AUTO-ADAPTATION: A NEW PHENOMENON OBSERVED IN A BACTERIAL POPULATION

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It has been found that, in the presence of certain α -hydroxy acids, a glycineless mutant of *Escherichia coli* will give rise to various partially reverted cell types. This effect has a chemical specificity in that only α -hydroxy acids of a particular steric configuration are active. The phenomenon also has a biological specificity, in that the active acids have no analogous effect on other mutants of *E. coli* requiring different amino acids. The conditions under which these partially independent strains arise have been given in previous publications, which also include a description of their general characteristics (Wright, 1951, 1953a).

The purpose of the present paper is to begin an analysis of the behavior of these bacteria growing in a minimal medium. The independent but slowly growing strains are referred to as fast, intermediate, or slow synthesizers, depending upon their rate of growth in minimal medium, and hence upon their rate of glycine and serine synthesis. All of the partially reverted cell types, like their entirely dependent parental mutant, can grow at the wild type rate in minimal media if supplied with either amino acid. The growth characteristics of individual strains (as tested on minimal medium) are reproducible for months if the strains are maintained on complete medium. In minimal medium, however, these cultures exhibit progressive, stepwise changes in their rate of growth until a generation time of about 100 minutes (at 27 C) is attained, indicating a wild type culture. Having once attained a maximum rate in minimal medium, the organisms are stable and apparently normal wild types. The manner in which a slow or intermediate

synthesizer is converted into a fast synthesizer during growth in minimal medium is illustrated by the representative growth curve of figure 1. Following a long period of growth at a low rate, the culture consistently grows at stepwise increasing rates until the generation time of wild type is attained; about 7 "breaks" in the curve of strain 15 occur during this time (only 2 of them are shown here). The completed curves are always apparently composed of a series of sharply defined, logarithmic stages.

Evidence will be presented in this paper which supports the hypothesis that these progressive increases in growth rate are due predominantly not to a selection mechanism but to a mass transformation of the population at the critical times. The cells periodically transform themselves into types capable of synthesizing serine and glycine at stepwise increasing rates; this phenomenon has been called "auto-adaptation" for reasons which will become apparent later. The general implications of auto-adaptation, as well as its relationship to the conditions under which the partially reverted strains originally arose, will be discussed in another publication.

METHODS

Media. The minimal medium used throughout had the following composition per liter of distilled water: KH_2PO_4 , 2.0 g; $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 2.0 g; $(\text{NH}_4)_2\text{SO}_4$, 1.0 g; MgSO_4 , 0.2 g; NaCl , 1.0 g; glycerol, 5.0 g. A description of the flasks in which the cultures were grown has been given previously (Wright, 1953a). Cell density was determined with a Klett-Summerson spectrophotometer. The scale reading of this instrument is directly proportional to the concentration of the substance determined, so that plotting the log of this reading as a function of time gives a straight line during logarithmic growth.

Correlation of viable count and optical density. Since the growth curve of strain 15 in figure 1, for example, was obtained by measuring cell density optically, the apparent changes in growth

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Figure 1. Strain 15 growing in minimal medium at 27 C. Five breaks occur between rate 3 and wild type rate; only the final rate of wild type cells is shown here. The ratio of A to B cells as determined by plating is indicated at three points during the transformation of the culture.

Figure 2. Strain 16 growing in minimal medium at 37 C. Cell density is followed optically (open circles) and by means of viable count (solid circles). The position of the curves relative to each other is of course arbitrary.

rate observed could have been interpreted as due to changes in the density of individual cells or in cell size rather than to changes in cell number. Thus, optical density determinations were correlated with viable count. To establish this

correlation, a culture was used which would undergo an apparent increase and decrease in growth rate while growing in minimal medium. (Decreases in growth rate will be discussed later.) Every hour during the early and most critical period of the experiment, a density determination of the culture was recorded, and two independent dilution series prepared. The viable counts obtained from each dilution series were averaged, and this value averaged with the corresponding one obtained an hour later. These viable count values and the Klett readings are plotted against time in figure 2. Each viable count value corresponds to a cell density half way between two density determinations. By plating diluted cell samples to minimal agar, it could be shown that the bacterial colonies responsible for the viable count determinations were actually all slow synthesizers.

It can be seen from figure 2 that the viable count curve and the cell density curve are parallel until the culture reaches a cell density of about 115, or 1.5×10^8 cells per ml; the curves thereafter diverge in such a way as to show that beyond this density, cell numbers are increasing at a higher rate than would be apparent from following cell density with a spectrophotometer.

Thus, below a density of about 1.5×10^8 cells per ml, optical density can be taken as a true indication of cell numbers. If log viable count is plotted against log density, a straight line is obtained up to the critical cell density, whereafter the curve slopes upwards. An experiment was carried out to determine whether the change in slope of this curve was due to alterations in cell density during growth, for example, or whether it could be attributed to an optical phenomenon unrelated to changes in the individual cells. At the end of the experiment described above, a series of dilutions was prepared from the fully grown culture. When viable counts and density determinations were made for each dilution, and their respective log values plotted, the curve coincided with the corresponding one obtained from the growing culture. An optical phenomenon, rather than a change in the composition of the individual cells during growth, therefore is responsible for the increased slope. It is known in fact that very dense solutions must be diluted appropriately in order to make accurate determinations of optical density.

EXPERIMENTAL RESULTS

INDIRECT ANALYSIS

Significance of the transition period. Two cell types (with respect to their rate of growth into colonies on minimal agar) can be detected in a culture of partially reverted cells when it suddenly increases its rate of growth (figure 1). One explanation for this change in growth rate is that the dominating cell type present at growth rate 3 (cell type B) has been selected for out of a population largely consisting of cells growing with a generation time represented by rate 1 (cell type A). We can assume, for the sake of the following argument, that cell type A mutates to cell type B at a relatively low frequency since the growth of A cells continues for 3 days before a rate increase occurs. The minority type B cells arising in the culture by mutation grow at a higher rate than the type A cells and, therefore, eventually dominate the culture. The respective generation times of the two cell types can be determined, and with these values it is possible to calculate the number of cells which theoretically should be present in the culture at any time, assuming the conditions stated above (table 1). By plotting the total number of cells against time, one can obtain a theoretical curve repre-

senting the growth of a mixed culture containing type A cells (generation time 40 hours) and type B cells (generation time 8 hours). Table 1 arbitrarily begins with a ratio of 2.0 type A cells to 0.03 type B cells. This ratio is a convenient starting point since it occurs shortly before the growth rate of the culture begins to increase from the initial rate representing the generation time of A cells. The assumption of a particular mutation frequency has little effect on the type of curve obtained; as the frequency becomes lower, the initial increase in slope of the computed curve merely will be shifted farther to the right. Experimentally, the establishment of the new growth rate occurs within a period of about 8

TABLE 1

The theoretical ratio of A to B cells during growth in mixed culture, assuming the respective generation times found experimentally. If the total number of cells is plotted against time, a smooth curve is obtained over a 70 hour period between the initial and final growth rates, representing the generation times of A and B cells, respectively

HOURL	RATIO TYPE A/B CELLS	TOTAL, A + B CELLS
0	2.00/0.03	2.03
10	2.36/0.07	2.43
20	2.80/0.18	2.98
30	3.32/0.43	3.75
40	4.00/0.96	4.96
48	4.60/1.92	6.52
56	5.28/3.84	9.12
60	5.63/5.55	11.2
70	6.70/13.6	20.3
80	8.00/32.8	40.8

hours, and the generation time of type A cells is 40 hours; the number of new type B cells arising by mutation during this time is clearly negligible when compared to the new type B cells arising as the result of cell multiplication. Thus, new type B cells arising by mutation may be neglected in the calculations. The theoretical curve obtained represents the entire transition period of a culture beginning with a generation time of 40 hours and ending with one of 8 hours. The ratio of A/B cells at various times can be seen from table 1. By comparing such a curve, representing the result of simple selection, with the one of strain 15 in figure 1, a striking difference becomes apparent. In the theoretical curve the transition period lasts about 70 hours, and

in the experimental curve it is 7 hours in length. This comparison suggests that the type B cells arise about 10 times faster than could be expected on the basis of a simple selection hypothesis. Had they arisen *en masse* in some degree, the short transition period could be explained.

A similar kind of analysis can be made with the curve in figure 1 by extrapolating back the line for rate 3. Thus, it is possible to determine the approximate number of type B cells present at some previous hour (knowing the relationship between optical density and viable count), provided that the type B cells (of rate 3) arose predominantly from the growth of previously existing type B cells in the culture. By such an extra-

cells arose *en masse* shortly before they dominated the culture.

"Glycine effect" in liquid cultures. What is the effect of a high rate of growth during and prior to a rate increase upon the nature of these "breaks"? Figure 3 shows the behavior of a control culture of strain 14A as well as that of a culture to which has been added a small amount of glycine. The utilization of glycine took place from hour 67 to hour 78 and served to increase the rate of growth during this time. At about the 84th hour a portion of this culture was transferred to a new flask of minimal medium, in order to make observations of the third break possible (indicated by an arrow). The two cul-

Figure 3. Strain 14A growing at 27 C in minimal medium (broken line) and in the presence of a small amount of glycine (solid line).

pulation, it can be determined that at hour 66, for example, there should have been two type A cells to one type B cell. As can be demonstrated from table 1, when the ratio of type A to type B cells is 2:1, the culture should be about half-way transformed from the generation time of A to that of B. This was found also to be true experimentally (see below). Thus, according to a simple selection mechanism, enough B cells should be present at hour 66 to produce a half transformed culture—i.e., some 12 hours before the culture actually is half transformed. This discrepancy indicates that the ratio of type A to type B cells at hour 66 was much greater than 2:1; in fact, plating experiments showed that it was greater than 100:1. Therefore it may be concluded also from this analysis that type B

tures paralleled each other in growth rate and in the time of their third break at hour 89; this is a strong indication that both cultures underwent the same extent of transformation prior to this time, in spite of the fact that one of them had used glycine. The lag prior to glycine utilization perhaps can be explained by arguing that the growth rate in the absence of glycine is very low, and time is necessary to build up not only the enzymes for maximum glycine utilization but all the enzymes and metabolic intermediates necessary for growth at wild type rate. It may be concluded that, within this range at least, a higher rate of growth and greater density of cells do not affect the time or sharpness of the subsequent breaks.

However, these cultures behave very differ-

ently following more abundant growth produced by the presence of high concentration of glycine, serine, or yeast extract (Wright, 1950). Since cultures of 14A at 27 C (generation time 40 hours) do not exhibit their first rate increase until the 69th hour from the time of inoculation, there is ample opportunity to vary the rate and extent of growth before the characteristic transformations take place. During this early growth period different concentrations of glycine, serine, or yeast extract were supplied to such cultures in various experiments. A typical experiment was carried out in the following way: At hour 0,

or "break" at hour 68 to 69, the behavior of the two cultures at equal population densities could be compared. Cultures with the history of x^1 are referred to as 145 glycine (figure 4), for example, depending upon the optical density reached by culture x before transfer (x^1).

Cultures grown to cell densities lower than 145 in the presence of glycine or serine are not affected in their subsequent behavior in minimal medium. If grown in the presence of yeast extract, however, a cell density of 425 can be reached before the subsequent breaks are influenced. These two densities, 145 and 425,

Figure 4. Strain 14A growing in a minimal medium at 27 C after no exposure to glycine (control) and after growth to the various indicated densities in the presence of glycine.

two cultures (x and y) of strain 14A were started in minimal medium and incubated at 27 C overnight. At hour 15, 0.15 ml of 0.1 M glycine was added to culture x . On the following day (hour 48), the density of culture y was less than 20, whereas that of culture x was 145, and two consecutive readings indicated that all the glycine had been used, and that the generation time of the cells was again about 40 hours. One ml of this dense culture then was transferred to a flask of fresh minimal medium (x^1). By the 60th hour from the time of inoculation of the two original flasks, x and y , flasks x^1 and y had attained approximately equal densities (about 20 to 40). Thus, by the time of the expected rate increase

respectively, are "critical", meaning that the breaks are inhibited only slightly (e.g., 145 glycine in figure 4). Growth to higher densities in the two kinds of media prevents the subsequent breaks, and the growth rate of such cultures is decreased (225 glycine in figure 4) following abundant growth.² It can be seen also in this figure that growth to a cell density of 110 in the

² The effect of abundant growth in the presence of glycine in inhibiting the cells' ability to synthesize glycine may be analogous to the depression of the ability of *Escherichia coli* cells to synthesize methionine after growth in its presence (Wijesundera and Woods, 1953; and Cohn, Cohen, and Monod, 1953).

presence of glycine had no effect on the breaks: this culture behaved like the control. The inhibitory effect of yeast extract in high concentrations on the transformations of a slow to a fast synthesizer may account for the stability of the former type on yeast agar slants. A comparison of the critical density of glycine to that of yeast extract indicates that not only a high growth rate is required to inhibit the breaks, but rather the presence of the glycine or serine molecule (specifically) in a particular concentration. This aspect of the glycine and yeast extract effect will be dealt with again in the discussion.

When a culture breaks downward as the result of previous abundant growth in the presence of glycine, the subsequent low rate of growth can be maintained for more than 50 hours before the culture again undergoes increases in growth rate. Such cultures always eventually begin the typical transformations. More frequently than not, cultures forced to decrease their rate of growth do so at the same time as the control culture increases its growth rate (see 225 glycine, figure 4); they seem to be affected—in a negative way—at the very time of the normal breaks. It seems strange that such cultures fail to exhibit the lower rate of growth continuously after being transferred to the new medium, rather than first growing at a higher rate. Apparently there is some kind of “delayed effect” on these cultures due to previous growth to the required densities in yeast extract or glycine. At present, no interpretation can be offered for this peculiar effect.

Significance of the “glycine effect” in relation to a selection mechanism. An attempt will be made to interpret the glycine effect in terms of the selection of type B from a population of type A cells, a possible mechanism for the rate changes which has been described earlier and found unsatisfactory. A fair amount of growth can take place in the presence of glycine (figures 3 and 4) before a break and yet not affect the time or strength of the break. Since the break is caused by a change in ratio of type A to type B cells (see below), and since the break is unaffected, it follows that during glycine utilization the change in proportion of A to B cells occurs at the same rate as it does in minimal medium. That is, in order to produce a break at the normal time, B cells would have to be selected from A

cells as quickly in the presence of glycine as in minimal medium alone. It would be a peculiar kind of selection mechanism that operates at the same rate in the presence of glycine, when the generation times of both types A and B are 2 hours, as in minimal medium, when the generation time of type A is 40 hours and that of type B is 8 hours.

Suddenly, by growth to the respective critical densities with yeast extract or glycine, the subsequent breaks in the curve are inhibited. After a “breakdown” has occurred, platings reveal the presence of only type A cells as might be expected from the generation time indicated by density determinations. Thus, the normal ratio at this time between type A and B cells has been altered. It can be assumed that under these conditions type A cells are selected for rather than type B cells. However, this assumption is at variance with the one made in the previous argument, which was that type B cells would be selected for as fast in the presence of yeast extract or glycine as in a minimal medium. Thus, in order to uphold the selection hypothesis, it would be necessary to argue that at low glycine concentrations type B is selected for, while at higher concentrations type A has an advantage over type B. While such a situation is not impossible, it seems unlikely; another interpretation will be suggested in the discussion.

DIRECT ANALYSIS

Glycine effect on solid media. The experiments with liquid cultures described above demonstrated that the growth of the partially reverted strains, as well as conversions to increasingly independent types, can be suppressed in minimal medium if the strain was grown previously in the presence of glycine, serine, or yeast extract. The experiments suggested that this effect of glycine utilization on the future behavior of the cells in a glycine-free medium operates on the whole population and not by means of a selection mechanism. The most striking evidence for this conclusion is that the whole phenomenon can be observed on an agar plate where the cells are fixed spatially and selection is limited. This was shown in the following way: A minimal agar plate is spread with a suspension of the partially reverted strain, the plate dried, and a drop of a glycine solution placed in a hole made in the center of the plate. After a day's incubation, a cir-

cular area of growth appears around the region of glycine diffusion. By the second day, however, the cells on the glycine-free medium have grown to about the same extent as the cells in the center of the plate, which have by then depleted the plate of glycine.³ At the end of the second day a circle of inhibition of growth begins in the area of previous glycine utilization. This region of

inhibition since "mutations" do not occur among the cells which had used glycine. Only secondary colonies are visible in these photographs which were taken on the first four days of incubation (figure 5). The primary colonies (not visible in photograph) are extremely numerous in contrast to the sporadic and relatively low incidence of reverted secondary colonies, which probably is

Figure 5. The "glycine effect" on solid media. Pictures were taken of plates on the first four days of incubation at 35 C. See text for further explanation.

sparse growth thereafter becomes pronounced increasingly in contrast to the outer regions of the plate. By this time "mutations" to more independent cell types appear over the rest of the plate to the extent that after about two days the new (primary) colonies almost cover the initial layer of growth, leaving a bare central area of

³ Much denser growth can occur on these plates, showing that growth does not stop because of an exhaustion of the carbon source.

due to their suppression by the primary colonies and by the original cells spread on the plate. It was found in reconstruction experiments designed to examine the conditions under which the reversion frequency of the dependent mutant was determined that colony formation of 80 to 90 per cent of the partially reverted cells was suppressed on minimal agar by the presence of the dependent parental population (Wright, 1953a).

This demonstration of the glycine effect on solid media affords direct evidence that the inhibition of growth and mutations to more independent types affects the individual cells of the population and does not operate via a selection mechanism. As mentioned previously, partially reverted cells which have been inhibited by glycine utilization can "re-adapt" by growing in a minimal medium, and again undergo the typical transformations. The implications of the glycine effect in relation to the specific suppression of the phenotypic expression of a mutation are discussed elsewhere (Wright, 1953b).

Analysis of cell types present during a break. It seemed possible to analyze directly the nature

TABLE 2

Two experiments with strain 15 growing in minimal medium at 27 C. The ratio of A to B cells was followed during transformation by plating to minimal agar at the times indicated. These results are plotted in figure 6. The letters A-D refer to representative photographs for experiment I; they are shown in figure 7

PICTURE	EXPERIMENT I		EXPERIMENT II	
	Hr after inoc.	Ratio A/B	Hr after inoc.	Ratio A/B
A	73	26.0	72	78.0
	75	8.0	75	16.0
B	77	5.2	78	13.0
	79	3.5	81	8.0
C	81	2.5	83	6.5
	83	2.3	84	5.0
D	84	1.2	86	4.8
	85	0.9	88	3.1

of the transition of a slow to a fast synthesizer in minimal media by plating frequently from cultures during the time interval when the growth rate was changing. This method was used with strain 15 shown in figure 1. Before and during the 78 hour break, samples of the culture growing in minimal medium were diluted and plated to minimal agar. When such plates were incubated at 34 C for three days, two sizes of colonies were easily distinguished as the picture of figure 7 indicates (described in detail below). It was hoped that the changing proportion between the two types of colonies on the plates prepared at various times might truly represent the proportions of the two types of bacteria presumably present in the culture; one responsible for growth rate 1, and the other for

growth rate 3 (see figure 1). Apparently there was such a correlation because the maximum rate of change of proportion of the small (type A) to the large (type B) colonies occurred on plates prepared from cultures undergoing transformation, as determined by density measurements. The observed correlation strongly suggested that the two types of colonies do in fact (arise from and) truly represent two distinct types of bacteria present in the culture. The two kinds of cells might be expected to continue to grow on the minimal agar plates at their own characteris-

Figure 6. Two experiments giving the ratio of A to B cells, determined by plating to minimal agar at various times during the transformation of culture 15 in liquid minimal medium. See table 2.

tic rates, although probably not constant ones. In fact, it is highly probable that increases in growth rate must have occurred on the plates because it is doubtful whether type A cells could have produced visible colonies in three days since the generation time of these cells (initially) was about 40 hours at 27 C.⁴

Table 2 presents data for two similar experiments (I and II) with strain 15, the results of which are plotted with two ordinates in figure 6

⁴ Because A and B cell types are so unstable and short lived in a transforming culture, it would be impractical if not impossible to do meaningful reconstruction experiments with them.

in order to show the similarity of the shape of the curves with time. The first experiment will be discussed in detail. The proportion of small (A) to large (B) colonies from two plates was calculated and averaged; the hours indicated correspond to the number of hours after inocula-

2, between hours 78 and 84, is a transition stage with respect to cell types between the initial and final rates of a mixed culture containing these two types of cells. That is, there are roughly equal numbers of A and B cells during this period.

Figure 7. Representative plates showing the ratio of A to B colonies during the transformation period of strain 15 growing in minimal medium. The letters refer to table 2.

tion of the culture from which the plates were prepared. The letters A to D to the left of table 2, referring to experiment I, indicate the hours for which pictures of representative plates were taken; they are shown in figure 7. A small colony presumably would represent the progeny of a type A bacterium growing at rate 1, and a large colony would arise from a type B cell which grows at rate 3 (figure 1). The intermediate rate

It is clear from table 2, as well as from figure 6, that the proportion of the two colony types changes with time in favor of the faster growing cells. This is hardly surprising; it would be the inevitable result of the differences in growth rate of the two types of cells in a liquid culture. However, the following considerations will show that the observed rate of change is incompatible with a (previously outlined) "mutation-selection"

mechanism, and that a mass transformation of type A to type B cells might well have occurred shortly before the first break in the growth curve is observed. Knowing the generation times of the two cell types, it is possible to calculate (with data similar to those in table 1) the theoretical change with time of the ratio of the two cell types growing in mixed culture. These values were plotted in figure 8 (theory for selection), starting with an experimentally found initial ratio of 26A:1B; this theoretical curve

Figure 8. The ratio of A to B cells during transformation; (a) experimentally (same curve as for experiment I in figure 6); (b) theoretically, on the assumption of simple selection; and (c) theoretically, on the assumption of a mass conversion.

can be compared directly to curve I of figure 6, which has been redrawn in figure 8 for sake of comparison (experimental). Theoretically, about 18 hours of growth are necessary to go from a ratio of 26A:1B to a ratio of 8A:1B. Experimentally, this change occurs in about 2 hours, or 9 times as fast as could be expected on the basis of selection alone. Again, the evidence points towards a mass conversion of the population to account for the nature of the observed rate increases.

Mathematical analysis of the mass conversion.

It seemed of interest to have a more quantitative expression of these transformations; thus far, it has been possible to state only that they occur about 10 times faster than they would were selection alone responsible. What percentage of the population is transformed over a given time period, and at what rate? Professor K. Linderström-Lang has kindly analyzed the growth curve of a partially reverted culture (strain 15, figure 1) with the following results:

It is assumed that cell type A (generation time 40 hours) transforms at a constant rate into cell type B (generation time 8 hours). The number of A cells at any given time, t , will depend upon their growth constant, K_A ($= \log 2/40$), and upon the rate at which they are being transformed, K_T :

$$A = A_0 e^{(K_A - K_T)t}$$

A_0 is the number of A cells present at the time transformation begins. The number of B cells at any given time will depend upon their growth constant, K_B ($= \log 2/8$), and upon the rate at which they are arising due to the transformation of A cells:

$$B = A_0 \frac{K_T}{(K_T - K_A) + K_B} (e^{K_B t} - e^{(K_A - K_T)t})$$

If K_T is assumed to be 0.03, and if the number of A and B cells is determined over a 15 hour period, a theoretical curve is obtained with a transition period similar to that found experimentally (see figure 9). This is actually a perfectly smooth curve; moreover, it can be shown that the largest change in slope occurs at hour 3.6—i.e., in the middle of the transition period between the two cell types. Nevertheless, it is possible to draw three reasonably straight lines through the calculated points, producing a curve similar to the experimental one in figure 1. (The number of points calculated was purposely similar to the number determined experimentally.) Furthermore, the changing ratio of A to B cells during the transition period is remarkably similar to that found experimentally. This can be seen from figure 8, in which both the experimental and theoretical ratios (theory for mass transformation) are plotted against time. The ratio of cell types is indicated also at various times in both figures 1 and 9 to facilitate their direct comparison with the respective curves.

In many respects, the fit between theoretical

and experimental curves is good so that one is tempted to say that the experimental points should be fitted by a smooth curve, and that the determinations are not accurate enough to justify the drawing of three straight lines. In this case, it could be concluded that there are two cell types, A and B, and that A transforms to B at a rate of about 3×10^{-2} per hour, or 1.2 per generation time of A cells (= 40 hr).

On the basis of many experiments, however, it is felt that there is some justification for drawing three straight lines. If there actually

shown clearly that the conversion is extremely rapid, and, as concluded previously by an extrapolation of rate 3, that the cells begin to transform only after growing in minimal medium for more than 60 hours. The possibility that these conversions are under the control of an inhibitor or conversion factor will be discussed below.

DISCUSSION

Partially reverted strains of a glycineless *E. coli* mutant have been observed to grow at stepwise increasing rates in a minimal medium. On

Figure 9. A theoretical curve for mass conversion of A to B cells. Their calculated ratio is indicated at three points during transformation. This curve should be compared to the experimental one of figure 1.

are two "breaks" in the curve, it is necessary to assume the existence of a third cell type during the transition period, growing with a generation time intermediate between that of A and B. (In this case, the intermediate cell type would be indistinguishable from and scored as a type B colony on the minimal agar plates.) It must be assumed further that there are two very fast transformations, one from A to the intermediate type, and the other from this latter type to B, giving a minimum over-all conversion rate (A to B) of 3×10^{-2} per hour.

Whether two or three cell types actually are involved in this transformation, it has been

a number of independent grounds, it has been concluded that the rate increases are brought about not by a selection mechanism but by mass conversions of the population into new cell types. The simple interpretation which has been suggested for the phenomenon (auto-adaptation) is that one cell type (A) acquires the ability to transform itself at a high rate into another cell type (B) which is more efficient at glycine and serine biosynthesis. Part of the evidence for this interpretation rests upon experiments in which small samples of an auto-adapting culture are removed, diluted by a factor of about 10^6 , and plated to minimal agar. After such a dilu-

tion, it is doubtful that the behavior of the cells on the minimal agar plate could be influenced by an inhibitor or conversion factor present in the original culture. In other words, the ability of a cell to produce a small (A) or large (B) colony on the minimal agar is one possessed by the cell at the time of its removal from the original culture. Following the ratio of A to B cells, by plating at various times during auto-adaptation, gives values which are remarkably similar to corresponding ratios calculated on the basis of a theoretical mass conversion. This correlation renders improbable the interpretation, for example, that an inhibitor present in the culture suppresses existing B cells until a critical time at which the inhibition is overcome by a substance being produced by the cells (A or B). B cells, by definition, give a large colony on minimal agar after a certain period of incubation. If such cells were present (but suppressed), they should have been released from inhibition by the diluting and plating procedure, and hence been detected long before the mass conversion rather than at a time and at such a rate as to fully account for it.

Let us consider the possibility that a conversion factor is accumulated by cell type A, released into the medium, and at a critical concentration causes the conversion of A to B cells. The fact that the ratio of the two cell types changes with time at a rate which can account fully for the transformation suggests that the cells are "fixed" or transform at a much lower rate when they are removed from the culture, diluted, and plated. In fact, accumulation of conversion factor would be expected to proceed more slowly on the agar plates, due to diffusion away from the colonies and to the relatively lower concentration of cells. As pointed out previously, A and B cells do continue to transform (at different and probably lower rates) after being plated, for it is by their further transformed progeny that they are recognized.

It is useful to formulate a general working hypothesis in trying to understand auto-adaptation and its inhibition by glycine. This hypothesis is tentative, and its primary function is to correlate in the simplest way the various facts so far observed, as well as to suggest specific experimental approaches for a further understanding of the problem.

Due to the cells' metabolism in minimal me-

dium, a conversion factor is built up to a critical level, at which point A cells become B cells at the very high rate of 1.2 per generation of A cells. Let us assume that this conversion factor is a precursor of glycine, and that the cells have a partial genetic block in the conversion of this precursor, X, to glycine:

During growth of the cells in minimal medium, the concentration of X is built up to a critical level which induces the formation of more enzyme, E_x . [Recent observations have suggested that "constitutive" enzymes also may be formed adaptively in response to their substrates (Ephrussi and Slonimski, 1950; Vogel and Davis, 1952).] The inducing action of X could be on the enzyme (E_x) directly or upon a gene (G_x) controlling the formation of E_x (Wright, 1950). As X accumulates, it could affect a subunit of the gene, for example, at which point the culture is transformed to cells (type B) capable of synthesizing glycine at a higher rate, i.e., by a more efficient conversion of X to glycine. This process periodically repeats itself until synthesis of these amino acids no longer limits the growth of the cells (wild type). Such an interpretation has points of similarity to Hinshelwood's explanation of drug resistance (Hinshelwood, 1946).

With such a view it is possible to interpret the inhibitory effect of glycine utilization on the partially reverted cells. When the cells are given small amounts of glycine, it is all converted to metabolites necessary for growth. If supplied in excess, however, glycine interferes with the normal function or accumulation of substrate X and hence the enzyme it induces, E_x . It has been observed often that accumulation of precursors is suppressed if the microbial mutants are supplied with an excess of the required growth factor (Davis, 1951). A possible mechanism which could explain such an action would involve the accumulation of an intermediate (Y) which could inhibit competitively the further conversion of one of its normal products. Direct evidence has been given recently for a competitive inhibition within a single biosynthetic pathway (Davis,

1952). Whether or not X is involved directly in the competition, growth and hence the normal rate of accumulation of X would be depressed, resulting in an interference with the normal time of a mass conversion.

Thus, by some as yet unknown mechanism the presence of excess glycine could in effect "de-adapt" the cells, presumably by removing or competing with the adaptive substrate, X. When the glycine is removed and the cells allowed to metabolize in a minimal medium, they re-adapt and build up the concentration of X to a level at which mass conversions can again occur. Cross-feeding experiments recently have given support to the kind of interpretation outlined above. It has been demonstrated that auto-adapting cells accumulate a factor to which glycine inhibited cells are sensitive. Identification of the factor is in progress and will be reported later.

The fact that low glycine concentrations do not affect the future behavior of the partially reverted cells in minimal medium made it possible to detect them as they were arising by partial reversion of the fully dependent mutant in the presence of glycine (Wright, 1953a). Another observation also can be explained by the general interpretation suggested above. It was noted that the degree of inhibition produced by a given amount of growth in the presence of glycine could be attained only by three times as much growth in the presence of yeast extract. It is known that there are more substances in yeast extract than glycine and serine which will satisfy a mutant requiring these amino acids (Hungate, 1946). Thus, in the presence of yeast extract the amino acid requirement of the partially reverted cells is relieved by the utilization of vitamins or peptides, for example. Being only distantly related to the biosynthetic pathway concerned with glycine synthesis, these substances have little effect on the concentrations of X and E_x . Thus, in order to reach inhibitory concentrations of glycine and serine, it is not surprising that three times as much growth must occur with yeast extract than with glycine or serine alone.

It does not seem possible or profitable at this stage of the problem to try to classify the mechanism underlying auto-adaptation, other than to describe some of its general characteristics. The partially reverted strains growing in minimal

medium apparently exhibit a mixture of characteristics observed for mutations and for enzymatic adaptations. The regularity in time and degree of the changes observed, the stepwise, quantitative acquisition of a biosynthetic ability, as well as the proportion of the population affected by these changes suggest an adaptive phenomenon. Moreover, the acquired enzymatic ability can be reversed by a mechanism which is understood most easily in terms of a "de-adaptation" phenomenon. On the other hand, auto-adaptation has features which could be called qualitative, in that conversion of a slow to a faster growing cell type occurs in a discontinuous, discrete step; rather than a continuous spectrum of cell types, there are two distinct types which can be distinguished easily by colony size on minimal agar. The growth characteristics of the intermediate cell types present in a transforming culture are stable and heritable when the cells are removed from the transforming culture and maintained on "complete" media. Furthermore, the conversion concerns a constitutive pathway and occurs "spontaneously" in the sense that it is not induced by an externally supplied substrate. These features are reminiscent of mutations.

Whatever the chemical nature of the conversion factor, it is produced by and acts upon a culture initially composed of a homogeneous population. Thus, the culture adapts itself, or auto-adapts, whether in the general sense of the culture as a whole becoming more efficient at growing in a minimal medium, or in a stricter sense, implying a mechanism which may be analogous to enzymatic adaptations.

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SUMMARY

The behavior of partially reverted strains of a glycineless mutant of *Escherichia coli* has been

analyzed. When cultured in a minimal medium, the cells grow at stepwise increasing rates until the need for serine and glycine no longer limits their growth. The curves are always composed of a series of sharply defined logarithmic stages, regardless of whether the cell density is followed optically or by means of viable count. A rate increase has been attributed to a mass conversion of the population, rather than to a selection mechanism, on a number of independent grounds. Two of them are: (1) A culture of partially reverted cells growing at rate A can establish a higher growth rate, B, about ten times faster than would be expected if cells growing at rate B were selected for in a culture consisting predominantly of cells growing at rate A. (2) By plating frequently to minimal agar during a change in growth rate of a partially reverted culture, it is possible to follow the ratio of (the easily distinguished) type A and type B cells. The over-all change in ratio of the two cell types takes place about ten times faster than could be expected on the basis of selection of the faster growing type B cells.

A mathematical analysis of the growth curves of a transforming culture has shown that A cells undergo conversion to B cells at a rate of about 3×10^{-2} per hour, or 1.2 per generation of A cells.

This phenomenon has been called "auto-adaptation" and resembles an enzymatic adaptation in that a large proportion of the population presumably acquires an increased biosynthetic ability in a relatively short time. Unlike the commonly described adaptive enzymes, this biosynthetic ability is not formed in response to an externally supplied substrate. It is assumed that more enzyme is produced in a discontinuous way as a result of the metabolic activity of cells already in possession of low levels of this enzyme. A possible interpretation is that the level of a substrate (a precursor to glycine) responsible for the production of more enzyme is built up during the cells' metabolism, and it is this periodic accumulation which results in stepwise increasing levels of enzymatic activity. Such cultures have another feature which may be analogous to the usual adapted culture: they can be "de-adapted".

"De-adaptation" is brought about by supplying the cells with an excess of their growth limiting factor, glycine, the utilization of which presumably results in the removal or inhibition of the adaptive substrate and its enzyme.

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