THE NATURE OF THE FACTOR INHIBITING THE FERMENTATION OF A SUGAR IN THE MUTABILE AND PARACOLON FORMS OF *BACILLUS NEAPOLITANUS* EMMERICH.

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In a previous paper (Stewart, 1926), we have seen that the mutabile form of *B. neapolitanus* Emmerich is a heterozygote with a Mendelian formula IiFF, in which F represents a unit character for the fermentation of a particular sugar, *e.g.* lactose, I represents a factor for the inhibition of F, and i represents the absence of I in a pair of allelomorphs. Similarly paracolon has a formula IIFF.

A mutabile varying to lactose, if grown on a plate containing lactose with neutral red, will form white colonies, which after a definite period exhibit papillae, and these papillae then become red from the fermentation of lactose. In some strains the colonies remain white until the plate dries up, in other words the dominance of I over i is complete, but in the majority of strains the colonies, while originally white, do under a certain condition gradually assume a pink tinge, which is however not so vivid as the red of the homozygous colon form which has a formula iiFF. The condition under which this pinking occurs is that the plate should be sparsely sown. On a plate which is at all crowded the colonies remain white, while on a sister plate thinly sown from the same bacterial emulsion they become pink. Nevertheless on both the crowded and the thinly sown plate papillae appear and become red with the same regularity and at the same stage of their development.

The following are two examples of this phenomenon:

Bacterium TAB 1678, subrace 4336, mutabile.

Day 1st. Lactose plate 4355 thickly sown; 4356 thinly sown.
Days 2nd and 3rd. Colonies on both plates white; 4355 crowded.
Day 4th. 4355, colonies white; 4356, white with pink centres.
Day 5th. 4355, white colonies with dew drop papillae; 4356, white with pink centres.
Day 6th. 4355, white with papillae turning red; 4356, pink white with papillae.
Day 7th. 4355, white with red papillae; 4356, pink with papillae turning red.
Day 8th. 4355, white with red papillae; 4356, pink with red papillae.

Bacterium AK 3797. *B. dysenteriae* Flexner, showing Mendelian variation to saccharose.

Day 1st. Saccharose plate 4908 thickly sown; 4909 thinly sown.
Day 2nd. Colonies on both plates white; 4908 crowded.
Day 3rd. 4908, colonies white; 4909, colonies white with pink centres.
Day 10th. 4908, colonies white with red papillae; 4909, red with red papillae.

In the prevention of the pinking of the colonies on the crowded plate we are clearly not dealing with a deficiency of lactose, since the papillae become red even on the crowded plates. Nor are we dealing with a simple chemical neutralisation outside the bodies of the bacteria of acid which has already been formed, since any free neutralising agent would emphatically prevent the development of acidity in the minute papillae if it were present in sufficient strength to neutralise the large colonies.

We are therefore driven to conclude that the inhibitory factor I is present in greater concentration in the crowded than in the thinly sown plate, but that it acts by inhibiting the vital power of lactose splitting only in the body of the bacterium which produces it.

There are three facts to be reconciled: (1) On sparsely sown plates the inhibitory factor is not present in sufficient strength to inhibit completely the fermentation of the sugar. (2) On the crowded plates it is sufficiently concentrated to prevent fermentation in the colonies. (3) Nevertheless fermentation occurs equally in the papillae of crowded and uncrowded plates.

These three facts can only be reconciled if we regard the inhibitory factor as an internal secretion elaborated in the body of the non-sugar fermenting bacterium. This secretion can pass out of the body of the bacterium into the surrounding medium, and it is clear that on the thinly sown plate diffusion will be more rapid than on the crowded plate. There will therefore always be a higher concentration of this secretion in the bodies of the bacteria on the crowded than on the thinly sown plate, and the inhibition will be complete on the former, while it may be incomplete on the latter.

Secondly the secretion acts only within the body of the bacterium which produces it, and has no power of diffusing into the bodies of other bacteria, hence even on the crowded plate, with a concentrated inhibitory secretion in the medium, the homozygous recessive forms in the papillae are not inhibited from fermentation of the sugar concerned.

This internal secretion is clearly comparable to those internal secretions of higher animals to which the term chalone is applied, and we can find a striking similarity between its action and that of the ovarian internal secretion of the hen (Sharpey-Schafer, 1924–26, pp. 7 and 385). In the fowl as in other birds the female is heterozygous for sex, producing ova one half of which bear the male character and one half the female. The male sexual characters, both primary and secondary, are potentially present in the female, but are held inhibited by an inhibitory factor. This inhibitory internal secretion or chalone is provided by the ovary. If the ovary is completely removed the hen acquires the secondary sexual characters of the male, and in some cases even the primary characters as well, while if new ovarian tissue is now grafted on such a bird the male characters recede. The inhibitory factor for the male characters is therefore derived from the primary female character, in regard to which the hen is heterozygous, and her Mendelian formula can be written IiMM, where M represents the factor for the male characters and I a factor for their inhibition.

In the same way the mutabile form of a *B. neapolitanus* Emmerich, and of other species, is heterozygous for a factor inhibiting sugar fermentation. When this factor is present in full strength inhibition is complete, and although the factor cannot be extirpated experimentally as in the fowl, nevertheless it can be weakened by artificial culture on thinly sown solid media. When this has been accomplished the inhibited character of sugar fermentation makes its appearance in the pinking of the colonies.

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