A REVIEW OF SOME RECENT WORK ON PAPILLARY VARIATION IN BACTERIA AND BACTERIAL CYTOLOGY

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CONTENTS

Introduction

Bacterial papillae are raised spots which make their appearance on the colonies of agar plates or of other cultures on solid media. They are essentially daughter colonies which arise when the parent colony has ceased to grow. In their early stages they measure about 0·05 mm. in diameter and in many species of bacteria they grow no larger. In other species, however, favourable variation may take place in the papillae resulting in their active growth. These papillary variations have been studied especially in the coli-typhoid-dysentery group. They are strictly heritable and may occur in many characters such as sugar reactions, pigmentation, shape and size of the bacteria, capsulation, shape and consistence of the colonies. The changes in sugar reactions are most easily observed and recorded, and strains of bacteria can be found which vary regularly every few days in their power of fermenting lactose, saccharose, maltose or dulcite. Of such variable bacteria the best known is *Bact. coli-mutabile* (Neisser, 1906; Massini, 1907), a coliform organism which forms white, non-lactose-fermenting colonies on lactose neutral-red plates. After two or three days papillae appear on the colonies, which increase in size, form a red, lactose-fermenting core and grow out over the surface of the parent colony. Subcultures from red papillae yield separate red and white colonies. The white colonies continue to form red papillae, but the red race breeds true and does not revert to white even after prolonged growth on non-lactose media.

In the allied *Bact. paracolon* the small white papillae remain unchanged except in very rare cases, when, under a strong stimulus, as for example on first isolation or after long growth and drying on a plate, one or two papillae may become red and enlarge. Subcultures from these rare red papillae yield *Bact. coli-mutabile* either red alone, or red and white (Stewart, 1927, pp. 45, 49). The derived *Bact. coli-mutabile* has on one occasion thrown a reversionary *Bact. paracolon*.

Technique of pure line cultures

Work on such a subject must be conducted with pure lines each derived from a single organism. Such pure lines may be established by one of two methods: (1) single-cell culture, or (2) careful plating.

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(1) Regarding the first method Bernhardt (1915) writes: 'In our first experiments with dysentery bacteria we used Burri's single-cell method to verify our results but later we have convinced ourselves in agreement with other workers, that we were able to arrive at the same results by the ordinary methods of bacteriology, viz. sufficient dilution of the original material and plating in series.'

(2) Plating by the two-plate method, with a right-angled glass rod, from a carefully prepared and shaken suspension, gives well-isolated colonies. Experiments with a mixture of about equal numbers of red and white bacteria prove conclusively that such colonies are derived each from one cell only (Stewart, 1942).

Agglutination fortunately provides a method of checking whether two lines are related or not, since in *Bact. paracolon* and *Bact. coli-mutabile* an antiserum to one strain agglutinates that strain only. For example (Stewart, 1927):

Antiserum to *Bact. coli-mutabile*, race 249, white, from patient F.S. agglutinated:

Bact. coli-mutabile, 249, white, from F.S. (the antigen)	to 1 in 5000
Bact. coli-mutabile, 249, red, from F.S.	to 1 in 5000
Bact. paracolon, 3674, from F.S.	to 1 in 0
Bact. coli-communis, from F.S.	to 1 in 80

Antiserum to *Bact. paracolon*, race 1678, from patient T.A.B. agglutinated:

Bact. paracolon, 1678, from T.A.B. (the antigen)	to 1 in 2500
Bact. coli-mutabile, 1678, white, subline B, derived from above	to 1 in 2500
Bact. coli-mutabile, 1678, red, subline E, derived from above	to 1 in 500
Bact. paracolon, 1678, subline G, reversion from subline B	to 1 in 5000
Bact. coli-mutabile, 419, white, from patient I.B.	to 1 in 0
Bact. coli-mutabile, 419, red, from patient I.B.	to 1 in 0
Bact. coli-communis, stock	to 1 in 0

LACTASE IN THE WHITE FORM OF *BACT. COLI-MUTABILE*

These three forms therefore, *Bact. paracolon*, *Bact. coli-mutabile* white and *Bact. coli-mutabile* red, constitute together a single species with a central unstable white variant between a stable white on the one hand and a stable red on the other, an arrangement which suggests that the unstable white is in some way comparable to a heterozygote between two homozygous forms. If this is so then the negative character (non-lactose fermenter) being dominant over the positive must be due not to absence of lactase but to a factor which inhibits its action. That this is actually the case has been proved by Deere, Dulaney & Michelson (1939), who have demonstrated the presence of lactase in the white form of *Bact. coli-mutabile* after drying by vacuum distillation, and Deere (1939) suggests that the cause of the inhibition may be that the white cells are impermeable to lactose, while the lactase system is intracellular. The red cells, on the other hand, are lactose fermenters because they have become permeable to the sugar.

Such a supposition would also explain the fact that in many strains both of *Bact. paracolon* and of *Bact. coli-mutabile*, white colonies go red in the centre after one or two days' growth, but subcultures from these red centres are again white. The reddening would be due to the death of the older cells and the liberation of lactase into the medium.

Conditions governing variation

Variation from white to red does not depend on length of exposure to lactose. It takes place at that time in the life of a colony when the logarithmic phase has ended, provided the colony is then exposed to lactose, but irrespective of the length of that exposure. This statement rests on the following observations: (1) If the logarithmic phase is kept going by frequent subculture, variation in the chain of bacteria transmitted from culture to culture is postponed indefinitely, although in each individual culture it takes place after the completion of the logarithmic phase (Stewart, 1927; Deere *et al.* 1939). (2) If the logarithmic phase is cut short by massive inoculation or by starvation on a poor medium variation takes place early. (3) If the bacterium is plated on lactose-free agar and the colonies are allowed to grow to that age at which small red papillae would appear on a lactose culture of the same strain, and lactose solution is pipetted into small wells cut in the agar so that the sugar diffuses through to the colonies without disturbing them, then red papillae appear after a much reduced period of lactose exposure (Stewart, 1927, 1928).

The variation is discontinuous. In a culture of *Bact. coli-mutabile* in lactose peptone water no lactose whatever is consumed until the red variants appear (Stewart, 1927; Deere *et al.* 1936). In similar cultures of *Bact. paracolon* lactose is not consumed until the end of the second, third or fourth week. No red variants are then present and lactose consumption may be due to lactase liberated from dead cells.

Is variation spontaneous or induced and adaptive?

Does the variation white to red take place only on the appropriate sugar, e.g. lactose, or may it also take place on sugar-free media? Henderson Smith and more recently Lewis (1934) hold that the variation takes place spontaneously on any medium in a small proportion of the cells, about 1 in 100,000, and that these few red cells remain hidden until they come in contact with lactose, when they quickly overgrow the non-variant white cells. They consider therefore that the variation is not adaptive and that red overgrows white by natural selection. To demonstrate the hidden reds Lewis uses a synthetic medium with lactose which allows the growth of red but not of white. On plates of this medium he spreads many millions of white cells from non-lactose agar slopes. Colonies then appear in the proportion of one colony to 100,000 cells of the inoculum. These colonies are all red and Lewis claims that their parent cells underwent variation on the non-lactose medium. This, however, is not necessarily true as the same picture would result if the small proportion of cells had varied early after falling on the lactose medium. White bacteria on this synthetic medium, although they do not multiply sufficiently to form visible colonies, do not die off. They remain as a thin surface film in which variation would certainly be hastened since the logarithmic phase is prevented by crowding and starvation.

The present writer examined three samples of the white form from plain agar, by subculture every 12 hr. in large volumes of lactose-peptone water.* The samples consisted

* This method depends on the fact stated above that if subcultures of the white form in lactose peptone water are made in series every 12 hr. (to keep the logarithmic phase going), variation does not take place in the chain although it takes place in each individual culture after the 12 hr. limit. On the other hand, if the original inoculum contains even a very small proportion of red cells (1 in 250,000) they will manifest themselves after three or four subcultures by overgrowing the whites in the lactose medium. By this means red cells present in the inoculum and their descendants can be distinguished from variants formed during the experiment.

of 2,500,000, 2,850,000 and 80,000,000 bacteria but no red forms could be found (Stewart, 1942). He therefore concludes that variation takes place only on the appropriate sugar.

The variation therefore seems to be a heritable adaptation to environment by which lactase, present in the cell but inhibited, is freed from inhibition. The inhibition probably consists of resistance by the bacterial cytoplasm to the entry of lactose (Deere, 1939). In the variants this resistance is lost, the sugar is allowed to enter the cell and come in contact with the intracellular lactase system. Now if lactose cannot enter the cell in the white form how can it act to produce the variation? We can only suppose that in the autogamic phase even the white cell is permeable for the time being. Otherwise Deere's very attractive hypothesis falls to the ground.

UNIT CHARACTERS

In papillary variation different characters act as definite distinct units which can be built up as if they were bricks. For example: (1) One strain of *Bact. coli-mutabile* threw a red race on lactose and a pigmented yellow race on non-lactose. From the original white (non-lactose-fermenting) unpigmented strain, there could be formed red unpigmented, white pigmented and red pigmented strains. (2) A race of *Bact. dysenteriae* Flexner on maltose threw a pale red (feeble maltose fermenter) strain which in its turn threw a dark red strain. The three races plated together were quite distinct and without any gradation.

MARGINAL VARIATION

Although it is the rule that the red races of variable bacteria do not revert to white, yet there are a very few exceptional strains in which red constantly throws white. This reversion does not, however, take place in papillae but at the growing edge of the colony. It is therefore an example of the second or marginal type of bacterial variation, which differs from papillary variation in the fact that it takes place during and not after the logarithmic phase and will occur on any medium. The rough variation takes place at the margin. The present writer has met only two strains which revert from red to white, namely one *Bact. dysenteriae* Flexner, race A.K. on maltose (Stewart, 1927), and a strain of *Bact. coli-mutabile* in the collection of the Pathological Department, Cambridge.

HYPOTHESIS INTRODUCING AUTOGAMIC CONJUGATION

Papillae, as we have seen, represent daughter colonies which arise at the end of the logarithmic phase and which, if undisturbed by variation, remain at rest. It is not unreasonable to suggest that such resting daughter colonies may have their origin from an autogamic conjugation (that is the reunion of two nuclei which have divided without separation of their respective cells) since in other protists a wave of vegetative multiplication is followed by conjugation and a pause.

Autogamic conjugation was described by Schaudinn in *B. bütschlii* and *B. sporonema* (1902, 1903) and a similar appearance with a different interpretation by Dobell in *B. flexilis* and *B. spirogyra* (1908). In Schaudinn's bacilli the partition formed in the presporing division is reabsorbed, the chromidia of the two sister cells mix, then come together and coalesce to form the spores. In Dobell's *B. flexilis* the presporing division is only indicated by an annular groove but in the greater number of other bacilli it is

complete and ends in the formation of two cells each one-half the length of an ordinary vegetative cell. Dobell considered the complete division primitive and the incomplete as merely division manqué. Nevertheless the processes leading up to the presporing division in all forms are different from those leading to a vegetative division since the results differ. Dobell's view gives no explanation of the difference. The same author demonstrated well-defined nuclei in a number of large bacteria, their shape varying, some rounded, others filamentous, while a few were dispersed in numerous chromidia.

Cytology of *Myxococcus xanthus*

Recently, Beebe (1941) has studied *Myxococcus xanthus* by Feulgen technique and describes 'a compact or condensed nucleus: in the vegetative phase the nucleus is a single compact mass of nuclear protoplasm that divides prior to cell fission and is positive to nuclear dyes and Feulgen. In the transition phase it breaks up into four chromosomes stainable by gentian-violet and iron haematoxylin. In the prophase chromosomes are shown to be made up of chromeres. Autogamous fusion of chromatin material occurs before the mature spore has been formed and nuclear division, presumably meiotic takes place during germination of the spores.'

Nuclear apparatus of bacteria

Robinow (1942), using Feulgen technique with various stains and especially with Giemsa, studied *B. mycoides, B. proteus, Sarcinae* and other bacteria. His summary runs as follows: 'The structural unit of the nuclear apparatus of aerobic, spore-forming bacteria is a dumbbell-shaped body, giving a positive Feulgen reaction, and possessing a strong affinity for nuclear dyes. Resting spores contain one of these bodies, closely attached to, but distinct from, a rod of non-chromatinic protoplasm. Division forms of this body occur in a few spores. When germination begins, the dumbbell body enters the rod of protoplasm, where it soon becomes invisible. When it reappears at a later stage of the germination process, it has divided into two closely contiguous dumbbell bodies. Further divisions of the chromatinic bodies precede the divisions of the vegetative cell in a regular way. Each vegetative cell usually contains a pair of dumbbell bodies. Similar chromatinic bodies have been demonstrated in the cells of *Proteus vulgaris* and two Sarcinae.... It is concluded that the dumbbell bodies are comparable to the chromosomes of plant and animal cells.'

Robinow tells me in a personal communication that the nuclear pattern described by Beebe in *Myxococcus* as autogamic occurs also in such bacteria as *Bact. coli-communis* and *Proteus*, but he is not prepared at present to endorse Beebe's theoretical interpretation.

Summary

Some recent advances in the subject of bacterial variation and cytology are:

(1) The white race of *Bact. coli-mutabile* contains lactase, inhibited by some cause unknown (Deere *et al.* 1939). Deere (1939) suggests that the cells of the white race may be impermeable to lactose while the lactase system is intracellular.

(2) Variation from non-lactose-fermenter to lactose fermenter in *Bact. coli-mutabile* takes place on lactose only (Stewart, 1942).

(3) The nucleus of *Myxococcus xanthus* consists of definite chromatin masses. A process

resembling karyokinesis and autogamic conjugation precedes spore formation (Beebe, 1941).

(4) The nucleus of vegetative bacterial cells consists of a pair of dumbbell-shaped chromatinic bodies, which split longitudinally before cell division. The dumbbell bodies are comparable to the chromosomes of animals and plants (Robinow, 1942).

(5) Careful plating provides unquestionably pure lines in the coliform group (Stewart, 1942).

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