631. *Adaptive Behaviour of Coliform Bacteria to Certain Sugars.*

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Adaptation of a strain of *Bact. coli* to a cellobiose–ammonium sulphate medium and its complete adaptation to a corresponding glucose medium appear to be processes partially linked.

When *Bact. lactis aerogenes* is transferred between media containing glucose and inositol severally as carbon sources two types of behaviour are observable. In one, optimum adaptation to the two sugars can be held simultaneously; in the other, the two kinds of adaptation are to some extent incompatible and competitive.

The object of the experiments to be described was to clarify further the facts about the relations between the adaptive responses of bacteria to different carbon sources. Sometimes optimum-growth characteristics in one medium are quite compatible with those in another; sometimes two kinds of adaptation are incompatible and competitive, but the behaviour is of some complexity.

A preliminary investigation was carried out on the behaviour of several strains in inositol and cellobiose media, and the following two examples chosen for further study: (i) behaviour of *Bact. coli* (described as strain P34Type 1) in glucose and cellobiose media, and (ii) behaviour of *Bact. lactis aerogenes* in glucose and inositol media.

(i) *Behaviour of* Bact. coli *strain P34 Type 1 in Glucose and Cellobiose Media.*—The strain was transferred from nutrient broth to glucose to which asparagine in small quantity had been added. Subsequently it was subcultured daily in the glucose synthetic medium in the absence of asparagine. This medium contained, besides the carbon source, ammonium sulphate, magnesium sulphate, and phosphate buffer. After 20 subcultures in the glucose medium, when the mean generation time (the time taken for cell numbers to double in the logarithmic growth phase) was approximately stable, the strain was inoculated into a cellobiose medium and its mean generation time in this sugar measured at intervals; serial subculture in the glucose medium was also continued, and the mean generation time of the strain determined after every few subcultures. Experimental methods were as described in *J.*, 1938, 1930; 1939, 1683; 1943, 208; 1953, 663. At intervals the bacteria which had been transferred to cellobiose were re-transferred to glucose, and their mean generation time was determined. Fig. 1 shows the results obtained.

The mean generation times in the glucose cultures uninterrupted by passage through cellobiose remained fairly constant until the 48th subculture when the value fell by approximately 20%. The strain on first cultivation in cellobiose (after 20 subcultures in glucose) showed a mean generation time of 360 min., which during the next seven subcultures in this sugar fell rapidly to a value of 40 min., and then did not alter on repeated cultivation.

The mean generation time re-measured in glucose after a number of intervening passages through cellobiose fell together with that in cellobiose itself to a low and stable value. As usual it was reproducible and definite, and is the best general criterion of

bacterial growth rate. The value for the strain in glucose itself, however, did not reach a value of 40 min. until the 50th subculture in this sugar, but on subsequent passage through cellobiose it too fell to the same figure. From this it appears that there can be some change in the organisation of the cell which results in a more rapid reduction in the glucose

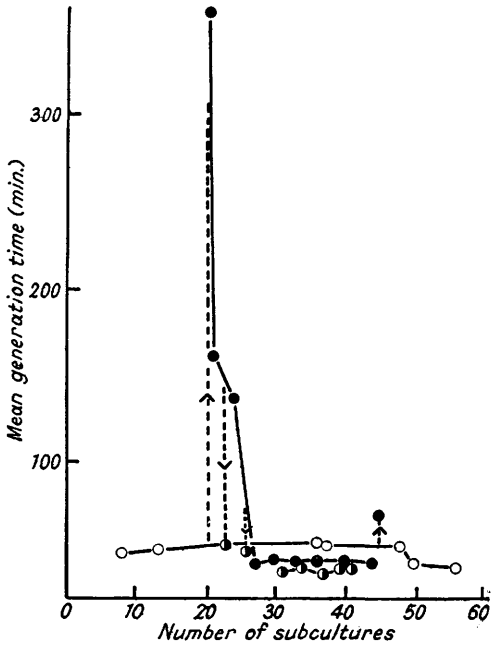


FIG. 1. Bact. coli *in glucose and cellobiose.*

○ *M.g.t. in glucose.*
● *M.g.t. in cellobiose.*
◑ *M.g.t. in glucose after intervening passages through cellobiose.*

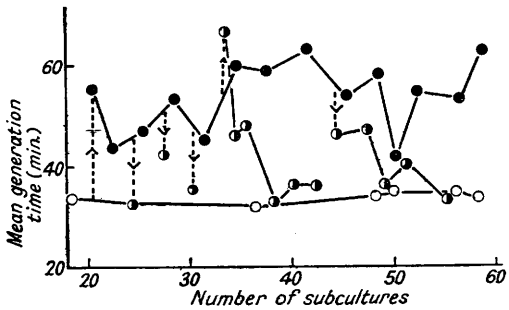


FIG. 2. Bact. lactis aerogenes *in glucose and inositol.*

○ *M.g.t. in glucose.*
● *M.g.t. in inositol.*
◑ *M.g.t. in glucose after intervening passages through inositol.*

FIG. 3.

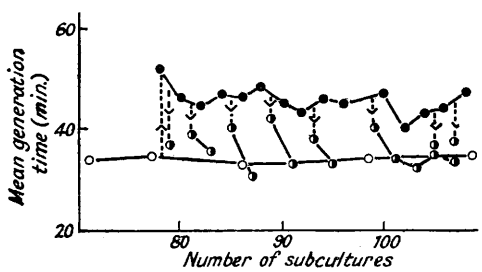
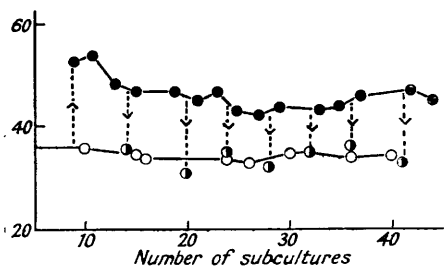


FIG. 4.



FIGS. 3 and 4. Bact. lactis aerogenes *in glucose and inositol.*

○ *M.g.t. in glucose.* ● *M.g.t. in inositol.*
◑ *M.g.t. in glucose after intervening passages through glucose.*

Continuous lines show serial subculture in the carbon source in question. Discontinuous lines show points at which transference is made from one carbon source to another.

mean generation time than would have occurred had the bacteria not been cultivated in the other sugar.

Since the mean generation time in glucose and that in cellobiose appear to be related to one another and since the mean generation time in glucose falls eventually to a stable value, it is expected that upon repeated passage through glucose efficient utilisation of cellobiose will become characteristic of the strain. In support of this it is observed that after 45 passages through glucose (just before the glucose mean generation time decreases)

the mean generation time of the strain on first cultivation in cellobiose is 68 min., which is considerably below the value of 360 min. obtained at an earlier stage in the passage through glucose.

(ii) *Behaviour of* Bact. lactis aerogenes *in Glucose and Inositol Media.—Bact. lactis aerogenes* was transferred from nutrient broth to a glucose synthetic medium and after a number of serial subcultures was inoculated into inositol. The mean generation time was measured at intervals in this medium, and also in glucose into which it was reinoculated from the inositol from time to time. The strain was also maintained in glucose and the mean generation time determined every few subcultures.

The mean generation time in glucose remained relatively steady at 33—37 min. At the 20th subculture the strain was inoculated into inositol and during repeated subculture the mean generation time fluctuated between 43 and 64 min. When the strain was reinoculated back into glucose after passage through inositol, it was found that the mean generation time was considerably higher than for cultures which had not passed through inositol (*i.e.*, passage through inositol had impaired growth in glucose). On repeated subculture in the glucose, however, this sub-optimal growth rate was restored to its normal value (see Fig. 2).

After 78 serial daily subcultures in glucose the strain was again transferred to inositol and the same procedure followed. In this case (Fig. 3) the fluctuations were smaller than in the earlier test, being in the range 40—52 min., and the loss in efficiency in the glucose utilisation, after passage through inositol, also less.

The whole series was later repeated in order to obtain more information about this apparent incompatibility of optimum adaptation to glucose and inositol. The same strain of *Bact. lactis aerogenes*, which had been maintained in nutrient broth in the meantime, was transferred to the glucose synthetic medium and the procedure of the earlier experiments again followed. Fig. 4 shows the results obtained. In this case very little fluctuation was observed after the first two subcultures, and the passage through inositol did not result in a decrease in the efficiency of utilisation of the glucose. There was found to be no difference in the characteristics of this series when subcultured every 12 hr. It was thought possible that the large fluctuation in the mean generation time in inositol, first observed, might be due to variations in the amount of iron in the medium, this metal having been found in some cases to have an effect on growth rate. The addition of the iron in this case should remove the fluctuation. It was found, however, that when the amount of iron was controlled at about $2\frac{1}{2}$ parts per million there was no change in behaviour.

From this it appears that *Bact. lactis aerogenes* can show two distinct types of behaviour: one where growth in inositol is incompatible with optimum growth in glucose and another where the two are compatible. It appears almost certain from a study of Figs. 2, 3, and 4 that the existence of the fluctuations in inositol is related to the modification of the properties in glucose.

The experiments were repeated after fresh cultivation of the strain in broth in order to ascertain whether, after growth in a medium containing a full complement of foodstuffs, the incompatible behaviour might again be observed on transfer of the strain to glucose and then to inositol. Complete compatibility was, however, again observed.

Discussion

These results could doubtless be translated into suitable assumptions about successive mutations in both directions and about relative growth rates of mutant and non-mutant types. Some of the assumptions would, however, not be very probable. A continuous series of successive linked mutations would have to occur leading to more efficient utilisation of glucose and cellobiose by the *Bact. coli* strain.

It is at least a tenable opinion that the alternative is more probable, namely, that training to cellobiose involves a general reorganisation of the enzyme system of the cell, which is not only compatible with optimum growth in glucose but actually hastens its attainment. This matter will not be debated in detail here, since, on the one hand, the issue of mutation and adaptation is best settled by other arguments and, on the other

hand, the immediate object of this paper is simply to add to our descriptive knowledge of what might be called the adaptive patterns of bacterial strains. The example of the *Bact. coli* strain with cellobiose and glucose belongs to the class where there is an ultimate complete compatibility of two types of adaptation.

The example of the *Bact. lactis aerogenes* with glucose and inostiol shows that in some circumstances optimum behaviour in one sugar may be incompatible with that in another. But this was not consistently so.

In order to explain why a given strain of bacteria shows more than one type of behaviour it is again neither necessary nor profitable to consider the problem in terms of a complexity of assumed mutations and back-mutations. The variation in the conditions in a broth culture can result in a variation in the properties of the strain of bacteria dependent upon their history. Now the only difference between the bacteria showing the first type of behaviour (Fig. 2) and those showing the second (Fig. 4) is their time of cultivation in nutrient broth, and the difference in the response to cultivation in inositol must be due to the culture in the broth.

The difference in behaviour probably arises when varying conditions in the nutrient-broth cultures affect the enzyme balance of the cells to different degrees. The properties of the strain are determined by the particular enzyme balance in the cells at the time of their transfer from the nutrient broth to the synthetic medium.

Upon long repeated passage through glucose the bacteria became more stable in their behaviour, smaller fluctuations and a lower degree of incompatibility being observed. Thus the compatible type seems to be the more stable of the two conditions. It is probable that, since a change from incompatible to compatible behaviour occurs on repeated cultivation in glucose, the incompatible type is associated with some degree of unbalance in the enzyme equilibrium of the cell brought about by conditions in the broth cultures.

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