NATURAL VARIATION OF *B. ACIDI LACTICI* WITH RESPECT TO THE PRODUCTION OF GAS FROM CARBOHYDRATES.

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Introduction.

Variations in bacteria may be said to fall into two classes (1) those which are temporary, and dependent on the environment, the bacteria tending to return to the original type when subculture is made on ordinary media; and (2) those which are fixed, and persist after repeated subcultures on ordinary indifferent media.

Although, for most purposes, this classification is useful, it is not always easy to decide to which class a given variety belongs. The altered culture may rapidly revert to the original type, because it consists of a mixture of the new variant and the original form, and ordinary media may exercise a selection which rapidly reduces the new variant to an imperceptible minority, or may lead to its complete extermination; while on the other hand growth on the medium on which the variant first appeared, favours the new form.

There may be a mixture of varieties of this kind in a culture which springs from a single variant bacillus, for though the majority of the bacilli produced belongs to the new variety, a small proportion of the original type often occurs by reversion in the course of successive generations.

That the proportion of variants in a culture depends on the medium on which the culture has been made and on the age of the culture was shown by Penfold (1911) in the case of cultures of the *Bacillus typhosus*

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in dulcite-peptone water in which the proportion of bacilli capable of fermenting dulcite increased progressively.

Penfold also found that the variety of *B. coli* which had lost the power of producing gas from sugars after growth on chloracetic agar, did not remain constant till selection on the latter medium had been repeated. This tendency to revert in the carlier phases of the variant culture, indicated the presence of a mixture of the individuals which produced gas, and of those which did not, and perhaps also of intermediate variants.

The intermediate strains of the bacillus whose occurrence is about to be recorded in this paper, appear to have consisted of similar mixtures, though derived from colonies which in all probability arose from single bacilli.

Only that variety which remains constant on ordinary culture media can be considered as a true fixed variety, but even in this case the constituents of the medium may have a special selective action in some direction, and unless no atavist variant bacilli make their appearance in the course of growth the strain may revert to the original type.

The tendency of a variant to revert in culture, therefore, depends on the occurrence of individual bacilli of the original type and also on the favouring of these forms by the culture medium, but, frequently, atavistic bacilli may occur without an obvious change in the culture because the medium does not afford them any preferential treatment. On the other hand, if the particular medium is strongly selective for any particular type, the culture will in time take on the character of this type if individuals of this variety occur, even in small numbers.

Variations which remain constant in artificial culture have been described affecting many of the characters and biochemical properties of bacteria, but I propose here to mention only those relating to the fermentation of the carbohydrates.

Variations in fermentation.

Among the characters which are used for distinguishing allied forms of bacteria of the coli-typhoid group the production of acid, or acid and gas from sugars and alcohols, has proved to be of great service since these characters are as a rule uniform for known pathogenic bacilli both when the organism is first isolated and even after long growth and repeated subcultures on ordinary laboratory media.

Variations in fermentation reactions are not only of practical importance for diagnostic purposes, but are also of great theoretical interest,

since they affect some of the characters, which have usually been reckoned among the more permanent features of bacteria, and concern the physiological processes intimately connected with the life and growth of the bacteria.

Varieties showing changes in the fermentation characters during artificial culture have been described and fully tested of recent years.

Hiss (1904) found that *B. dysenteriae* Type *Y* could acquire the power to ferment maltose by growing on a medium containing that sugar.

Twort (1907) produced similar changes in *B. typhosus* as regards dulcite and lactose.

Lentz (1909) and others have made similar observations.

Arkwright (1909) recorded natural and cultural changes in the fermentation properties of the meningococcus, which occurred independently of growth on sugars.

Penfold (1911, 1912) studied variations of this type in detail and has summarised the work on bacterial variation generally. He also confirmed and extended the work of Massini (1907) on *B. coli mutabile.*

Baerthlein (1912) correlated changes in the sugar reactions with variations in the type of colony on agar plates.

These examples, from amongst a large number of observations, illustrate for the most part the variations which have been observed in the direction of acquiring additional characters.

Other observations, which are no less striking, have been made on the loss or partial suppression of some characters.

Scheierbeck (1900) showed that streptococci occurring in milk vary very much in the amount of acid which they produce in a given time, and he was able by growing cultures in milk containing carbolic acid to obtain strains with widely different acid-forming powers, which remained constant after frequent subculture.

Penfold (1911, 1912) showed that it was possible to suppress to a very large extent the gas-forming property of *B. coli, B. enteritidis* (Gaertner) and *B. acidi lactici* by growing these organisms on agar containing chloracetic acid. The new variety in this case while forming acid from the same sugars and alcohols as the original strain had lost the property of forming gas from sugars, but retained it for the alcohols. He proved the identity of the two varieties by means of serological tests. The variants remained constant through numerous subcultures.

Revis (1911) by growing *B. coli* (Escherich) on 0·1 % malachite green broth succeeded in suppressing the gas-forming function for alcohols and sugars, the variant still producing acid from the same sugars and alcohols as the original strain.

Natural variations.

Reference to the writings of workers on the subject of variation of bacteria, shows that while under special conditions definite and fixed varieties can be observed to occur, still there is little direct evidence to show that similar variation takes place under natural conditions, *e.g.* in the human body or in the outside world.

Colonies of the same organism but of dissimilar appearance occasionally appear on the original plates used for isolation, but when subcultures are made these differences usually disappear.

There is however some evidence (Baerthlein 1912) that fixed varieties which can be distinguished by the appearance of the colonies may be observed on the original plates inoculated with the stools of a patient.

The proof that these different types of colony consist of the same organism, is often imperfect, and in many of these cases proof of recent origin from a single strain is impossible and the same patient may have been infected from two different sources.

The acid-producing variety of *B. coli mutabile* which first appears on lactose agar subcultures has not as yet been conclusively demonstrated in the faeces. It is therefore only known to arise under artificial conditions.

Penfold (1912) however showed that the lactose-fermenting variants which are produced by *B. coli mutabile* closely resemble those lactose fermenters which commonly occur in the faeces.

It is more frequently in later subcultures that variations have been noticed, and since the best evidence of variation has been obtained by dealing with pure cultures from a single colony, most attention has been turned in this direction.

The natural varieties of pathogenic bacteria which may be isolated from different patients or in different epidemics, *e.g.* the morphological varieties of the *B. diphtheriae* described by Dale (1910) and the well-known subgroups of the mannite-fermenting type of the *B. dysenteriae*, belong to a different category, since it cannot be known how long their special characters have been acquired nor whether they first appeared in the patient from whom they were isolated.

In this connection must be mentioned the two following observations:

Bock (1906) found that three strains of *B. suipestifer* from different laboratories formed no gas from glucose, but as regards agglutination behaved like normal strains. He used malachite green agar ($\frac{1}{400}$ and $\frac{1}{800}$) for differentiation but does not state whether these strains had grown on this medium.

Bainbridge (1909) examined a bacillus which had been sent to him as a strain of *B. suipestifer* and found that it never formed gas from carbohydrates, although it formed acid from the same media as other strains, and in all other respects, including agglutination, resembled the standard *B. suipestifer*.

Nothing is known of the circumstances under which these varieties have arisen.

When bacteria have been met with, which present characters suggesting that they are natural variants of well-known types, direct evidence of the origin of the variant from the type form is usually absent or unconvincing. For instance, strains of bacteria belonging to the coli-typhoid family which resemble members of the *B. coli* group in producing acid from lactose, and in not liquefying gelatine, but which produce no gas from the sugars from which acid is formed, have been described by a few writers.

Mair (1906) recorded two strains of a coliform bacillus isolated from the urine of two patients which formed acid but no gas from glucose and lactose.

Wilson (1908) retested Mair's strains. He also examined 50 varieties of coliform organisms from the urine of cases of cystitis, pyelitis etc. Three of these latter strains formed acid but no gas from glucose. Including Mair's two bacilli he investigated five such strains. Of these five, two formed acid from the same sugars and alcohols as *B. acidi lactici*, with the exception of adonite, and also like it formed indole in broth. One of the two latter strains formed acid and gas from mannite and sorbite, but only acid from glucose, lactose and other sugars, and therefore resembled Penfold's chloracetic variants.

I have been able to find a record of only one instance in which two varieties of a bacillus, which differ in little except in the production and non-production of gas, have been isolated from the same patient under circumstances which make it practically certain that the two varieties are in reality of identical origin and have become differentiated in the body of the patient.

The case referred to is that described by Sörensen (1912) who at different times isolated two strains of a coliform bacillus from the urine of a glycosuric patient. The two strains gave identical cultural reactions except that the one (Strain I) formed gas from all the sugars and alcohols from which acid was produced, and the other (Strain III *a*) formed acid only and no gas from these substances. (See Table I.)

TABLE I.

Sörensen's Bacillus	I	III *a*	III *b*	III *c*	IV
Glucose	A. & G.	A.	A.	A. & G.	A. & G.
Galactose			A.	A. & G.	A. & G.
Fructose			A.	A. & G.	A. & G.
Mannose			A.	A. & G.	A. & G.
Starch			A.	A. & G.	A. & G.
Maltose			A.	A. & G.	A. & G.
Lactose	A. & G.	A.	A. & G.	A. & G.	A. & G.
Cane sugar	A. & G.	A.	A.	A. & G.	A. & G.
Inulin			A.	A. & G.	A. & G.
Raffinose			A.	A. & G.	A. & G.
Mannite			A.	A. & G.	A. & G.
Milk	A.	A.	A.	A. & clot	A. & clot

There was evidence moreover that these two varieties exhibited the same differences in the patient's bladder. On the occasions on which the gas-forming variety (I) was isolated, the patient's bladder contained gas, and when the variety (III *a*) which did not form gas in culture was present, the gas in the bladder was not observed.

Sörensen showed the identity of the two strains by fermentation and cultural reactions but not by serum tests. Some differences were observed in the growth in broth. The gas-former grew with a slimy surface film on the medium and the bacilli grew in long threads, whereas the slimy character was absent in the other variety and the bacilli were shorter.

After growth for a month in artificial culture the variety of bacillus which at first formed no gas suddenly reverted to the gas-forming type (III *c*) as a sequel to growth on a 2% glucose medium.

At one period a culture (III *b*) with intermediate characters occurred in the course of cultivation on artificial media. This intermediate variety was distinguished by the formation of gas from lactose, but not from glucose or other sugars nor from alcohols.

At a later date the patient's bladder was again found to contain gas, and a gas-forming strain (IV) like Strain III *c* in every particular was isolated.

Strains I, III *c* and IV resembled each other in that they formed a slimy pellicle on broth; III *c* and IV differed from the other strains by clotting milk.

Natural variation of a bacillus of the *B. acidi lactici* group.

The bacillus which is the subject of the present communication was isolated from the urine of a man of 79 years who was suffering from an enlarged prostate and a variable degree of cystitis.

This bacillus, which belongs to one of the subgroups of *B. coli*, has been isolated from the urine every time that it has been examined, *i.e.* eight times in the 11 months from Feb. 1912 to 16 Jan. 1913. Though the bacillus presents variations in its characters, it is believed to be essentially the same throughout.

The urine has been passed into a sterile vessel and examined immediately. At first both staphylococci and streptococci were present as well as the coliform bacillus, but no streptococci were found at the last seven examinations and only a few staphylococcus colonies appeared on the cultures.

The deposit obtained by centrifuging the urine was examined in stained films and by plating out on agar. The bacillus was always found in large numbers by both methods.

Characters of the bacillus.

The bacillus isolated at the first examination of the urine in February 1912 had the same characters as the *B. acidi lactici* (Hueppe) as described by MacConkey (1909) except as regards motility. The typical *B. acidi lactici* is non-motile, but the bacillus now described is slightly motile in the sense that a few motile individuals can be found in six hours' and 24 hours' broth cultures. It therefore more accurately corresponds to No. 1 on MacConkey's list. It may perhaps for purposes of classification be called a motile variety of *B. acidi lactici*.

In morphology the bacillus varies in length but in young agar cultures it is frequently very short and almost coccal.

The tests used on each occasion on which it has been isolated have been, growth on gelatin, litmus milk, peptone-water containing 1 % of glucose, cane-sugar, lactose, dulcite, mannite, adonite or inulin, and Ehrlich's test for indole in broth cultures.

On the original agar plates used for isolation, the colonies have usually been very small and translucent for 24 to 48 hours, but later and in subcultures large semi-opaque flat colonies have developed.

On the last seven occasions during the four months Sept. 1912 to Jan. 16, 1913, on which the bacillus has been isolated, several colonies have been examined from each specimen by the above mentioned tests. A few colonies have been grown on a more extended series of carbohydrates, glucosides and alcohols, as will be detailed later. (See Table III.)

The result of these examinations has been that two distinct varieties of the bacillus have been isolated, and in addition cultures showing intermediate characters have frequently been obtained by picking off single colonies from the original agar plates.

TABLE II.

Varieties of bacillus.

	I	II	III	IV
Glucose	A. G.	A.	A.	A. G. S.
Lactose	A. G.	A.	A. G.	A. G.
Cane sugar	–	–	–	–
Inulin	–	–	–	–
Mannite	A. G.	A.	A. G.	A. G.
Dulcite	–	–	–	–
Adonite	A. G.	A.	A. G.	A. G.
Milk	A. C.	A. C.	A. C.	A. C.
Indole	+	+	+	+
Motility	+S.	+S.	+S.	+S.
Gelatin	–	–	–	–

A. = acid. A. G. = acid and gas. A. C. = acid and clot. + = indole positive. +S. = slight motility. – = no change in reaction of carbohydrates, or no liquefaction of gelatine.

I and II represent the two extreme varieties, III and IV the intermediate varieties.

Colonies on the original plates have, as a rule, been uniform in appearance and, when some difference in size or opacity has been noticed, these differences were not correlated with a different behaviour on test media.

No coliform bacillus giving other reactions has ever been found and the only other bacteria discovered have been staphylococci and previously to September 1912 also streptococci.

As is shown in Table II the only difference between the varieties has been in the production of gas, except perhaps some difference in

length of the bacilli and occasionally in the size of the colonies in subcultures.

Cultures of variety I sometimes show a longer form of bacillus than is found in similar cultures of variety II.

The two extreme varieties (I and II) have almost always bred quite true in artificial culture both when frequently subcultured and when left without subculture for upwards of three months on agar.

Evidence of an irregular change in an old agar culture has been noticed once and has latterly been obtained by growth in special media.

The intermediate varieties differed from the extremes only in details of the gas-producing function. They differed somewhat among themselves as regards the same character, some forms producing no gas from glucose but a full amount from lactose, while others produced a very little gas from glucose which was not increased after a week's incubation.

Some colonies produced only a very little gas from mannite.

TABLE III.

	I	I *a*	II	A	B	C
Glucose	A. G.	A. G.	A.	A.	A.	A. G.
Laevulose	A. G.	A. G.	A.	A.	A.	A. G.
Galactose	A. G.	A. G.	A.	A.	A.	A.
Inulin	–	–	–	–	–	–
Dextrin	A. G. S.	A. G.?	A. G.?	A. G.?	A. G.?	A. G.?
Maltose	A. G.	A. G.	A.	A.	A. G. S.	A. G.
Lactose	A. G.	A. G.	A.	A.	A. G. S.	A. G.
Cane sugar	–	–	–	–	–	–
Raffinose	–	–	–	–	–	–
Arabinose	A. G.	A. G.	A.	A.	A. G. A.	A. G.
Salicin	A. G.	A. G.	A.	A.	A. G.	A. G.
Amygdalin	–	–	–	–	–	–
Isodulcite	A. G. S.	A. G. S.	A.	A. G.	A.	A.
Mannite	A. G.	A. G.	A.	A.	A.	A. G.
Dulcite	–	–	–	–	–	–
Sorbite	A. G.	A. G.	A.	A.	A. G. S.	A. G.
Adonite	A. G.	A. G.	A.	A.	A. G. S.	A. G.
Erythrite	A.?	–	–	–	–	–
Inosit	–	–	–	–	–	–
Glycerine	A. G.	A. G.	A.	A.	A. G.	A. G.
Milk	A. C.	A. C.	A. C.	A. C.	A. C.	
Indole	+	+	+	+	+	+
Motility	+S.	+S.	+S.	+S.	+S.	+S.
Gelatin	–	–	–	–	–	–

A = acid. A. G. = acid and gas. A. G. S. = acid and slight gas. A. C. = acid and clot. Indole + = indole formed. Motility + S. = slight motility. Gelatine – = no liquefaction.

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Moreover these intermediate forms showed a great tendency to change into variety I which produced a full amount of gas, or occasionally into variety II.

Three strains, namely I and I *a*, which formed gas, isolated respectively in February and Sept. 1912, and II, a strain which did not produce gas, which was isolated in Sept. 1912 at the same time as I *a*, were inoculated on 22nd December, 1912 into peptone water tubes containing 20 different carbohydrates, glucosides and alcohols. (See Table III.)

No differences between the three strains were found except as regards the production of gas. Strains I and I *a* however always produced gas when acid was formed, whilst strain II produced only acid and never gas from any of the substances with the possible exception of dextrin, but in all the dextrin tubes small bubbles of gas appeared and the acid reaction was slight even in the case of Strains I and I *a*.

All the strains formed acid and clot in milk, produced indole in broth, and grew on gelatine at room temperature without causing liquefaction.

At the same time three intermediate strains, A, B and C, which had been recently isolated (on Dec. 9, 1912), were inoculated into peptone water containing the same 20 test materials. These latter strains proved to be identical with the three strains I, II and I *a* as regards their acid-forming properties, but varied amongst themselves as to gas production.

Strain A formed no gas, B very little gas and C much gas when grown on glucose peptone water immediately after isolation.

Serum tests.

Serum experiments were also made in order to test the identity of the three strains I, II and I *a*. Three rabbits were inoculated with pure cultures of the different strains, and their sera used for agglutination tests and also for testing the absorption of agglutinins by the different strains. The results as seen in Tables IV and V showed no differences of any note between the three strains. No sign of agglutination appeared with normal rabbit's serum in dilutions of $\frac{1}{200}$ to $\frac{1}{12800}$, nor in 0·85 % salt solution. The agglutination was carried out at the room temperature and was complete in 20 hours.

For comparison three latoratory strains of *B. acidi lactici* (Hueppe) were also tested as regards agglutination. With serum prepared from

TABLE IV.

Agglutination.

Serum I	1/400	1/800	1/1600	1/3200	1/6400	1/12800	(Control salt solution)
Bac. I	⫴	⫴	⫴	⫴	⫴	‡	–
Bac. II	⫴	⫴	⫴	⫴	⫴	‡	±
Bac. I *a*	⫴	⫴	⫴	‡	+	–	±
Ser. I a							
Bac. I	⫴	⫴	⫴	⫴	⫴	‡	–
Bac. II	⫴	⫴	⫴	⫴	⫴	‡	–
Bac. I *a*	⫴	⫴	⫴	⫴	‡	±	–
Ser. II							
Bac. I	⫴	⫴	⫴	⫴	⫴	+	–
Bac. II	⫴	⫴	⫴	⫴	⫴	‡	–
Bac. I *a*	⫴	⫴	⫴	⫴	‡	‡	–
Bac. ac. l. 1	–	–	–	–	–	–	–
Bac. ac. l. 2	–	–	–	–	–	–	–
Bac. ac. l. 3	–	–	–	–	–	–	–

⫴ = complete. ‡ = nearly complete. + = marked. ± = very slight.
– = no agglutination.

TABLE V.

Agglutination after absorption of agglutinins.

Ser. I (abs. with Bac. I)	1/800	1/1600	1/3200	1/6400	1/12800
Bac. I	±	–	–	–	–
Bac. II	+	+	–	–	–
Bac. I *a*	±	±	–	–	–
Ser. I (abs. with Bac. II)					
Bac. I	+	±	–	–	–
Bac. II	+	+	±	–	–
Bac. I *a*	±	±	–	–	–

TABLE VI.

Ser. II (abs. with Bac. I)	1/800	1/1600	1/3200	1/6400	1/12800
Bac. I	⫴	‡	+	±	–
Bac. II	⫴	⫴	‡	–	–
Bac. I *a*	‡	‡	+	–	–
Ser. II (abs. with Bac. II)					
Bac. I	⫴	⫴	‡	–	–
Bac. II	⫴	⫴	‡	±	–
Bac. I *a*	‡	‡	+	–	–

(Bac. I and I *a*, gas-formers. Bac. II, non-gas-former.)

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Bacillus I all three strains agglutinated fairly well in dilution of $\frac{1}{8000}$ and very slightly in $\frac{1}{32000}$. With serum prepared from *Bacillus II* no agglutination occurred in a dilution of $\frac{1}{800}$ nor in higher dilutions.

The agglutination of typical laboratory cultures of *B. acidi lactici* was therefore slight with Serum I and very slight or absent with Serum II. This evidence as far as it goes points to a closer relationship between the three strains I, II and I *a* than between any of them and the laboratory strains.

The two sera I and II prepared with strains I and II respectively were used for absorption experiments and each serum was absorbed with each of the two bacilli separately. The results given in Tables V and VI show an approximately equal absorption by the different bacilli, of the agglutinins for all three strains.

The complete uniformity of the sugar tests as regards acid production and the strong confirmation of the identity of the three strains given by the agglutination tests leave little room for doubt as to the identity of origin of the varieties which form gas and those which do not.

It appears almost conclusively proved that they all originated from a single strain.

Transformation of one variety into another.

As a further test of the identity of the two varieties, attempts were made by culture to transform one variety into the other.

After many failures it was eventually found possible to transform the non-gas-forming variety into the variety which produced gas from sugars. Thus completing the proof of the essential unity of the two varieties.

Cultures of the two extreme varieties remained constant for over four months when subcultured at irregular intervals on agar or broth. In order to obtain information as to the media which favoured either variety, mixtures of the two forms were made by introducing drops of broth cultures of the two varieties into tubes of broth in different proportions, and subculturing daily after incubation into glucose and lactose peptone water. On subculture from mixed cultures after 18 days incubation gas was produced from lactose but little or none from glucose. When 1 % glucose tubes which had been inoculated with mixtures in broth, were incubated and subcultured daily in glucose and lactose, after incubation for 48 hours, only acid and no gas were produced in the sugars. A similar result was obtained in subcultures after four days, but on the fifth day the subcultures failed to grow.

Glucose tubes containing pure cultures of the gas-former were also sterile after four days incubation. This and similar experiments showed that in glucose cultures the gas-former died out in four days to a week at 37° C., but the non-gas-former lived seven to ten days under the same conditions.

In lactose tubes inoculated with a pure culture of the gas-former, subcultures grew and showed production of acid and gas after at least eight days; and after at least five days in the case of mixed cultures, subcultures gave similar results.

It was found too that cultures of the intermediate varieties which when first isolated formed acid and no gas from glucose but acid and gas from lactose, changed so as to produce acid and gas from both sugars after growth in broth for two or three days, but after growth in glucose peptone water for a similar period sometimes no gas was formed from either sugar.

In accordance with these results attempts were made to procure a variation in the gas-former by plating out after three or four days' growth on glucose peptone water, and examining a number of colonies in expectation that if a non-gas-forming variant occurred it would be encouraged in the acid glucose culture in preference to the gas-former.

No such variant could be demonstrated by these means. Attempts were also made, so far without success, to select out a gas-former from a culture of the variety II by prolonged incubation in weak (0·05 %) glucose broth or in ordinary broth, and also by repeated subcultures in ordinary broth continued for over a month.

Penfold (1911, 1912) found that his chloracetate variant of *B. coli* which produced no gas from glucose readily produced gas from sodium formate, showing that in all probability the peculiarity of this variant consisted in a defect in its power of making formic acid from the sugar.

He found, moreover, that by growing *B. typhosus* and his chloracetate variant together in glucose peptone water gas was formed which he attributed to the breaking down of the formic acid made by the *B. typhosus*.

Harden and Penfold (1912) subsequently confirmed this view by showing that the amount of formic acid produced by the chloracetate variant was much less than that produced by the original strain of *B. coli*.

In order to find out whether my gas-former (II) had similar characteristics as regards gas formation from formates, cultures were made with a loop of a broth culture of variety II (1) together with

B. typhosus in glucose peptone water, (2) in peptone water and in broth containing sodium formate with the following results:—

The gas-forming variety (I) produced abundant gas from peptone water containing sodium formate in 24 hours.

Variety II which did not form gas from sugars or alcohols was shown to be a slow gas-former from sodium formate. (See Table VII.)

Similar results have been obtained in broth containing larger percentages of formate.

TABLE VII.

One loop of broth culture of II (non-gas-former) added to each tube and incubated at 37° C.

Tube		Time	Result
1	1 % glucose peptone water + five drops of a *B. typhosus* culture	7 days	Acid, no gas
2	0·5 % sodium formate in peptone water	5 ,,	No gas
3	1·0 % ,, ,, ,, ,, ,,	5 ,,	Some gas
4	2·0 % ,, ,, ,, ,, ,,	5 ,,	No gas
5	1·0 % ,, ,, ,, broth	2 ,,	Some gas

TABLE VIII.

Cultures of variety II (non-gas-former) in media containing sodium formate subcultured to lactose.

Date of culture	% formate	Date of subculture to lactose	Result in lactose
24/1/13	2 % in peptone water	29/1/13	Acid on 30/1/13
		31/1/13	,, ,, 1/2/13
,,	1 % ,, ,,	29/1/13	,, ,, 30/1/13
		31/1/13	,, ,, 1/2/13
,,	0·5 % ,, ,,	29/1/13	,, ,, 30/1/13
		31/1/13	,, ,, 1/2/13
,,	1 % in broth	29/1/13	Acid and gas on 30/1/13 (small quantity)
		31/1/13	Acid and gas on 1/2/13 (large quantity)

It seemed possible that this late yield of gas was due to a "training" or selection of the bacilli which acted on the formate under suitable conditions of growth, especially in the absence of acid which appears to inhibit or destroy the gas-formers as shown above.

The cultures of II in formate (see Table VIII) were therefore subcultured to lactose peptone water with the following results:—

The 1 % formate broth was also plated out on agar on the 31st Jan. and after incubation showed colonies of different sizes. Five large colonies when inoculated into lactose broth gave acid and no gas as before, but of nine small colonies inoculated into lactose peptone water, eight gave acid and gas after incubation for 24 hours. The gas-forming property still remained after subculture on ordinary nutrient broth.

These experiments appear to show that the defect in variety II associated with its inability to form gas is not a want of power to make formic acid but to an inability to split the formic acid formed under ordinary conditions of culture in glucose peptone water, and that the power to produce gas from formates may be acquired in a neutral solution of sodium formate in broth.

The new variant (II *a*) of variety II was examined by inoculation of the formate broth culture into a series of sugars and alcohols, and the identity of its reactions with those given by variety I was shown.

Further work is being done on these varieties.

Discussion of results.

There is no direct evidence in the case of the bacillus described above as to which variety should be considered the original parent and which the more recent variant. However, the fact that the gas-former corresponds in almost all its characters with the *B. acidi lactici* which is very commonly met with in the faeces, makes it more probable that this is the parent form.

Variation in bacteria which is shown by the loss of some property has been attributed to a general lowering of the functional activity of the bacteria concerned, in which one function has been suppressed before the remainder, because it has been more recently acquired or is less essential to life.

Thus Scheierbeck's variant streptococci were produced by the inhibiting agent—carbolic acid—and the impaired acid-forming function of the bacteria was associated with slower growth.

Such an explanation, if cogent, would perhaps lessen the importance of lost characters from the theoretical standpoint of evolution, though for the practical purpose of diagnosis the loss and the acquisition of a characteristic are equally important.

The loss or impairment of a function may however be associated with a gain in another direction as in the case of Scheierbeck's streptococcus cultures which produced less acid during the first period

of growth, but continued to grow for a longer time in the cultures and eventually formed a higher percentage of acid than the more rapid acid-formers.

Penfold's variant on chloracetic-agar which had lost its power to form gas from sugars, grew in larger colonies on the selective medium than the original type, indicating an increased vigour rather than a diminished one.

One of the varieties of the bacillus which is the subject of this paper, had no power of forming gas from sugars or alcohols, and in some instances appeared to grow in smaller colonies on agar than certain races of the gas-forming variety. When however cultures of the two varieties were mixed in broth and the mixture plated out on agar the gas-former grew in smaller colonies than the variety whose power to form gas was in abeyance. This was shown by inoculating a number of the small and of the large colonies into glucose peptone water.

Moreover, the variety which did not form gas survived for a considerably longer period in glucose peptone water than the gas-former.

The suggestion, therefore, that a general weakness is the rule in the case of strains of bacteria which show loss of function in one direction, is by no means supported by the evidence in all cases, and the apparent loss may be fully compensated in other directions.

Some special features of the varieties described above remain to be mentioned.

(1) The power of producing gas from alcohols is absent in the case of those strains which have not this function in regard to sugars. In this respect the anaerogenic strain resembles those produced by Revis by cultivating *B. coli* on malachite-green, and also differs from Penfold's variant in that it does not readily produce gas from formates.

(2) The intermediate strains which I obtained from colonies on the original plates mostly showed the remarkable characteristic that they produced a full amount of gas from lactose in 24 hours, but none or very little from glucose in the same time. This behaviour is difficult to explain since *a priori* it seems probable that the lactose is first split into glucose and galactose before these sugars are further acted on with the production of acid and gas.

The suggestion that the gas was formed from the galactose split off from the lactose appeared to be negatived by the fact that when grown in galactose and in glucose, these strains yielded very small amounts of gas from both these sugars, but from lactose at the same time a large yield was obtained.

This observation however does not stand alone. Penfold (1911, 1912) found that during the selection of his chloracetate varieties, the power to form gas from glucose disappeared before the same function as regards lactose.

Revis also had the same experience. Sörensen too noted a somewhat similar phenomenon, as is shown in Table I.

(3) The relative proportions of the different varieties obtained from the samples of urine varied somewhat on different occasions. (See Table IX.)

Thus of 70 colonies picked off the plates made on the 8th December, 21 formed full gas, and 24 formed acid only, whereas 25 showed intermediate characters.

TABLE IX.

Number of colonies examined on each occasion.

Sample of urine... / Date...	R. 1 12/9/12	R. 2 20/9/12	R. 3 10/10/12	R. 4 31/10/12	R. 5 8/12/12	R. 6 31/12/12	R. 7 17/1/13	Total
Full gas I	4	3	4	3	21	5	4	44
No gas II	2	1	4	3	24	15	14	63
Intermediate III. (No gas from glucose)	3	0	1	0	0	1	3	8
Intermediate IV. (Some gas from glucose)	3	2	0	0	25	3	9	42
	12	6	9	6	70	24	30	157

Of a total of 157 colonies picked off the original plates on the last seven occasions, 44 produced a large amount of gas from glucose, lactose and mannite; 63 formed no gas from any of these three substances and 50 were intermediate in character. Of these latter eight produced no gas from glucose, while the lactose tubes showed a large amount of gas in the Durham's tubes. The remainder formed very little gas from glucose but a large amount from lactose. In each case the gas production was judged by the gas collected in the small Durham's tubes.

It seems unlikely that so large a proportion as 50 isolated colonies out of 157 (31·8 %) were formed from two bacilli of different varieties. These intermediate forms must therefore be regarded as having arisen from single bacilli of intermediate character or from bacilli which at once gave rise to individuals of varying type.

(4) It has not been found possible to maintain the intermediate strains constant. When plated on agar from broth, or glucose or lactose

peptone water cultures, the colonies when inoculated into sugar media usually conform to one or the other extreme type of variety, but occasionally a culture showing intermediate characters has been obtained after plating out the culture three times in succession.

CONCLUSIONS.

1. A bacillus belonging to the *B. acidi lactici* group has been repeatedly isolated during 11 months from the urine of one patient, and no other Gram-negative bacillus has been found in the same urine during this period.

2. The bacillus has occurred in two varieties which differed as regards gas-formation only. Variety I formed gas from sugars and alcohols and variety II formed acid and no gas from the same sugars and alcohols.

3. The two varieties gave identical serum reactions both as regards agglutination and absorption of agglutinins with specific sera prepared from rabbits immunised with the respective varieties.

4. Intermediate varieties as regards gas production also occurred, but were not constant when subcultured.

5. Varieties I and II remained constant in their characters after four months' subculture on broth and agar.

6. Variety II which at first did not produce gas from sugars was induced to do so by first growing in a solution of sodium formate in broth.

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