosus* B. threw off mutants as shown by papillae-formation, when grown on raffinose agar, while *B. Aertryck* did not do so. This appears to be the only cultural distinction between the two varieties.

I am indebted to Dr F. A. Bainbridge for 29 strains of the food-poisoning group which I classified according to this test into two groups, viz. (1) *B. paratyphosus* B. and (2) *B. Aertryck.*

The strains were plated on raffinose agar and observed daily. Significant differences between the colonies of the different strains did not appear until the 5th day and they became more marked by the 7th and 9th days. The intensity of papillae-formation, and time of appearance of the same, are indicated in the following Table V. None of the strains showed papillae of acid reaction though neutral red was present in the plates.

The points to be noted in the table are:

(1) The four strains of *B. enteritidis* Gaertner produced in three cases papillated colonies, being in this respect like *B. paratyphosus* B.

The test is, therefore, not strictly specific for *B. paratyphosus* B.

(2) All the pure *B. paratyphosus* B. strains produced papillae except "*Murray, original*"; the number and size of the papillae were, however, very various in the case of the different strains and also of the same strain at different times.

(3) One strain only of *B. Aertryck* showed papillae, viz. "*S. P. Wassermann*" and in this case they were of great number and large size; strain "*Victoria*" showed one papillated colony only, on a crowded plate. See illustrative photographs (Plate III, figs. 5, 6, 7).

A mixed group of 10 of the above strains were plated out again on raffinose agar, and all gave the same result except that strain "*M.* 5" showed 20 % of its colonies papillated.

Conclusions:

(1) Theoretical. The raffinose mutation is not strictly specific for *B. paratyphosus* B. as *B. enteritidis* Gaertner may show it, and occasional strains of *B. Aertryck* also.

(2) The raffinose mutation is however the only cultural means of helping to distinguish between *B. paratyphosus* B. and *B. Aertryck*. It will but rarely err in separating these two varieties and, having regard to the difficulty and occasional impossibility of the absorption agglutination test, it has a distinct practical value.

Interconversion of B. Aertryck and B. paratyphosus B. in respect of the mutation test.

"*M.* 5" which showed up to 20 % of papillated colonies and "*Victoria*" which showed only one colony with papillae were sub-cultured on to agar from the smooth interpapillary area of papillated colonies, and in this way pure strains were obtained, all the colonies of which produced papillae. Similarly strains of *B. paratyphosus* B. had sub-cultures taken from the papillae, and these on plating readily yielded strains which were unable to produce papillae.

Dr Bainbridge, who intended to see if alteration of the mutation test in the case of these strains was accompanied by alteration of the absorption agglutination tests, unfortunately never had the opportunity to carry this out.

TABLE V.

To determine the value of the raffinose mutation in separating B. paratyphosus B. from B. Aertryck.

The following 29 strains, all being members of the food-poisoning group, were obtained from Dr Bainbridge in order to divide them into two groups, viz. *B. paratyphosus* B. and *B. Aertryck*. After having classified them the results were compared with Dr Bainbridge's results obtained by the Castellani absorption test.

Name of strain	Condition of raffinose plate on the 5th, 7th and 9th day of incubation: 5th day of incubation	7th day of incubation	9th day of incubation	Intensity of papillae formation	Source of strain	Classification according to Castellani's method by Dr Bainbridge	Remarks
Trautmann 5	Definite papillae seen with a hand lens	As last observed ...	A few naked-eye papillae present	+	Healthy man	*B. paratyphosus* B.	—
Danyz R ...	,, ,, ,,	Small naked-eye papillae present	As last observed ...	+	Stock strain	*B. enteritidis* of Gaertner	—
Potter ...	,, ,, ,,	,, ,, ,,	Many papillae of moderate size present on all the larger colonies	+ +	From bone abscess after paratyphoid fever	*B. paratyphosus* B.	—
Danyz H ...	,, ,, ,,	Papillae only visible with a hand lens	A few naked-eye papillae seen on all the larger colonies	+	Stock strain	*B. enteritidis* of Gaertner	—
Prigge 9 ...	Many naked-eye papillae present	As last observed ...	Up to 40 or 50 papillae present on larger colonies with naked eye	+ + +	Carrier case	*B. paratyphosus* B.	—
Prigge 5 ...	A few naked-eye papillae present	Many definite naked-eye papillae	Up to 30 naked-eye papillae present on single colonies	+ + +	,, ,,	,, ,,	—
Savage W ...	Many large naked-eye papillae present	,, ,, ,,	Every colony covered with large well-marked papillae	+ + +	From case of paratyphoid fever	,, ,, ,,	Platedfrequently on the medium and always found papillated to the same intense degree
MacWeeney	,, ,, ,,	,, ,, ,,	60 % of the colonies show numerous and large papillae	+ + +	,, ,,	,, ,, ,,	
Trautmann 2	No papillae present	No papillae ...	No papillae ...	0	From food ...	*B. Aertryck* ...	—

Murrow ...	,, ,, ,,	,, ,, ...	,, ,, ...	0	From case of food poisoning	,, ,, ...	—
Eichom ...	,, ,, ,,	,, ,, ...	,, ,, ...	0	... ...	*B. enteritidis* of Gaertner	—
W. 9. B. ...	,, ,, ,,	,, ,, ...	,, ,, ...	0	From food ...	*B. Aertryck* ...	—
Trautmann 1	,, ,, ,,	,, ,, ...	,, ,, ...	0	From case of food poisoning	,, ,, ...	—
Trautmann 3	,, ,, ,,	,, ,, ...	,, ,, ...	0	,, ,,	,, ,, ...	—
M. 5	,, ,, ,,	,, ,, ...	,, ,, ...	0	Stock strain	,, ,, ...	—
840	,, ,, ,,	,, ,, ...	,, ,, ...	0	From healthy pig	,, ,, ...	—
Prigge 6 ...	,, ,, ,,	Many definite naked-eye papillae present	Many papillae ...	+ + +	From healthy man	*B. paratyphosus* B.	—
Victoria ...	,, ,, ,,	No papillae ...	No papillae except on one colony	0	Fatal food poisoning case	*B. Aertryck* ...	—
McAlister ...	No naked-eye papillae present	No naked-eye papillae	A few papillae got on a few colonies. They are small in size	+	From water	*B. paratyphosus* B.	—
Fowler 3 ...	,, ,, ,,	Many naked-eye papillae on all the colonies	As last observed ...	+ +	From case of paratyphoid fever	,, ,, ,,	—
Savage *c* ...	,, ,, ,,	Many large naked-eye papillae on all the colonies	,, ,, ...	+ + +	,, ,,	,, ,, ,,	—
Prigge 4 ...	,, ,, ,,	No papillae present	No papillae ...	0	Healthy man	*B. Aertryck* ...	—
Murray Original	,, ,, ,,	,, ,, ,,	,, ,, ...	0	Doubtful case	*B. paratyphosus* B.	—
W	,, ,, ,,	Papillae formation well marked	As last observed ...	+ + +	*B. typhi murium*	*B. enteritidis* of Gaertner	—
S. P. Wassermann	,, ,, ,,	,, ,, ,,	,, ,, ...	+ + +	Unknown ...	*B. Aertryck* ...	—
Prigge 1 ...	,, ,, ,,	,, ,, ,,	,, ,, ...	+ + +	Case of paratyphoid fever	*B. paratyphosus* B.	—
Fowler 1 ...	,, ,, ,,	,, ,, ,,	,, ,, ...	0	Food poisoning	*B. Aertryck* ...	—
O'Brien ...	,, ,, ,,	,, ,, ,,	,, ,, ...	0	Guinea pig epizootic	,, ,, ...	—
Rommeler liver	,, ,, ,,	,, ,, ,,	,, ,, ...	0	Healthy liver	,, ,, ...	—

Saccharose results.

There is not yet sufficient information forthcoming to enable us to determine to what extent the saccharose mutation of intestinal bacteria is specific for them, but six strains of saccharose mutating bacteria have been very shortly described by Thaysen (1911) and with the few tests he described he could classify them into two groups, so that preliminary observations suggest that the saccharose mutation is not very specific.

B. typhosus and dulcite.

B. typhosus has been shown by the author to produce papillae on dulcite agar. Some strains produce the papillae early, in which case they are of acid reaction if a suitable indicator is present in the agar.

The process here is, however, not a mutation in the strictest sense, since the fermenting power so acquired tends to disappear unless the growth on dulcite media is continued for two or three months.

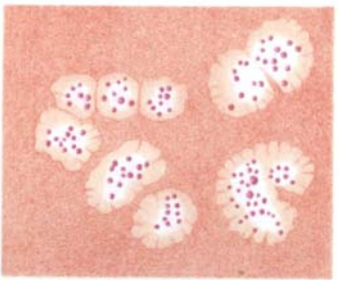
All typhoid strains agree in being able to throw off dulcite-fermenting variants readily. Of one batch of 14 strains tested by the writer all of them produced acidity within four days in the second sub-culture tubes of litmus-dulcite-peptone water in which they were grown. Many other strains were tested since and have behaved in the same way.

It may be quite definitely accepted that the ready power to throw off dulcite-fermenting variants is a character of the *B. typhosus*. This character has been shown, however, to belong to certain water bacteria (Meyers Coplan 1909) and to Morgan's *No.* 1 *Bacillus* (Twort (personal)), so that while it is possessed by all typhoid strains it is deprived of much of its specific value by being common to some other bacteria.

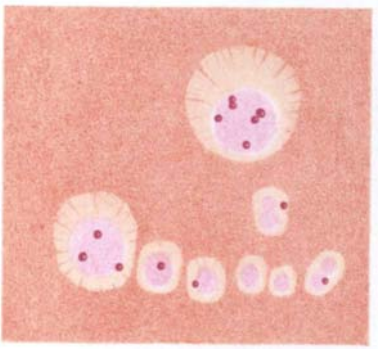
The specificity of the chloracetic acid mutation.

The addition of sodium monochloracetate to agar medium has been shown (Penfold 1911) to cause many intestinal organisms to form papillae on their colonies (see photographs 8 and 9); these papillae are more highly resistant to chloracetate of soda than the original strain. This sudden acquirement of resistance to the medium may be reasonably looked upon as a mutation, but unlike the mutations previously dealt with, the strain is still able to produce papillae on the same medium in the presence of higher concentrations of the salt.

B. coli Escherich, *B. enteritidis* Gaertner, *B. paratyphosus* B., *B. paratyphosus* A., *B. Grünthal* and many other organisms have papillae-forming



A lactose mutating strain grown six days on neutral-red lactose-agar plate. Strain IV in Table I. Papillae numerous.



Colonies of strain 19 on a neutral-red lactose-agar plate, grown nine days at 37° C. See Table I. Papillae few.

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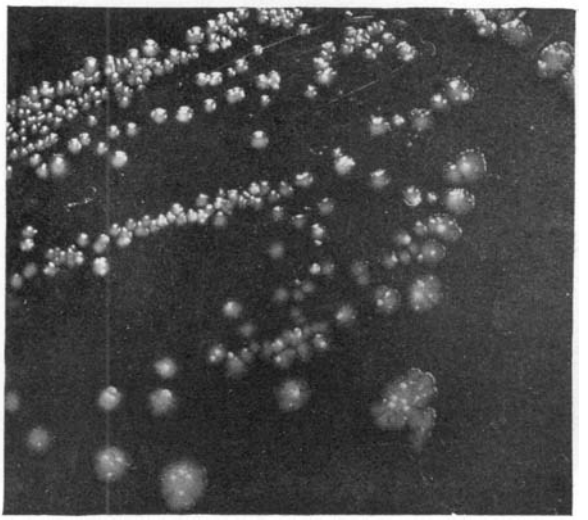

Fig. 1. *B. coli mutabile*, strain "6"; plate seven days old.

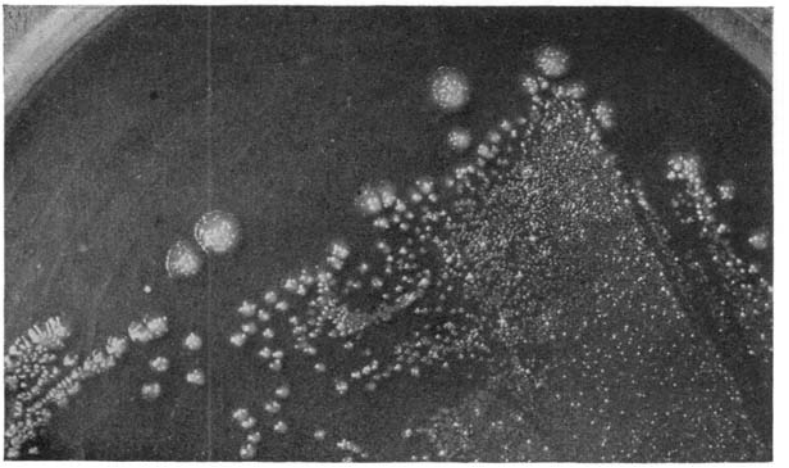

Fig. 2. *B. coli mutabile*, strain "2"; plate three days old.

PLATE II shows photographs of lactose agar plates of four of the strains described in the paper as lactose mutators. All the plates were incubated at 37° C. during the time indicated.

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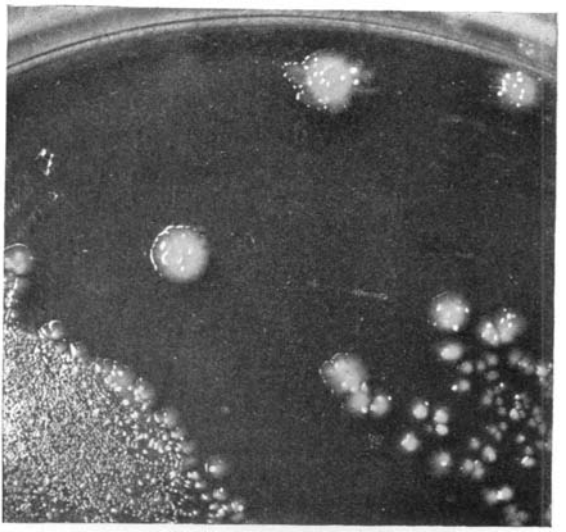

Fig. 3. *B. coli mutabile*, strain "4"; plate two days old.

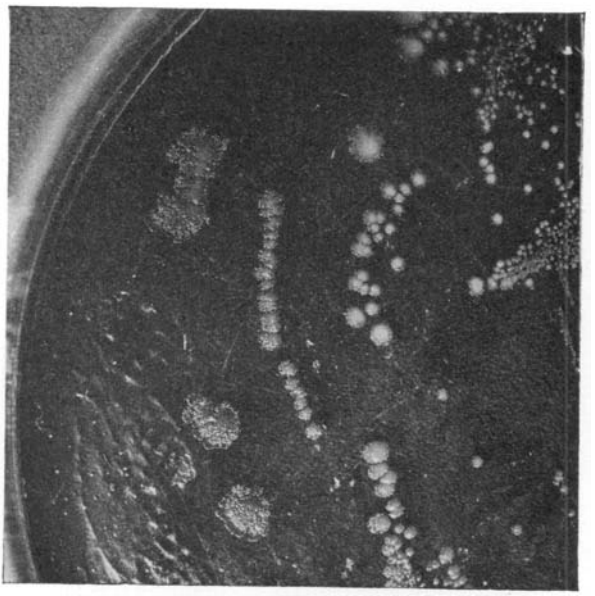

Fig. 4. *B. coli mutabile*, strain "24"; plate seven days old.

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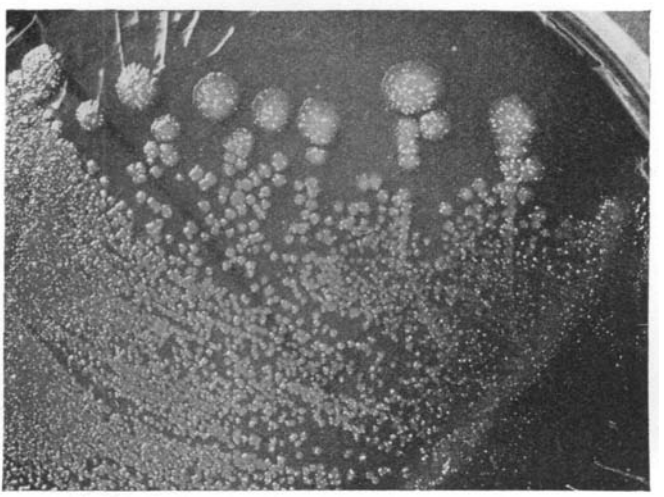

Fig. 5. *B. paratyphosus* B. grown for nine days on raffinose agar at 37° C. A typical strain. Observe characteristic papillae.

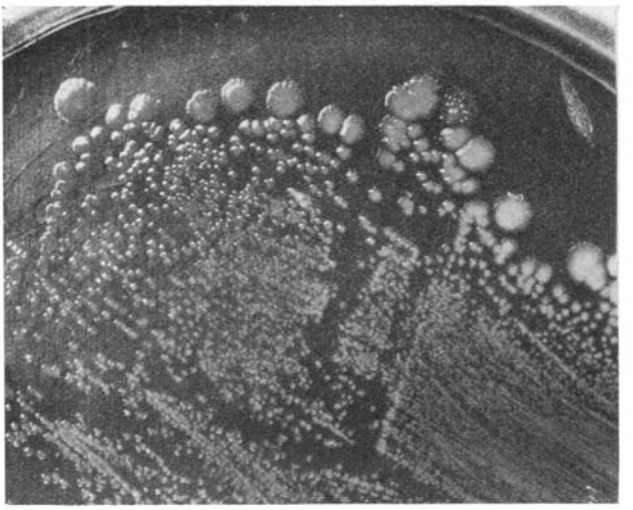

Fig. 6. *B. suipestifer*, strain "Victoria" grown on raffinose agar. The strain appears atypical since one of its colonies is shown to be papillated.

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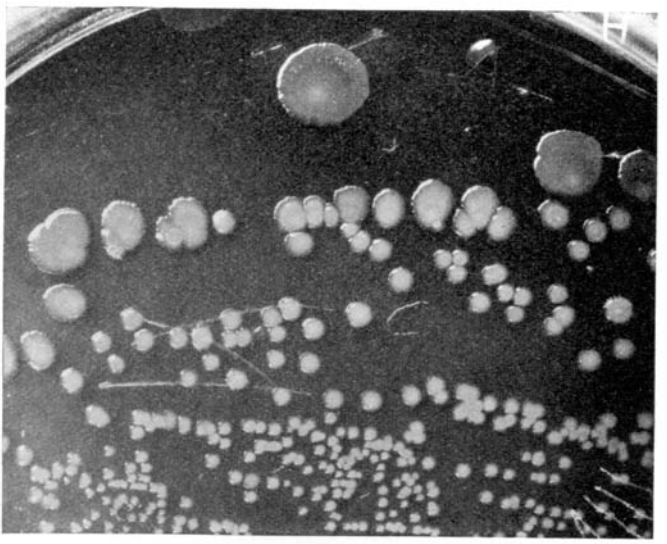

Fig. 7. *B. suipestifer*; showed typical smooth colonies only on raffinose agar though the plate had been incubated nine days.

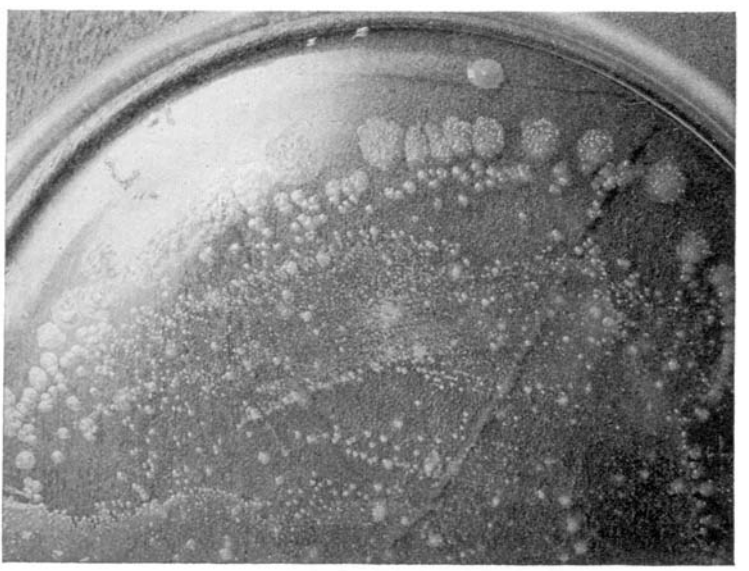

Fig. 8. *B. coli* Escherich after seven days growth on chloracetic acid agar.

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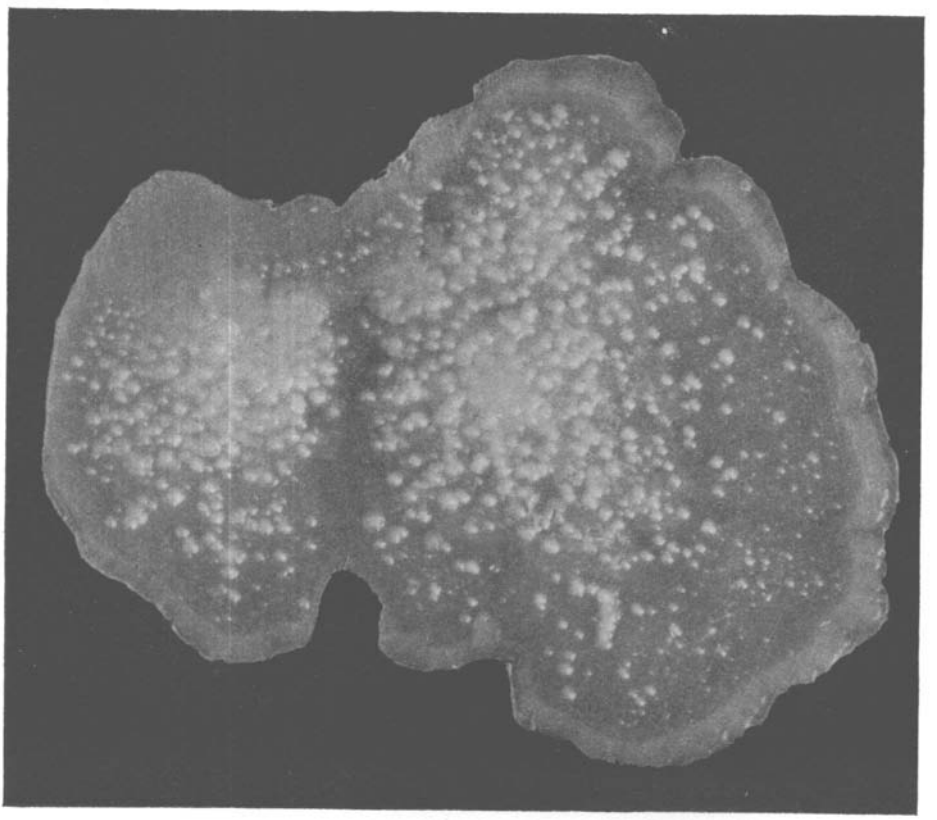

Fig. 9. *B. coli* Escherich on chloracetic acid agar after twelve days growth. One large colony magnified about four times and presenting an enormous number of papillae.

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power on this medium, but none of the typhoid strains (nine) examined possessed it.

The degree of specificity in this instance is apparently small.

Inability to vary quickly in particular directions is probably just as characteristic of the species as ability to vary. It took Twort two years to produce by constant growth on lactose a lactose-fermenting variant of *B. typhosus*. I tried rapid sub-culture on lactose-peptone water of a number of strains of *B. typhosus*, carrying on the process as far as the fiftieth generation in some cases, without obtaining the slightest sign of a new strain. I have also tried monthly and fortnightly sub-cultures with a large number of strains, and found that 18 months' growth on lactose failed to make any of them lactose-fermenters. We may therefore state that *B. typhosus* as a species is unable to produce readily lactose-fermenting variants and any organism readily taking on the power to ferment lactose is not *B. typhosus*. As previously mentioned not one of the eight strains of *B. typhosus* grown on chloracetic agar could produce papillae.

Similarly I have tried to obtain a saccharose-fermenting variant from a *B. coli* Escherich strain, but in spite of sub-culture on saccharose-peptone water twice weekly for over seven months, I found such a variant did not arise.

Eight months' growth of *B. enteritidis* Gaertner on milk was tried in the case of six separate series with the hope of removing or diminishing the preliminary period of acidity by which the organism is characterized when grown on milk; no change, however, occurred in this or any other property of the organism.

Single cell culture (Burri's method).

This method has been applied several times by me to strains of *B. coli mutabile* and I have readily succeeded in getting pure cultures from single cells. These cultures grown on lactose throw off the lactose-fermenting variants in exactly the same way as strains which have been obtained by the ordinary plate method.

GENERAL CONCLUSIONS.

The greatest degree of specificity of which we can conceive is that a particular property shall belong to every member of a species and shall belong to no other species or individual strain of such species. This degree of specificity is not, however, known to be shown by any

bacterial variation process. The nearest approach to it is the *iso-dulcite* mutation of the *B. typhosus* we have before mentioned, which fails, however, in so far as a very few other organisms have been found which show the same adaptability to this medium.

Nearly all strains of *B. paratyphosus* B. mutate on *raffinose* agar, but *B. enteritidis* Gaertner may do so and rarely the *B. Aertryck* (or *B. suipestifer*).

In respect of the *lactose* mutation it appears to be a common property of many bacterial species and has practically no specific differentiating value at all.

It is yet premature to say whether every member of any species possesses this property of mutating when grown on lactose.

The power to vary quickly in respect of *dulcite* appears to belong to every strain of *B. typhosus*.

The inability to vary quickly in respect of certain carbohydrates would appear to be as characteristic of certain species as the power to vary.

It will be noticed that the above examples of varying degrees of specificity of variation processes are taken entirely from the intestinal group of organisms. They are, therefore, not at variance with, though very distinct from, the haphazard variations that have been described in the case of the streptococci.

In sending this paper to the press I desire to thank Dr Ledingham for much helpful criticism in the course of this research, Dr Reid of Ealing for the excellent photographs he took of the illustrative plates and Miss Rhodes for the coloured drawings.

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