134. *The Adaptation of Some* Bact. coli *Strains to Utilise Sucrose.*

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Two strains of *Bact. coli*, initially showing very long lags and slow growth rates, on transfer to a medium with sucrose as the carbon source become adapted to grow without lag and at an optimum rate not only by serial subculture in a sucrose medium but also by long-continued passage through glucose. The disappearance of the lag in the sucrose is attended by the nearly simultaneous attainment of a further stage in the adaptation to glucose.

The fact that towards the end of the lag in sucrose considerable increases in cell mass without multiplication of numbers occurs, taken in conjunction with other kinds of evidence, shows that this is not a case of mutation and selection, but of adaptations where the glucose- and sucrose-utilising mechanisms are partially linked.

The well-known capacity of bacterial cells to develop increased powers of assimilating carbon sources such as sugars when repeatedly cultured in their presence is not only explicable in terms of two alternative hypotheses, but may possibly even depend upon the one or the other of two corresponding mechanisms according to circumstances. These are, as repeatedly discussed in the literature, on the one hand, direct modification of enzymatic constitution, when the cell substance is continuously synthesised in new media, and, on the other hand, chance mutations in parts of the cell material which give new forms selectively favoured by the appropriate environment. Before a satisfactorily clear idea can be formed of the relative importance of the two modes of response, extensive experimental results are needed. This paper describes investigations on the adaptation of some strains of *Bact. coli*.

Adaptation of Two Strains of Bact. coli *to Sucrose.*—One strain (g) was an *Escherichia coli* obtained from the National Collection of Type Cultures, and the other (b) was obtained from Dr. R. L. Vollum (Radcliffe Infirmary, Oxford). Both required a training process (gradual elimination of an asparagine enrichment of the growth medium) before they would grow without lag on transfer from broth to the usual synthetic medium employed in this work (glucose, ammonium sulphate, phosphate buffer, magnesium sulphate, as given by Gladstone, Fildes, and Richardson, *Brit. J. Exp. Path.*, 1935, **16**, 335). The two strains were chosen from a large number tested because they showed on transfer to a medium in which sucrose replaced glucose very long lags, and revealed the need for extensive adaptation to the sucrose.

On serial subculture in the sucrose medium (each subculture corresponding to about ten successive cell divisions) the lag fell eventually to zero, and the mean generation time (time taken for the cell number to double in the logarithmic growth phase, *i.e.*, inverse measure of growth rate), after showing erratic fluctuations, fell to a steady and constant limit. This adjustment process is illustrated for strain (b) in Fig. 1, and resembles that frequently met in previous studies of other examples.

It was observed, however, that if the parent culture in glucose, from which the original transfer to sucrose was made, was continuously subcultured in the glucose medium itself, and at intervals tested by the transfer of samples to sucrose, complete adaptation to the latter sugar was eventually acquired. This, at first sight, might seem to suggest that the serial subculture in glucose was somehow leading to an enrichment of the population with sucrose-utilising mutants, the origin of which was due to chance.

But it was also observed that the sucrose-adaptation was accompanied by the nearly though not quite simultaneous attainment of a further stage of adaptation of the cells to the basal glucose–ammonium sulphate medium itself. This was revealed, not by the lag, which was already zero but by the mean generation time, which quite soon after the appearance of the sucrose adaptation showed a further fall to 40 min. with one of the strains and to 36 min. with the other. These various observations are illustrated in Tables 1 and 2 and in Fig. 2.

Thus there is not only the possibility that sucrose-utilising mutants have appeared, but also the possibility that in the final stages of adaptation to the ammonium sulphate–glucose medium an enzyme has developed which, as well as causing a more efficient metabolism of glucose, is capable of dealing with sucrose.

FIG. 1. *Serial passage through sucrose.*

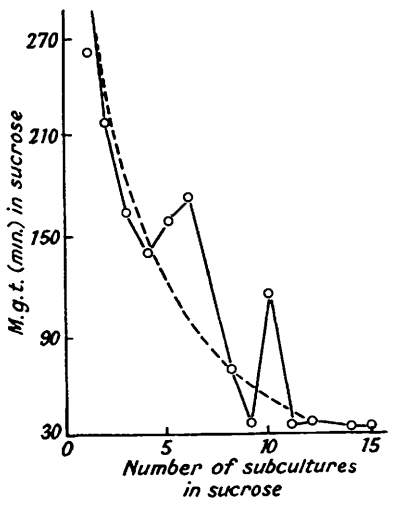
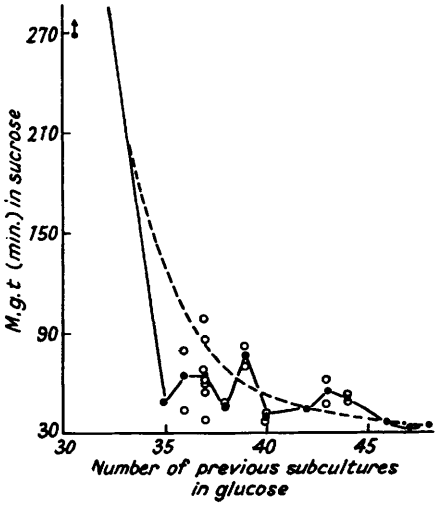


FIG. 2. *Serial passage through glucose.*



○, *individual values*; ●, *average values.*

TABLE 1. *Behaviour of strain (b) on serial passage through glucose.*

Strain (b) is *Bact. coli* (P 81 Type 2); ΔL is the amount by which the lag in sucrose exceeds the (very short) lag in glucose; m.g.t. = mean generation time.

Number of subcultures in glucose preceding test	M.g.t. in glucose, min.	M.g.t. in sucrose, min.	ΔL, hours (or as stated)
1—20	45—59, mean 50	160—354, mean 255	4—10 days
21—35	44—56, mean 50	45—330	214—2
36	54	39, 98, 62, 64, 63, 69, 65, 86, 63, 55	1·0
37	42	48	0·2
38	47	53, 83	1·5
39	41	42	0·5
40	46	45	1·8
42	46	51	2·0
43	42	52	1·2
45	36	36	0·0
46	33	34, 35	0·0
49	36	—	—
Cultures taken from single colonies of subculture 47		35, 34, 34, 35, 36, 34, 34, 34, 35, 34	—

TABLE 2. *Behaviour of strain (g) on serial passage through glucose.*

Strain (g.) is *Escherichia coli* of the National Collection of Type Cultures; ΔL is the amount by which the lag in sucrose exceeds that in glucose; m.g.t. = mean generation time.

Number of subcultures in glucose preceding test	M.g.t. in glucose, min.	M.g.t. in sucrose, min.	ΔL, hours (or as stated)
0	86	340	189
1—10	58 (mean)	75 (mean)	mostly >10 days
11—20	59 (mean)	—	mostly >8 days
21	59	—	<10
22	63	—	<10
23	56	37	0
25	—	38	0
27	44	—	—
29	40	—	—

Bacterial Mass and Bacterial Number.—If growth in sucrose depended upon the presence of mutants formed by chance in previous periods of multiplication, then the lag phase would be simply the time required for these special cells to multiply to a count comparable with the total inoculum. There would then be no detectable increase in the total bacterial mass without a corresponding increase in the cell number. If, on the other hand, the lag phase is the period in which intermediates are slowly accumulated and the steady state of a new series of growth reactions is established, then a large proportion of the cells may begin to increase in mass more or less at the same time, and a considerable synthesis of bacterial substance may precede any detectable multiplication.

Cell numbers are determinable by direct counting in a hæmacytometer chamber under the microscope, and bacterial mass has been shown to be measured by the turbidity of the culture. This has been shown by Monod for *Bact. coli* (" Recherches sur la Croissance des Cultures Bactériennes," Paris, Hermann & Cie, 1942) by direct comparison with dry weights, and by Baskett with the closely related *Bact. lactis aerogenes* (unpublished observations) by comparison with nitrogen content. Even if the proportionality is not linear (which in fact it normally is) the turbidity changes will at the very least show whether the culture as a whole is changing just before division sets in.

The strain (b) of *Bact. coli* was accordingly kept under observation during a long lag phase in the sucrose medium, and the initial stages of growth were studied with results shown in Fig. 3. Here the total bacterial mass for comparative purposes is expressed in terms of an equivalent number of cells of a constant *standard size.* This standard was

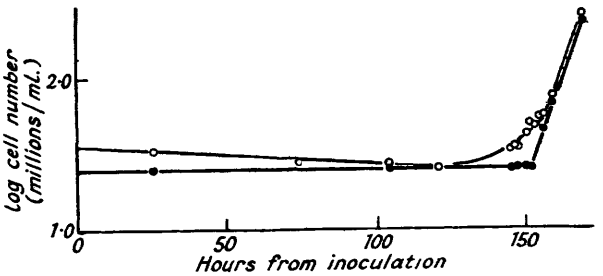


FIG. 3. *Bacterial mass and bacterial cell number.*
○, *mass*; ●, *number.*

taken to correspond to a time near the end of the lag phase. (Since there is a certain shrinkage or decay during the first few days after inoculation with a very inert culture, there is, as shown, a slight downward slope of the mass curve at first.)

It is seen that a considerable increase in mass does in fact precede the multiplication in numbers, and the *prima facie* conclusion is that a general modification of the bulk of the population has been in progress during the lag. If the ultimate growth were to be referred only to a minute proportion of pre-existent mutants, the bulk of the population remaining inert and incapable of multiplication, the general swelling in size which normally precedes division would not take place. The fresh bacterial mass would be undetectable until the mutants reached a detectable count. The general change in the properties of the culture before numbers increase shows that most of the cells are in fact changing in some way. Since wholesale division ensues soon after, the hypothesis that the lag has been a period of chemical change in them all is almost impossible to escape. The mutation hypothesis will only be applicable in this particular example if a minute proportion of mutants present in the culture are assumed able to provide, in the form of diffusible substances, intermediates, or metabolites which can be used by the normal non-mutant bacteria for incorporation into their cell structure. Since, from the length of the lag, the initial number of mutants would be almost vanishingly small, the activity which would have to be ascribed to them would hardly be credible.

Statistical Spread of Growth Rates.—When the culture is fully adapted to sucrose, the growth rate is constant and reproducible, the measured values, for strain (b), of the m.g.t. being 35 ± 1 min. In the intermediate stages the enzymatic organisation of the cell is unstable, and this factor is reflected not only in an irregular fluctuation of the m.g.t. from one subculture to the next, but also in an irreproducibility of behaviour when similar

inocula are transferred to a new batch of medium. When the lag of strain (b) in sucrose had just fallen to about one hour (at a first transfer from glucose) ten culture tubes containing sucrose medium were inoculated with 0·1-ml. samples of the same glucose parent culture. The lag was in each case about one hour, but the ten values of the m.g.t. ranged from 39 to 98 min. (Table 1).

Discussion.—The fact that the cell mass increases before there is any detectable multiplication renders very probable the hypothesis of a general adaptive response of the whole population. If this view is accepted, the conclusion is that the final stages in the adaptation to the glucose–ammonium sulphate medium and the development of the sucrose adaptation are closely coupled.

The violent fluctuations in growth rate which occur just before the stable state is reached would in any event be difficult to account for by the hypothesis of mutation and selection, since as soon as the mutants are established they should show their own stable properties and would not subsequently fluctuate rapidly in numbers. The variations are certainly not due to experimental error, since they disappear as soon as the strain is stabilised, as is shown by the final entries in Table 2. The fluctuations are much more readily explicable by the adaptation theory. At first, the enzymatic organisation of the cell is unstable and slight variations in the conditions, for example in the size or age of the inoculum, lead to different growth characteristics, including the temporary reversions which the mutation theory could hardly explain at all.

To account for the scatter in the actual growth rates of similar inocula referred to in the previous section the mutation theory would have to postulate either a wide variation in the efficiency of existing mutants or a whole range of mutant types with differing growth rates. The first postulate is itself practically equivalent to an adaptation theory, and the second leads to difficulties of a special kind. The scatter would have to be explained by random variations in the numbers and types of mutants transferred in the inoculum. For these to be significant the actual numbers would have to be very small since otherwise a representative sample of all types would be transferred on each occasion. The lags, therefore, would be long. But they are in fact all about one hour, and leave no time for fresh selective processes to operate.

If, on the other hand, we postulate a period of very unstable adjustment in the course of the adaptation, then the behaviour of the various inocula will depend upon the exact amount of glucose or of intermediates carried over with the cells, the degree of thermal shock associated with transfer, the exact conditions of aeration in the new sucrose medium and so on. Thus the non-uniform growth characteristics will be due essentially to the instability of the organisation at the moment when one reaction pattern is in competition with another which is about to replace it.

The partial coupling of the sucrose and glucose adaptations is in itself interesting and is, in a general way, paralleled by some experiments in which *Bact. lactis aerogenes* was trained to glucose and to cellobiose, passage through the one improving the growth rate in the other. Two possibilities suggest themselves. The first is that during the passage through glucose the cells are exposed to minute concentrations of sucrose synthesised by their own enzymes, and so have the opportunity, especially during periods of glucose exhaustion, of metabolising sucrose itself. The second possibility, which is more general, is that during the final stages of adaptation to the glucose–ammonium sulphate medium there is some measure of reorganisation of the enzyme systems and that this is such as to permit an easier development of the sucrose-utilising mechanisms. Such a phenomenon might well prove to be rather common with closely related pairs of sugars.

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