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# Interactions during the growth of mutating populations of bacteria.

Adaptation in its most general sense means a satisfactory adjustment to the environment. It is a behavior frequently found among microorganisms. In the red bread mold, Neurospora, it has been possible to show that adaptation can involve genic mutation (1). A biochemical mutant of Neurospora, requiring a supply of leucine for growth, can back-mutate to dispense with that requirement and grow in the absence of leucine. Such adaptation is complicated by the fact that, in the heterocaryon formed by back-mutation, the deficient leucine-dependent nuclei may, under certain conditions have a selective advantage over the leucine-independent back-mutated nuclei. Attempts to discover the mechanism of this selection or competition of nuclei in Neurospora have not met with success.

Since there were clues in the literature that a similar selection in favor of biochemically deficient cells occurred among bacteria, it was thought that a mechanism which operated through culture medium might be more easily studied than one which acted through the cytoplasm of a heterocaryon. Therefore, an investigation of adaptation in biochemical mutants of bacteria was begun. The organism chosen was a mutant strain of Escherichia coli which requires a supply of histidine for growth. It was secured after x-radiation (2) and will be referred to as h-. After purification by repeated isolation from single colonies, a stock culture was established on agar medium containing histidine. The growth of this organism was studied in stationary test tubes each containing

10 ml. of a synthetic medium. Unless otherwise mentioned inocula consisted of from $10^5$ to $10^6$ cells and growth was measured as optical density.

On different concentrations of histidine the results shown in Fig. 1 were obtained. After 10 hours there is no growth in the absence of histidine but increasing amounts of growth occur with increasing concentrations of histidine. The upper curve is for an optimum concentration. After about 17 hours adaptation occurs. Cultures without any histidine show complete growth but with increasing concentrations of histidine adaptation is progressively less complete. At intermediate concentrations adaptation does not occur even after 7 days. When an adapted culture is washed and plated into agar and synthetic medium devoid of histidine, colonies were formed. These colonies, when isolated and transferred to liquid medium without histidine, grow like wild type E. coli, i.e. without the long initial period of the h- strain. These new histidine-independent cultures, which are called h+, also differ from h- cultures inasmuch as they will form colonies on agar devoid of histidine while h- cells, even after 7 days, will not form colonies visible to the naked eye. Partially adapted cultures can be shown in a similar way to contain smaller numbers of h+ cells. All of these h+ cells have arisen from the h- cells by "mutation". The adapted growth may be due to h+ cells already present in the rather large inoculum but it is also possible to have the same type of adaptation after the inoculation of a single h- cell into medium without histidine. Adaptation then is due to a change in the synthetic capacities of the cell or to mutation.

When the amounts of growth achieved after 11 hours and after 29 hours are plotted against histidine concentration the curves shown in Fig. 2 are obtained. The decreasing amounts of adaptation that can occur with increasing concentrations of histidine are clearly shown. This is the same type of relationship that was found in leucineless Neurospora. Since it can be shown, by plating out, that there are h+ cells in the incompletely adapted cultures the problem is to discover what prevents these cells from continuing growth. We might assume, as in the case of leucineless Neurospora, that the h+ organisms are prevented from growing by the presence of too many h- cells. We know that the proportion of h- cells in these cultures which never adapt is greater than 99 percent. There are progressively smaller numbers and percentages of h- cells as adaptation is more complete.

It is possible to eliminate the depression of growth which occurs on intermediate concentrations of histidine by decreasing the percentage of h- cells in the inoculum. When the inoculum consists almost entirely of h+ cells there is no depression of growth. Consequently we conclude that this depression is brought about by the h- cells. The proportion of h- and h+ cells remains about the same as in the inoculum when growth is allowed to take place in the presence of an optimum amount of histidine. Under these conditions, then, the growth characteristics of the h- and the h+ bacteria are the same. It is only on limiting concentrations of histidine that selection of the h+ cells takes place during adaptation and the ratio introduced in the inoculum changes. But even

though selection is in favor of the he cells we know that they are eventually inhibited by the h- bacteria.

We have been able to reveal some of the factors involved in this inhibition. Under the conditions of our experiments we know that acid production is proportional to growth. This results in varying reductions in the pH of the culture medium. After adaptation is complete hydrogen ion concentration is limiting, for when the pH is brought back to 7 growth resumes. The addition of histidine, or other components of the medium, will not bring about a reinitiation of growth. But the limiting pH is different for different histidine concentrations. It is highest at the intermediate concentrations where growth is most depressed. One hypothesis which explains this assumes that an inhibitor is formed to varying extents by the h- cells with maximum production at intermediate histidine concentrations. Where the least amount of growth has occurred after 24 hours (Fig. 2) and where the pH is decreased during growth only to 6.5, there would be the maximum amount of inhibitor. This situation would parallel the accumulation of precursers by some biochemical mutants of Neurospora which can occur only in intermediate concentrations of the required growth factor (3).

Although the inhibitor (s), has not been characterized there are several lines of evidence indicating that it exists. Cultures of h- bacteria, allowed to adapt on different histidine concentrations were sterile filtered, brought to pH 7 in the presence of an optimum amount of histidine and inoculated with either he or h-

bacteria. The amount of growth secured was a function of the histidine concentration on which the bacteria had been allowed to adapt. Culture filtrates from those concentrations which supported the least growth, although their pH was brought to 7 and they were supplemented with an optimum amount of histidine, allowed the least growth of the h+ and h- cells with which they were reinoculated. The amount of new growth supported was proportional to the amount of growth which had been allowed on the original histidine concentrations. Once again where the least new growth occurred the pH was brought to pH 6.5. The limitation of this new growth was not due to the presence of an inhibitor (s) which was in greatest concentration in those filtrates which had originally contained intermediate histidine concentrations. This inhibitory effect is not destroyed by heat. In another series of experiments the phosphate concentration and buffer capacity of the medium was increased. Nevertheless, at intermediate histidine concentrations the same amounts of growth were obtained on the different media - and this despite the fact that that the pH was decreased by growth to different extents.

This inhibition is a function of the inability of h- bacteria to synthesize histidine. One of the main physiological differences between h+ and h- cells is the probable accumulation of precurser in the latter, especially at intermediate histidine concentrations. Perhaps the inhibitor is in some way related to a histidine precurser. To speculate further would involve an unwise extrapolation of the data. It would also be inappropriate to discuss notions with

regard to the mutation from h- to h+ or of the mutation which also occurs from h+ to h-. Our knowledge is not yet extensive enough but a word of caution may be stated. These mutations may not be independent of histidine or the genes or factors controlling histidine synthesis are so unstable as to raise questions about calling changes that occur in them mutations in the ordinary genetic sense. We should be prepared in these new investigations on the genetics of microorganisms to encounter new phenomena and new concepts.

In conclusion, the growth of a bacterial culture is a population event. It may involve mutation in growth abilities and when competitions and interactions occur they may bring about rapid changes in the population. These studies on histidineless Escherichia coli confirm, in a sense, the work on leucineless Neurospora. They enable one to postulate that cells with biochemical deficiencies may be able to compete favorably with synthesizing cells in a way that does not involve differences in growth rate.

Fig. 1. The growth of histidineless Escherichia coli on different concentrations of histidine expressed as γ per ml.

Fig. 2. The effect of histidine concentration on the amount of growth produced by histidineless Escherichia coli before and after adaptation.

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