Variation in Bacillus coli.—*The Production of Two Permanent Varieties from one Original Strain by means of "Brilliant Green."*

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In a former communication* it was noted that a profound change in physiological activity could be brought about in some strains of *Bacillus coli* by growth in the presence of Malachite Green. This change consisted in the complete loss of power to produce gas in the usual test media, the action ceasing at the acid stage.

Similar experiments have been carried out using Brilliant Green in place of Malachite Green, and many interesting results have been obtained. The following is of particular interest in that two permanent varieties arose from one original organism under precisely similar circumstances. The method employed was exactly the same as in the former experiments. The culture used was repeatedly plated until it was evident that the final culture used had arisen from one single organism. This procedure is undoubtedly better and less liable to error than some of the more complicated methods which have been proposed (*cp.* Eisenberg†).

The properties of the original culture were :—

Milk	Coagulated in 48 hours.	
‡Lactose peptone water	+ + +	„ „
Sucrose „	——	
Adonitol „	——	
Dulcitol „	+ + + +	on 5th day.
Inulin „	——	
Glucose „	+ + +	in 48 hours.
Salicin „	+	on 5th day.
Mannitol „	+ + + +	in 48 hours.

This selected culture was then inoculated into nutrient broth (+I) containing 0·004 per cent. of Brilliant Green. Two different inoculations

* Revis, 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 192.

† Eisenberg, 'Cent. für Bakt.,' 1912, Abt. I, vol. 63, p. 305.

‡ The positive sign indicates the production of *acid* and *gas* in the test medium, using 5 c.c. medium and a gas tube $\frac{3}{16}$ inch diameter. The signs +, + +, + + +, and + + + + indicate $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ inch of gas in the tube. No change, thus ——.

were started in order to provide a control for any changes which might occur. This procedure has, however, been found subsequently to be of little avail, as in many cases in which two inoculations from the same cultures have been so started they have not behaved in the same manner under the same circumstances. In this particular case one culture experienced much greater difficulty in growing in the presence of Brilliant Green and eventually died out. The other which survived was finally plated out, when it had been trained to grow in broth containing 0·05 per cent. of the dye, and the organism was then developing well in it. The plating out was made on ordinary nutrient (+I) agar and the plates were kept at 20° C.

Two types of colony arose:—(A) very small, (B) large and arborescent. Both varieties grew rather slowly at 20° C. on the plates, as did also sub-cultures from them. They were tested in the usual peptone water media with the following results:—

(A)	Milk	Acid in 7 days, but no coagulation at all.
	Lactose peptone water ...	++ in 48 hours.
	Sucrose „ ...	——
	Adonitol „ ...	——
	Dulcitol „ ...	*A., sl. G., about the 20th day.
	Inulin „ ...	——
	Glucose „ ...	++ in 48 hours.
	Salicin „ ...	A., sl. G., on the 7th day.
	Mannitol „ ...	+ in 48 hours.

(B) was very similar, but milk coagulated in 48 hours, dulcitol was not attacked at all, and only A., sl. G. occurred in mannitol.

It is noteworthy that the organism which showed the greater vitality (judged by growth on agar) had suffered the greater loss of fermentative power. This has been found to be the rule in other cases. Both of these organisms (A and B) were carried on in Brilliant Green broth (0·05 per cent.) but (A) soon succumbed, while (B) grew as well as before. This will now be called Culture B.

The original culture in Brilliant Green broth from which these (A and B) were obtained was kept going in the same medium (Culture C) and an inoculation was also made from it into Malachite Green broth (Culture D). In all cases these cultures were re-inoculated into fresh tubes once a week and were all kept at 37° C.

* Indicates *acid* reaction, but only very small bubble of gas.

Culture C.

This was plated out several times at intervals of about two months, but only one type of organism was obtained in every case. This type coagulated milk usually in 48 hours, produced only A., sl. G. in lactose, A., sl. G. to +reaction in dulcitol (in 8 to 12 days), + +reaction in glucose and A., sl. G. to +reaction in mannitol (in 48 hours). In salicin, acid usually appeared about the 7th day. This variety was quite permanent. Every endeavour was made to restore the original activity of the organism, without success.

It does not differ markedly from the original culture, but tested side by side the difference is decided.

Culture D.

This was the inoculation of Culture C made into Malachite Green broth (0·05 per cent.), as stated. It underwent no greater physiological changes than did C, milk did not coagulate until about the 15th day in most cases, and in lactose never more than the A., sl. G. reaction was obtained. Physiologically the organism was the same as Culture C and was as permanent; culturally it showed marked differences. Its power to grow at 20° C. was greatly inhibited. No growth was apparent until six days had elapsed, and then a very watery viscid streak appeared. Growth at 37° C. was quite normal.

Culture B.

This organism, on the other hand, became profoundly modified. After several re-inoculations in B.G. broth it was plated out and the colonies obtained on testing in the usual media gave: Milk, acid 48 hours, coagulated in about five days; lactose, glucose, and mannitol, acid in 48 hours, but no gas at any time; dulcitol, not attacked at all; salicin, sometimes acid after several days and sometimes not attacked. Every attempt to restore the original activity failed, the above features being quite permanent. One colony of this type was then started in Malachite Green broth (0·05 per cent.), in which it soon grew well.

After several re-inoculations, the colonies, on plating out on agar (+I) at 20° C., came up very slowly and were watery and very large ($\frac{1}{2}$-inch diameter). On testing, acid production was delayed in lactose and mannitol till the 5th day, but glucose was rendered acid in 48 hours. Sub-culture on agar at 37° C. restored acid production in lactose to 48 hours, but not in mannitol, which was still delayed. Gelatin sub-cultures at 20° C. were rather weaker physiologically than the original culture from which they were taken.

It must be carefully noted that only changes in physiological activity had occurred. The ordinary growth of all the organisms described above was

quite normal and strong at 37° C. and was quite strong at 20° C., there being only a great tendency to delay in development. In every case before testing in the various peptone waters a strong growth was first obtained and heavy inoculations were used, and there was no trace of the dye stuff added to the test media with the culture. The effect which was produced in each case described above had been brought about by some impression made on the protoplasm as it was transmitted unchanged through successive cultivations on ordinary media.

The point which stands out clearly from the above results is that, from an original typical culture of *B. coli* obtained from a single cell, two strains have arisen, (1) a strain slightly modified by the dye stuff, but in a permanent manner *and refusing to be further affected*; (2) a strain gradually undergoing profound and increasing change in the same environment and resulting in an organism entirely different from the original culture, the strain being also of a permanent character.

It is important to notice that from one original organism there have arisen, by a simple process of cell division, at least two organisms, one of which is practically resistant to its environment, while the other has become greatly and progressively modified. It has been held that all such individuals should behave alike under similar circumstances, but it has been my constant experience that this is not the case. Failure to recognise this has no doubt led to the impression that organisms do not show variation.

Further, granting that these fermentative changes are brought about by enzymes present in the bacterial cell, it is evident that these are not an intrinsic and integral part of the protoplasmic substance. They may be entirely lost or greatly modified in activity, and, supposing that two enzymes at least are necessary to bring about the complete fermentation of the test substance, it is also evident that those which bring about the acid change may subsist while those which produce gas, etc., are completely lost.

Under these circumstances, it is not too much to suppose that in the life of the organism itself, the opposite phase may occur, and that, as under certain circumstances fermentative power is lost, so also, under some other set of circumstances, it may be acquired when it does not already exist.