The Reversion of Catalase during Growth of Yeast in Anaerobiosis

J. GORDIN KAPLAN

From the Laboratory of Cell Physiology, Dalhousie University, Halifax, Nova Scotia, Canada

ABSTRACT Growth of originally aerobic bakers' yeast under conditions of anaerobiosis caused a decrease in the total specific catalatic activity (patent plus cryptic) of one-half per generation. It is concluded that reversion of catalase was a dilution, rather than a destruction, of the intracellular enzyme. However, the specific patent (whole cell) catalase activity remained constant for one or more generations, and then declined at a considerably slower rate than did the total activity. Thus the cryptic factor diminished progressively during anaerobic growth; after seven or eight generations virtually all the catalase was patent; *i.e.*, the cryptic factor (the ratio of total enzyme to patent enzyme) was approximately unity. At this point, the basal level of enzyme was attained, and thereafter maintained by a basal synthesis, which produced only the patent, heat-stable, variety. Aerobic growth caused a significant, but much smaller, decline of both total catalase activity and of the cryptic factor. The data suggested that during reversion, the cryptic, heat-labile catalase became progressively converted to the patent, heat-resistant form. A model of these events is presented.

INTRODUCTION

An inducible enzyme is one whose rate of synthesis is increased greatly by the presence of a specific inducer. Chantrenne and Courtois (1) demonstrated that yeast catalase was an inducible enzyme, and that its inducer was oxygen, or possibly hydrogen peroxide formed by reduction of oxygen by cell metabolism.

When a population of previously "induced" cells is allowed to grow in absence of the inducer, the rate of synthesis of the inducible enzyme quickly falls, and the concentration of the enzyme within the growing cells is reduced to a minimum, the *basal level*; it is maintained at this level by a *basal synthesis*; that is, the low level synthesis which occurs in the absence of the specific inducer. The process whereby the high induced enzyme level becomes reduced

to the basal level has been called *enzymic reversion* (Pollock (2)). There are two principal mechanisms of enzymic reversion.

1. The enzyme is stable, and the total enzyme activity of the growing culture remains constant, but the level per cell becomes progressively reduced with each cell generation, as the enzyme is parcelled out to the daughter cells. Under these conditions, reversion is due to dilution of the intracellular enzyme, a process first demonstrated by Wainwright and Pollock in the case of bacterial nitratase (3), and by Rickenberg, Yanofsky, and Bonner (4) and Hogness, Cohn, and Monod (5) in the case of the β-galactosidase of *E. coli*.

2. The enzyme (or coenzyme) is unstable, and the total activity of the culture diminishes in the absence of the specific inducer. Spiegelman and Dunn (6) showed that the maltozymase levels of yeast decreased as the cells were induced to form galactozymase by the inducer galactose; indeed, this could occur even in the presence of maltose, the inducer of maltozymase. Since the decrease in maltozymase level was considerably less if an exogenous nitrogen source was added, they concluded that proteolysis was the cause of the loss of activity. Mandelstam showed that the decrease in lysine decarboxylase of *B. cadaveris* which followed withdrawal of the inducer, lysine, was not due to proteolysis but rather to loss of the coenzyme, pyridoxal phosphate (7).

Finally, one should note that the work of Wright and McNeil (8) suggests that the stability of an inducible enzyme may depend on the nature of the inducer, at least in the case of cells growing in the presence of the inducer. Under such conditions, the β-galactosidase of *E. coli* was considerably less stable when induced by galactose, a poor inducer, than when induced by melibiose, a relatively good inducer.

Catalase within the living, aerobically grown yeast cell exists in two distinct states: (*a*) that fraction whose activity is detectable *in situ* (patent enzyme) and is highly resistant to inactivation by heat, and (*b*) that fraction whose activity is cryptic and relatively sensitive to heat inactivation (9). However, in anaerobically grown yeast the cryptic, heat-labile form is absent; virtually all the catalase of anaerobic cells is patent (10, 11) and is of the relatively heat-resistant variety (9). Nevertheless, treatment of anaerobic cells with lytic agents, such as *n*-butanol (9, 10) or ultraviolet radiation (12), causes changes in properties of the intracellular catalase (*enzyme alteration* (13)) similar to those reported previously for the enzyme of aerobic yeast (14).

In order to account *both* for the *latency* of most of the catalase activity of aerobic yeast and for the *alteration* of its properties which followed cell lysis, an hypothesis was advanced which pictured all of the intracellular catalase adsorbed at an interface of the oil/water type in a partly unfolded state (13). This model was found to be consistent with the behaviour of crystalline liver catalase adsorbed at oil-water interfaces stabilized by various amphipathic substances (15). However, the fact that in anaerobic yeast one can demon-

strate catalase alteration in the absence of latent enzyme means that the interfacial hypothesis is no longer tenable in its original form.

The present experiments were undertaken in the hope that a study of the reversion of the catalase-rich aerobic cell to the catalase-poor anaerobic cell would yield information permitting construction of a more satisfactory model of the relations between cryptic and patent catalase.

EXPERIMENTAL

Fresh cakes of bakers' yeast (*S. cerevisiae*, Standard Brands) were washed, and cells were suspended in growth medium to a density of approximately 1.0 mg/ml (dry weight) indicated by means of the Klett photoelectric colorimeter. An equal volume of the medium was then added, reducing the cell density to half. The flask, which had a Klett tube grafted into its flank, was then stoppered, flushed with nitrogen, and CO_2 drawn off according to the method of Slonimski (16). Growth was at 37°C. Cell densities were determined at frequent intervals until the original cell density was restored (1.0 mg/ml). At this time, half of the culture was removed from the flask and an equal volume of fresh medium added; the flask was then stoppered and flushed with nitrogen as before, and restored to 37°. This operation was repeated for as many cell divisions as were required. This procedure for the collection of cells at succeeding generations was adapted from that of Rickenberg, Yanofsky, and Bonner (4). It is clear that this method of measuring mean generation time will have statistical validity only; some cells may not have divided during the interval, while others may have done so more than once.

The samples thus collected were centrifuged, washed once with M/100 phosphate buffer, pH 7.0, and resuspended in buffer. Part of this aliquot was assayed directly, to determine the whole cell, or patent, catalase activity. The remainder was treated with butanol as previously described (13), washed, and resuspended in buffer and assayed to determine the total catalase activity (patent plus cryptic).

Catalase assays were performed by means of a Beckman DU spectrophotometer, following the decrease in absorption of H_2O_2 at 240 mμ. Specific activities will be reported as log optical density change per milligram yeast per milliliter. Each liter of growth medium contained: 1.43 gm $MgSO_4$, 0.43 gm NaCl, 1.00 gm KH_2PO_4, 1.20 gm $(NH_4)_2SO_4$, 0.005 gm $FeCl_3$, 1 gm yeast extract (Nutritional Biochemical Company), and 50 gm D-glucose.

RESULTS

Fig. 1 shows the effect of six anaerobic cell divisions on A, the specific patent activity of catalase, B, the specific total activity, determined following butanol treatment, and C, the cryptic factor; *i.e.*, the ratio of B to A. Curve B shows that the total activity decreased by approximately one-half per cell per generation, suggesting that the reversion of catalase was a consequence of intracellular dilution during growth. Despite the dilution of total activity per cell, the patent activity of the cells remained constant during the first three

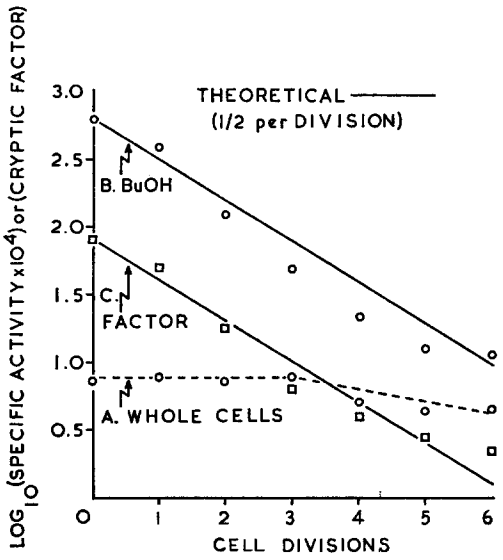

FIGURE 1. Semilog plot of the change during anaerobic growth of A, the specific patent activity, B, the specific total activity, and C, the cryptic factor (B/A).

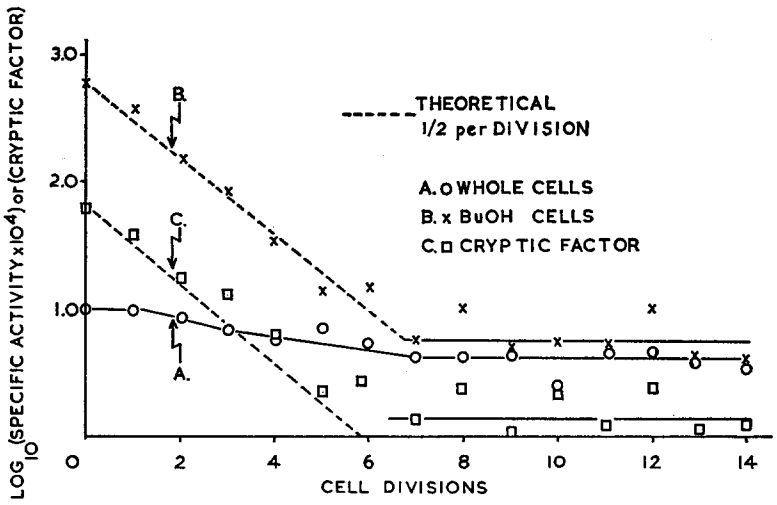

FIGURE 2. Semilog plot of the change during anaerobic growth of A, the specific patent activity, B, the specific total activity, and C, the cryptic factor (B/A).

anaerobic divisions (curve A); thereafter, the patent activity fell off by considerably less than one-half per cell per generation.

A series of experiments was undertaken in which, by prolonging the number of anaerobic divisions, the intracellular concentration of catalase was reduced to the basal level. Fig. 2 illustrates an experiment in which an originally

aerobic culture was grown anaerobically for fourteen generations. The patent activity (curve A) remained constant during the first division and thereafter fell off gradually, reaching its basal level at the seventh division; at this point the patent activity had been reduced by 2.4 times, or to about 40 per cent of the aerobic catalase level. In no experiment was the patent activity reduced to less than 30 per cent of the original aerobic level; in most it was reduced to about one-third.

Fig. 2, curve B, illustrates the decline in total activity per cell. The interrupted line was drawn on the supposition that the enzyme dilutes out by one-half per cell per division, and it is seen that the experimental points fit the theoretical curve reasonably well. Reversion thus seems not to involve

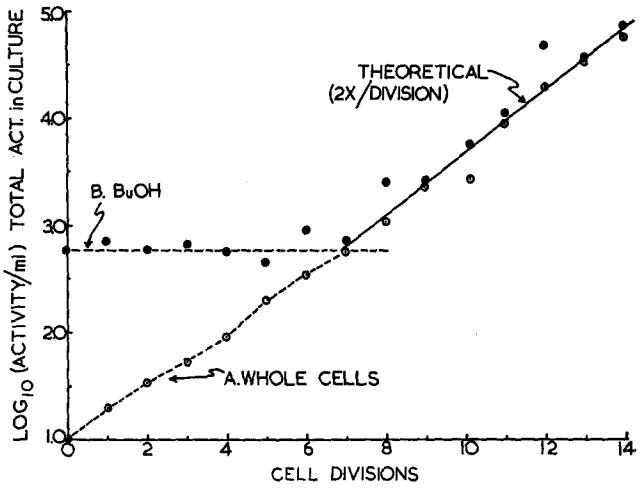

FIGURE 3. Semilog plot of the change in the activity per milliliter of culture of A, whole cells (patent activity) and B, butanol-treated cells (total activity). These are the same data as those of Fig. 2, corrected for the dilution of the original culture.

destruction of the enzyme, but rather a parcelling out of the enzyme, from mother to bud. At the seventh division, the total activity per cell reached the basal level, as in the case of the patent activity. At this point, the total enzyme content per cell was less than 1 per cent (generally about 0.5 per cent) that of the aerobic cells. The cryptic factor shown in curve C, was originally 58 and declined to 1.3 at the seventh division, and this is typical of most experiments. In this experiment (and in most), the cryptic factor diminished by somewhat less than one-half per division, in contrast with the results shown in Fig. 1 in which the patent activity remained constant for a greater number of generations than was ordinarily the case.

If the total specific activity (activity per cell) halved after each division, it is clear that the total catalase *in the culture* remained constant. If the specific

patent activity (patent activity per cell) diminished by less than one-half per division it is clear that the patent activity *in the culture* must have *increased* during the process of reversion. This is illustrated in Fig. 3, in which the specific activities of Fig. 2 have been multiplied by the factor by which the original culture was diluted. The activities thus presented represent the activity per milliliter of the original culture. The total activity, shown in curve B, remained constant for seven divisions; the patent activity, curve A, increased with each division, until after the seventh division virtually all the activity was in the patent fraction; that is, the cryptic activity had virtually

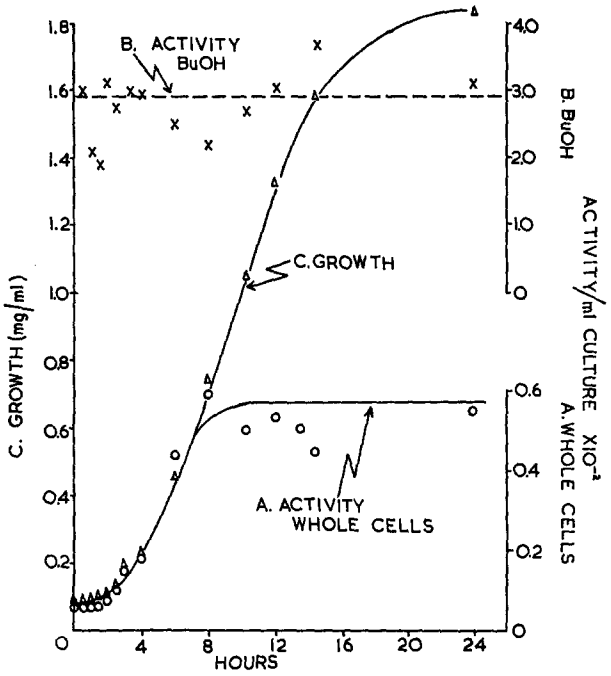

FIGURE 4. Changes with time of A, patent catalase activity, B, total catalase activity, and C, dry weight of yeast. Growth was under anaerobic conditions, at 37°C.

disappeared. Thereafter, as the basal synthesis proceeded, both the total and the patent activities (which were essentially the same) doubled in the culture with each generation, as shown by the solid line in Fig. 3.

If the increase in patent activity of the culture occurred while its total activity remained constant, as shown in Fig. 3, the probable cause of the phenomenon would be progressive conversion of the cryptic enzyme into the patent variety. That this is indeed the case is suggested by the kind of experiment represented in Fig. 4. Curve C represents the growth in anaerobiosis of an originally aerobic yeast suspension, and curve A represents the patent activity of the culture (*i.e.*, specific activity times milligrams of yeast per

milliliter of culture). Curve B shows the total activity of the culture, which remained more or less constant during growth. The close parallel in the rise of cell numbers and of the patent catalase of the culture (curves A and C) will be remarked; after 8 hours of growth, the patent activity tended to level off, for reasons which have not yet been elicited. If the culture was now diluted to its original cell density, once again the patent activity rose in parallel with the increase in cell mass. Usually a third dilution was required before essentially all the catalase activity of the culture was present in the patent form.

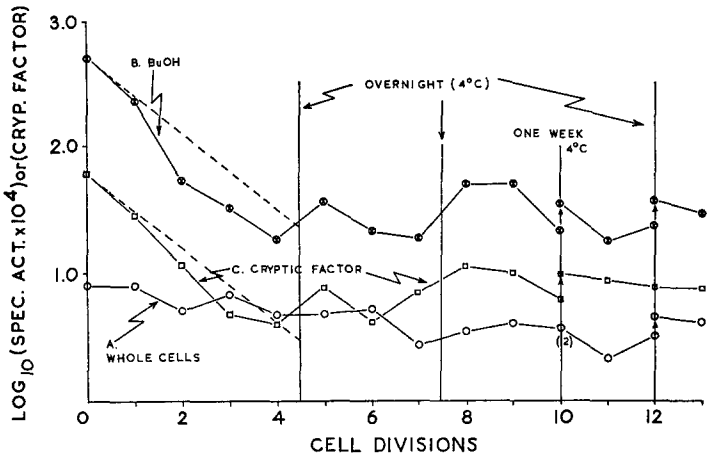

FIGURE 5. Variation, during aerobic growth, of A, patent specific catalase activity, B, specific total activity, and C, cryptic factor. The interrupted lines are drawn to decline at one-half per cell per division. Some destruction of catalase will be noted.

Growth under conditions of *aerobiosis* also reduced the catalase level of the yeast cell (and the magnitude of the cryptic factor), much as it does the activity of the respiratory enzymes (17). Fig. 5 represents such an experiment. Neither the total specific activity nor the patent specific activity fell to nearly the same extent as in anaerobic growth. There was a tendency for the cryptic activity to rise during periods when growth was stopped, even at 4°C; such periods are indicated by the vertical lines in the figure. I have never been able to reproduce in the laboratory the experimental conditions required to grow yeast cells having the high concentration of intracellular catalase characteristic of commercially produced bakers' yeast. Meyerhof (18) and Warburg (19) noted the same effect with respect to the respiratory rate of laboratory *versus* commercial bakers' yeast (see also Slonimski, 20).

DISCUSSION

During growth in the absence of oxygen, the total catalase content of previously induced (*i.e.*, aerobically grown) yeast diminished by one-half per

cell per division; therefore the total catalase of the culture remained constant. Reversion thus consisted of a process of intracellular dilution, as the enzyme was parcelled out from mother to bud. On the other hand, the patent activity per cell remained constant for one or more divisions, and then fell off by very much less than one-half per cell per division. Thus the total patent activity of the original culture doubled for one or more divisions and continued to increase during the process of reversion, until all the activity of the culture was in the patent fraction.

Diminution of the magnitude of the cryptic factor (or the magnitude of the *Euler effect* (21), as the phenomenon of the release of cryptic catalase activity has been called (22)) as a consequent of the growth of yeast, was reported by Yamahuzi *et al.* (23); they also found that in certain strains of yeast they were unable to demonstrate the Euler effect at all. Engel and Adler (24) reported that the catalase activity of *E. coli* B was unchanged following treatment of the cells with toluene, a treatment which greatly increases the activity of induced β-galactosidase; similarly, treatment of *E. coli* K-12 with butanol or toluene did not significantly change the catalase activity of the suspension (Kaplan, unpublished data). Clayton (25) has shown that treatment of aerobically grown cells of *Rhodopseudomonas spheroides* with toluene increased their catalase activity by a factor of slightly less than 3; treatment of the catalase-poor anaerobically grown cells did not increase their activity. Clayton interpreted his results in terms of a model in which the catalatic activity of the aerobic cells was limited by a permeability barrier which did not affect the activity of the enzyme in anaerobically grown cells. A simple permeability model of this kind is insufficient to account for the disappearance of the heat-labile component during reversion (9).

The progressive disappearance of the heat-labile, cryptic enzyme during anaerobic growth, and the correlation of the increase in mass of the anaerobic culture with the increase in its total patent activity (Fig. 4) suggest that the cryptic, heat-sensitive catalase was converted into the patent, heat-resistant form during reversion. Basal synthesis of new patent enzyme during reversion could not account quantitatively for the doubling of the patent activity of the culture during the first division(s) of reversion (Figs. 1 and 2, curves A).

After seven to eight anaerobic generations, the basal catalase level was reached; at this point the total catalatic activity was virtually all detectable *in situ*; that is, the cryptic factor was approximately unity. (It is interesting that Slonimski has found that eight anaerobic divisions were required to reach the basal level of cytochrome oxidase in yeast (26).) Thereafter, with each division the specific patent and total activities remained constant, and the patent and total activities of the culture doubled. The enzyme produced by basal synthesis differed in at least three respects from that produced by induction: (*a*) its activity was patent (10); (*b*) like the patent activity of the

aerobic and reverting cell, it was of the heat-resistant variety (9); (*c*) it was highly resistant to ultraviolet-induced alteration and photoinactivation (12).

As a result of the differences between the basal and the induced catalases, an interesting question now poses itself: does the basal synthesis go on during the divisions of reversion, or does it switch on only when the intracellular catalase has been diluted to the basal level? Let us assume in what follows that the basal level of enzyme is 2 units per cell; thus when a cell divides under basal conditions, both mother and daughter cells receive 1 unit of preformed

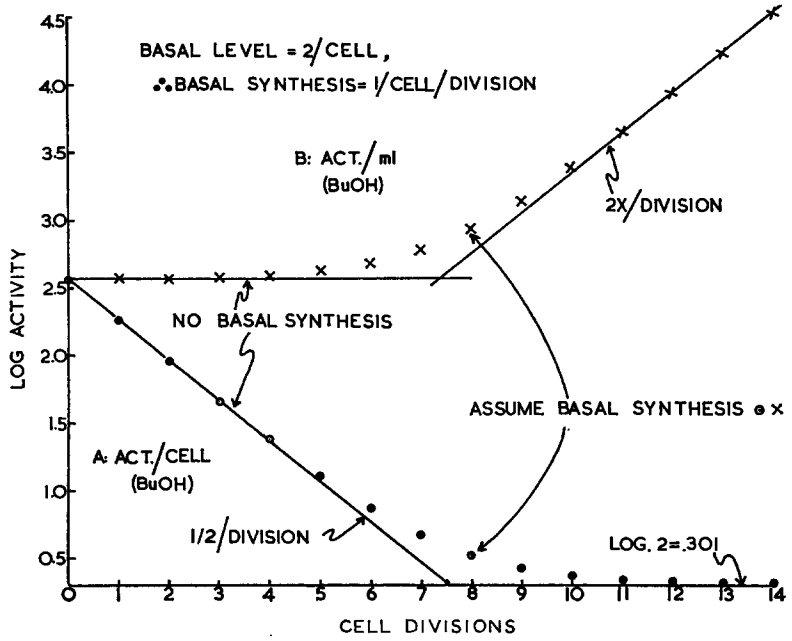

FIGURE 6. Expected variation in A, total specific activity and B, total activity per milliliter culture. The solid lines are drawn on the hypothesis that basal synthesis begins only when reversion is complete. The points are drawn on the hypothesis of a continuous basal synthesis.

enzyme and synthesize one new unit. In this way, the activity of the culture doubles with each division, but the activity of the cells remains constant at the basal level. Since there was usually a threefold decrease in specific patent activity during reversion, and since the cryptic factor was about 60 in the aerobic cells, it follows that the original total specific activity was 180 times the basal level, or 360 units. If there was no basal synthesis during the reversion phase of anaerobic growth, one would observe the decrease in specific total catalatic activity represented in Fig. 6 by the solid line of curve A; the total activity per cell would decline by one-half with each division, to reach the

basal level at the seventh or eighth division, remaining constant at that level thereafter. Similarly, as shown by the solid line in curve B, the total activity of the culture would remain constant until the seventh or eighth division, and abruptly change thereafter to double with each cell division.

If, on the other hand, a specific basal synthesis of one unit per division were taking place during reversion, one would obtain the data represented in Fig. 6 by the circles and crosses of curves A and B respectively. It will be noted that the contribution of basal synthesis to total enzyme levels of the cell and the culture would be too small during the early divisions of reversion to be detectable. Only at the sixth or seventh division would the deviation

TABLE I

Expected total specific activity under varying conditions of enzyme distribution and basal synthesis. Column 1, basal synthesis commences only when reversion is complete; equal distribution of enzyme at each generation. Column 2, enzyme is distributed two-thirds mother: one-third bud; basal synthesis commences when any cell reaches the basal level (2 units per cell). Column 3, basal synthesis goes on during reversion; equal distribution of enzyme.

Anaerobic divisions	No. of cells	Total specific activity		
		1	2	3
0	1	360	360	360
1	2	180	180	181
2	4	90	90	91.5
3	8	45	45	46.8
4	16	22.5	22.4	24.4
5	32	11.25	11.2	13.2
6	64	5.62	5.7	7.6
7	128	2.81	3.35	4.8
8	256	2.00	2.31	3.4
9	512	2.00	2.09	2.7
10	1024	2.00	2.01	2.3

from linearity become appreciable; at the eighth and ninth divisions, the catalase content of cell and culture would be higher than the basal level. Referring to Figs. 2 and 3, one notes that there was sufficient scatter of the experimental points of curves B to prevent a clear answer to the question posed above. The scatter in other experiments has been no less, and it is unlikely that this question can be answered by experiments of this kind. It is susceptible to attack by a more strictly biochemical approach.

The solid lines in Fig. 6 were drawn on the assumptions that basal synthesis did not occur until the basal level was reached and that the enzyme was distributed equally between mother and bud at each division. If the distribution of the enzyme during reversion was unequal, it is clear that some cells in the

population would reach the basal level in fewer generations, and consequently would commence basal synthesis earlier. If such were the case, the solid lines of Fig. 6 would not be significantly changed, as shown in Table I, in which the total specific activities of column 2 were calculated on the assumption that the distribution of enzyme during reversion was two-thirds mother:one-third daughter at each generation and that basal synthesis began when the basal level of 2 units per cell was reached. Only at the seventh and eighth divisions would there be a noticeable deviation from the specific activities on the assumption of equal distribution (column 1) and this deviation would be quite small.

An hypothesis accounting for the events occurring during reversion must be consistent with certain experimental observations: (*a*), there must be a difference of kind between the cryptic and patent enzymes; it is probably necessary, but not sufficient, to posit the existence of a permeability barrier to substrate (9); (*b*), enzyme latency must disappear progressively during growth in absence of inducer; (*c*), under basal conditions, synthesis must produce only the patent variety of catalase.

An admittedly speculative hypothesis which is consistent with these requirements and which seems to have some heuristic value supposes that patent catalase is bound to specific membrane sites; at most of these sites binding is reversible. The patent enzyme is stabilized against heat inactivation by virtue of being membrane-bound. The cryptic enzyme is concealed within the membrane, inaccessible to substrate. During reversion, new membrane sites are formed, and the distribution of enzyme between patent and cryptic fractions is governed by the equilibrium constant of the binding reaction. At certain of the sites, the binding is irreversible; it is the irreversible sites which are filled by basal synthesis.

While this hypothesis will be considered in a subsequent paper, it is interesting to note that in the case of yeast α-glucosidase (Robertson and Halvorson (27)) enzymic reversion was accompanied by release of bound enzyme from a particulate fraction. The release of bound α-glucosidase activity had the unusual consequence of *increasing* the apparent total activity during growth in the absence of inducer; thus, the cryptic factor rose during reversion (27). In a strain of *S. cerevisiae* isolated in this laboratory, the inducible β-glucosidase activity (determined after butanol treatment) declined during reversion by one-half per cell per division, but the specific patent activity declined at a much greater rate (28). In the case of this enzyme, whose patent activity seems to be limited by an energy-dependent, sterically specific transport system, the cryptic factor increased during reversion by more than twenty times.

On the other hand, the inducible β-galactosidase of a strain of *S. cerevisiae* isolated in this laboratory (28) and of a variety of strains of *E. coli* (Rickenberg,

cited in reference 29; Kaplan and Bonner, cited in reference 30; Holmes, Sheinin, and Crocker (31)) behaved during reversion like yeast catalase, the cryptic factor becoming unity when, or before, the basal level was attained. This was so because the patent activity declined far less during growth in the absence of inducer than did the total activity; for example, the former declined but three times and the latter about 200 times in the case of yeast catalase. It seems possible that since mechanisms which would reduce the latency of enzymes during reversion would be homeostatic, they might confer a selective advantage, at least under certain environmental conditions.

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