THE EFFECT OF FREE AMINO ACID POOL LEVELS ON THE INDUCED SYNTHESIS OF ENZYMES[1]

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A previous investigation (Halvorson and Spiegelman, 1952) demonstrated that analogues of amino acids which prevent incorporation from the free amino acid pool into protein also suppress the induced synthesis of maltase in yeast cells. The data obtained led to the conclusion that the quantitatively predominant pathway of induced enzyme formation in nongrowing cells involves the utilization of the internal free amino acids. The importance of amino acids in the formation of enzymes received independent support from work with other enzymatic systems and different biological material. Ushiba and Magasanik (1952) studied the adaptive utilization of myo-inositol by amino acid, purine, and pyrimidine auxotrophs of *Aerobacter aerogenes*. The response of enzymatic synthesis in resting cells of the mutant types to various kinds of supplementations suggested that the formation of enzymes from amino acids is involved. Pinsky and Stokes (1952) found that the presence of arginine, aspartic acid, and glutamic acid is necessary for the formation of hydrogenlyase in *Escherichia coli* and conclude that extensive synthesis from free amino acids accompanies enzyme formation. The same conclusion is derived by Monod, Pappenheimer, and Cohen-Bazire (1952) from an ingenious series of experiments with the β-galactosidase in *E. coli* to which we shall have reference later.

The experiments with yeast cited above established a correlation between the availability of the free amino acid pool and the capacity to synthesize enzyme. Control over protein synthesis from amino acids was achieved by interfering actively with the utilization of components of the amino acid pool by means of specific antagonists. It was of interest to extend these observations to see whether other experimental devices for making the free amino acid pool less utilizable also would lead to a decrease in enzyme synthesizing capacity.

A simple method of controlling the relative availability of the free amino acids in yeast is suggested by the investigations of Roine (1947) who demonstrated that the level of "free amino nitrogen" could be varied at will by subjecting cells alternately to nitrogen starvation and replenishment. These observations were confirmed and extended to several of the individual component amino acids by Halvorson and Spiegelman (1952).

The purpose of the present paper is to summarize experiments examining the effects of such variations in free amino acid pool levels on the capacity of yeast cells to synthesize maltase. The data are consistent with the conclusion that the free amino acids constitute the primary source of nitrogen for the fabrication of new enzyme molecules.

METHODS

Strain employed and conditions of growth. The yeast used is a diploid representative of *Saccharomyces cerevisiae* (strain K). It was grown in a complete medium prepared by adding the following to one liter of water: glucose, 40 g; peptone (Difco), 5 g; yeast extract (Anheuser-Busch no. 3), 2.5 g; $(NH_4)_2SO_4$, 6 g; $CaCO_2$, 0.25 g; $MgSO_4$, 0.25 g; and sodium lactate (60 per cent), 6 ml. Cells in the log phase were employed invariably and these were prepared by inoculating 500 ml of the complete medium with 0.2 ml of a 24 hour culture. The resulting suspension then was allowed to incubate unagitated at 30 C for 12 hr. Immediately prior to an experiment the cells were harvested by centrifugation, washed twice in chilled water, and suspended in the inducing medium to a density of 2.84 mg dry

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weight of cells per ml with the aid of a Klett-Summerson photoelectric colorimeter previously calibrated for this purpose. Unless otherwise specified the pH of the suspension was adjusted to 4.5.

Conditions of induction of enzyme. Inductions were carried out with cells suspended in a nitrogen-free synthetic medium modified after the one proposed by Burkholder (1943). The sources of nitrogen were omitted and 5.9 g of succinic acid were added per liter to increase the buffering capacity at pH 4.5. The inducing agent in these experiments was 3 per cent maltose.

Methods of following the synthesis of enzyme. Two methods were used for the continuous observation of the course of enzyme formation. In inductions carried out aerobically the usual two cup method (Umbreit, Burris, and Stauffer, 1950) was used, the level of enzyme being proportional to the $Q_{CO_2}^{O_2}$ above the endogenous. In anaerobic inductions the $Q_{CO_2}^{N_2}$ on maltose was taken as a measure of the enzymatic content. Since no endogenous source of energy is available anaerobically in yeast, an external initiating supply of energy was provided by the inclusion of 0.8 mg of glucose per cup in all anaerobic inductions. Observations on the progress of enzymatic synthesis were begun after all the glucose added was accounted for in terms of CO_2 output. This took between 40 and 50 minutes. Both methods of following enzymatic synthesis were checked by assay of maltase on dried cell preparations and yielded good agreement. The maltolytic activity of the dried cells was measured manometrically by the rate of glucose released using a maltose negative yeast strain, *Torula monosa* (Halvorson and Spiegelman, 1952).

Manometric measurements were carried out with conventional Warburg equipment at 30 C. Anaerobic conditions were established by flushing with nitrogen. Merck's maltose was purified further by recrystallization from 50 per cent alcohol.

Collection and analysis of free amino acid pools. The free amino acid pools were collected by the methods devised by Gale (1947). Their components were analyzed by the use of specific amino acid decarboxylases (Gale, 1945), microbiological analysis (Henderson *et al.*, 1948), and paper strip chromatography (McFarren, 1951). The details of the applications of these methods to yeast have been described previously (Halvorson and Spiegelman, 1952).

EXPERIMENTAL RESULTS

Microbiological analysis of the free amino acid pool. As a point of departure and in view of the paucity of the available information, it seemed desirable to establish the quantitative composition of the free amino acid pool for as many of the components as possible in the cells to be employed in the experiments. Table 1 summarizes the average of duplicate microbiological determinations for 16 of the component amino acids found in log phase yeast cells prepared according to the procedures described under Methods. The results agree with the data obtained by the decarboxylase (Taylor, 1947) and chromatographic analysis (Halvorson and Spiegelman, 1952; Bair and Rouser, 1952) of the free pools. Glutamic acid and lysine represent the major individual components of the pool. A number of investigators (Halvorson and Spiegelman, 1952; Bair and Rouser, 1952) have shown that under a variety of conditions the components of the

TABLE 1

Microbiological analysis of the free amino acid pool of yeast cells (strain K) in the log phase

A 25 ml suspension of cells in the log phase was prepared in water containing 50 mg dry cells per ml. This was placed in a boiling water bath for 20 minutes, cooled, and repeatedly centrifuged. The clear supernate was analyzed microbiologically. Five separate tubes were run for each amino acid analyzed, and duplicate samples were used. The average values and deviations from the mean are given.

AMINO ACID	μM/100 MG DRY CELLS
Leucine	0.51 ± 0.04
Valine	1.57 ± 0.13
Phenylalanine	0.34 ± 0.03
Tryptophan	0.027 ± 0.002
Glutamic acid	10.0 ± 0.71
Aspartic acid	3.6 ± 0.20
Histidine	0.97 ± 0.029
Lysine	4.3 ± 0.35
Isoleucine	0.64 ± 0.05
Proline	0.92 ± 0.02
Tyrosine	0.17 ± 0.01
Threonine	2.2 ± 0.10
Arginine	1.2 ± 0.10
Methionine	0.60 ± 0.02
Serine	3.67 ± 0.06
Cysteine	0.14 ± 0.02

free amino acid pool behave in a quantitatively similar manner. Thus, quite frequently the specific analysis of glutamic acid by means of a glutamic acid decarboxylase provides a convenient means of following the quantitative variation of the free amino acid pool level.

The effect of pool depletion on the capacity to synthesize enzyme. When yeast cells are allowed to metabolize glucose in a medium lacking nitrogen, the free amino acid pool is depleted rapidly as the amino acids are incorporated into protein. Experiments were undertaken to examine the effect of such depletion on the capacity of the cells to synthesize maltase when exposed to inductor. In order to minimize any irrelevant deficiencies which might occur as a consequence of starvation in a nonnutrient buffer, the depletions were carried out in the nitrogen-free synthetic medium employed for the inductions and described under Methods. Previous experiments (Halvorson and Spiegelman, 1952) have shown that this medium when supplemented by nitrogen supports maximal growth of the yeast cells.

TABLE 2

The influence of N starvation on the free amino acid pool and the capacity to synthesize maltase

Washed cells in the log phase were N starved in synthetic medium containing 3 per cent glucose. At the times indicated aliquots were withdrawn, washed, and resuspended in N-free synthetic medium containing 3 per cent maltose. The kinetics of aerobic adaptation to maltose were followed manometrically and the rates of enzymatic synthesis determined from the semilogarithmic plot of $Q^{8}_{O_2}$ against time in minutes. When maximal enzyme content was achieved, "fast-dried" cell preparations were made and the maltolytic activities determined by the *Torula monosa* assay system. The enzymatic activities are expressed as percentages of the zero time values. The glutamic acid (GA) concentrations were determined on parallel samples by the decarboxylase method.

DURATION OF NITROGEN STARVATION IN MINUTES	μM GA/100 MG DRY CELLS	RATE OF SYNTHESIS OF ENZYME	PER CENT CONTROL	PER CENT Q_{CO_2} (MAX) ON MALTOSE	
				Intact cell	Dried preparation
0	10.5	0.0228	100	100	100
150	2.1	0.0147	64	62.5	63.5
300	1.7	0.0118	54	44	49
600	1.1	0.0010	4.8	5.9	4.0

Washed cells in the log phase were suspended in the nitrogen-free synthetic medium containing 3 per cent glucose and incubated at 30 C with aeration. At intervals aliquots were removed for assay of the glutamic acid concentration of the free amino acid pool by the decarboxylase method. The ability to synthesize enzyme aerobically was determined concomitantly in terms of the rate of enzymatic synthesis as well as the final total yield of enzyme. The data obtained are summarized in table 2.

It is evident from the results that the depletion of the free amino acid pool which attends nitrogen starvation is accompanied by a lowering in the capacity to synthesize enzyme. The rate of enzyme formation and the final yield of enzyme are both decreased to a quantitatively similar extent.

Replenishment of the pool and the effect upon the capacity to synthesize enzyme. The next question to be analyzed was whether the correlation between free pool level and capacity to synthesize enzyme worked both ways. In particular it was necessary to examine whether replenishment of the pool in nitrogen starved cells would be accompanied by a parallel restoration of enzyme synthesizing capacity. By virtue of the fact that they possessed the most severely depleted pools and the lowest background in enzyme synthesizing capacity, cells subjected to prolonged starvation provided the most suitable material with which to study such questions.

Roine (1947) had demonstrated that an exposure of nitrogen starved cells to ammonia and glucose led to a rapid replenishment of soluble amino nitrogen. His findings indicated that the rate of replenishment of the free pool was such that it was restored virtually to its original level before much movement of the free amino acids into the protein fraction occurred. Restoration of a depleted pool would require the synthesis either directly by amination or secondarily by transamination of all components of the pool. For the purpose of the present experiments, it was desirable to survey various procedures for replenishing the free amino acid pool of prolonged nitrogen starved cells. Such a survey would provide methods for obtaining cells in which the free amino acid pool had been restored to different extents. The effects of these various levels of replenishment on the capacity to synthesize enzyme could be compared then.

Comparisons were made of the effectiveness of

ammonia and mixtures of amino acids as agents for replenishing the free pools. The relatively greater effectiveness of a replenishment carried out with the amino acid mixtures is illustrated in figure 1 which represents a descending chromatographic diagram developed with phenol-water for a short duration so as to retain all of the ninhydrin reacting components on the same strip. Ten hour nitrogen starved cells (column S) are compared with such cells replenished for ten minutes by exposure to ammonia (column N) and to casein hydrolyzate for the same period of time (column AA).

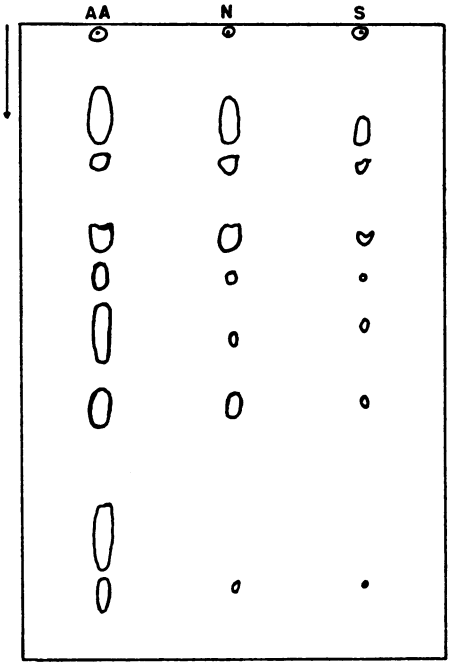

Figure 1. Comparison of replenishments of free amino acid pools with NH_4Cl and casein hydrolyzate.

Twelve hr nitrogen starved cells (column S) were replenished in one case by a 20 min exposure to 1 per cent NH_4Cl and 2 per cent glucose (column N) and in the other by a 20 min exposure to 1 per cent casein hydrolyzate and 2 per cent glucose (column AA). Free amino acid pools were collected in the usual manner and chromatogrammed in descending columns with phenol-water. The duration of exposure to solvent was such as to retain all the ninhydrin reacting components on the same strip.

The quantitative disparity between these two methods of replenishment was confirmed further by microbiological analysis of the free amino acid pools prepared from cells similarly starved and replenished. The results obtained and the details of the experimental procedures are summarized in table 3. A comparison of column AA of table 3 with the results of table 1 reveals that a 15 minute exposure to 0.5 per cent casein hydrolyzates restores virtually all components of the free amino acid pool to levels equal to or greater than those characterized by the log phase cell. On the other hand, with the exception of glutamic and aspartic acids, replenishment with ammonia for the same period of time fails to return the individual elements to pre-starvation levels. Indeed some of the components (e.g., histidine and methionine) show little if any increase within the 15 minute period of treatment.

The relatively poorer restorative capacity of short exposures to ammonia may reflect the necessity in such replenishment for the synthesis of the other free amino acids from the primary nitrogen acceptors by transamination and other similar reactions.

TABLE 3

A comparison of the effectiveness of ammonia and amino acid mixtures in replenishing the free amino acid pools of depleted cells

Suspensions of 12 hour N starved cells (column S) were exposed to 2 per cent glucose synthetic medium containing either 1 per cent NH_4Cl (column N) or 0.5 per cent enzymatic digest of casein (column AA) for 15 min at 30 C with aeration. The cells then were centrifuged, washed three times with chilled water, and free amino acid pool extracts prepared which then were analyzed microbiologically for their amino acid content.

AMINO ACID	μM/100 MG DRY CELLS		
	S	N	AA
Valine	0.68	1.40	2.36
Glutamic acid	3.1	10.9	15.6
Aspartic acid	0.62	2.70	3.30
Histidine	0.15	0.21	0.43
Lysine	1.78	1.76	7.9
Isoleucine	0.35	0.51	1.26
Proline	0.15	0.26	0.83
Serine	1.9	3.0	6.9
Methionine	0.13	0.14	0.54
Threonine	0.64	0.82	1.20
Arginine	0.36	0.58	2.07

In view of these findings, it was of some interest to compare the enzyme forming capacity of prolonged starved cells in which the pool had been replenished by these two different procedures. If pool levels did indeed represent the determining factor, it would be expected that the starved unreplenished cells would be capable of little or no enzyme synthesis and that the ammonia replenished cells would exhibit a lower capacity to synthesize enzyme than that attainable by those replenished with amino acid mixtures.

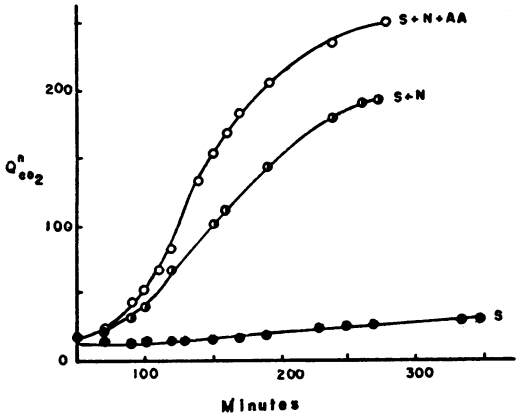

Figure 2. Comparison of NH_4Cl and casein hydrolyzate replenishment of the free amino acid pool on the capacity of cells to synthesize maltozymase.

Twelve hr nitrogen starved cells (curve S) were replenished in one case by 20 minutes' exposure to 1 per cent NH_4Cl and 2 per cent glucose (curve S + N) and in the other by 20 min exposure to the same mixture plus 1 per cent casein hydrolyzate (curve S + N + AA). Anaerobic synthesis of maltozymase in the presence of 3 per cent maltose is followed manometrically. 0.8 mg of glucose as an initiating energy supply is included in each cup. Observations of enzyme formation in this as well as in all other similar experiments are begun at 50 min, when the added glucose has been consumed.

To test these predictions the following type of experiment was done. Cells in the log phase were starved in the usual manner aerobically at 30 C for 12 hours in the presence of 6 per cent glucose. The resulting suspension following centrifugation and washing was divided into three aliquots, one of which was kept as an unreplenished control. To one 10 ml aliquot were added 0.5 ml of 40 per cent glucose and 1 ml of 10 per cent NH_4Cl and to a second the same plus 1 ml of 10 per cent enzymatic digest of casein. After 20 minutes of incubation at 30 C with aeration, the cells in the two suspensions were centrifuged, washed three times with chilled water, and resuspended in inducing medium. The capacities of these cells to synthesize maltase under anaerobic conditions with 3 per cent maltose as inductor are shown in figure 2. The starved cells (curve S) exhibit little capacity to form maltase. Those replenished by the 20 minute exposure to ammonia alone (curve S + N of figure 2) show a dramatic recovery from the starvation procedure. This recovery is, however, clearly superior if a mixture of amino acids is included in the replenishing mixture (S + N + AA). Instances in which the amino acids alone were employed as the replenishing agent gave results similar to those observed in the mixture of amino acids and ammonia.

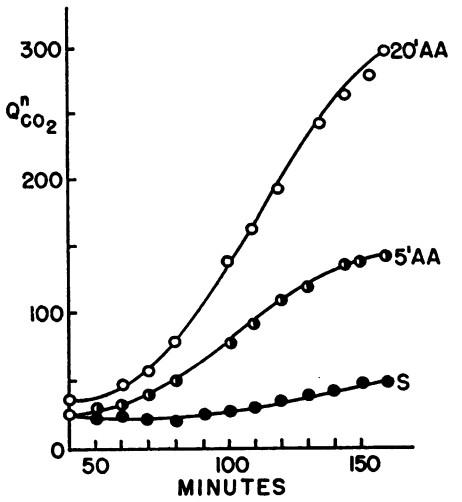

Figure 3. Effect of varying degrees of free amino acid pool replenishment on the capacity to synthesize maltozymase.

Twelve hr nitrogen starved cells (curve S) were exposed to 2 per cent glucose and 0.5 per cent enzymatic digest of casein for 5 min (curve 5'AA) and 20 min (curve 20'AA). Ability to form maltozymase anaerobically in the presence of 3 per cent maltose and 0.4 per cent glucose is described by the corresponding curves.

Experiments were next undertaken to attain different pool levels by varying the time of the exposure to the hydrolyzed casein and

examining the resultant effect on the capacity to synthesize maltozymase. In one series of such experiments, 12 hour nitrogen starved cells, prepared as described above, were exposed to 2 per cent glucose and 0.5 per cent enzymatic digest of casein and then incubated at 30 C with aeration. Samples were removed at 0 time as well as after 5 and 20 minutes. The cells were centrifuged, washed, and resuspended in the nitrogen-free synthetic buffer. The ability of these three samples to synthesize maltase anaerobically is shown in figure 3. From these results and other similar experiments, it appears that 15 minutes' exposure to 0.5 per cent of the amino acid mixture is sufficient to return enzyme synthesizing capacity to its maximal value.

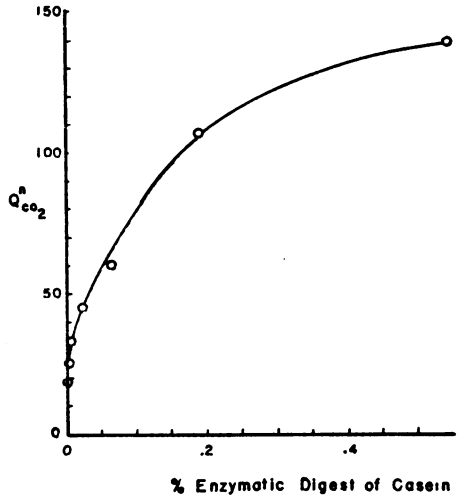

Figure 4. Effect of varying the concentration of amino acids in the replenishing mixture on the capacity to synthesize maltozymase.

Fifteen hr nitrogen starved cells were exposed for 15 min to the indicated concentrations of enzymatic digest of casein. The subsequent ability to synthesize maltozymase anaerobically was examined and the results plotted in terms of the $Q^N_{CO_2}$ value on maltose attained in 180 min.

In parallel replenishment experiments aliquots were removed for analysis of glutamic acid as a quantitative measure of pool replenishment. The results showed that the conditions required for maximal restoration of the free pool content and of enzyme synthesizing capacity are virtually identical. A 15 minute aerobic exposure to the 0.5 per cent amino acid 2 per cent glucose mixture brings the glutamic acid from starvation levels of between 1 to 3 μM per 100 mg to maximal levels which usually are near 16 μM per 100 mg of cells but in isolated instances can reach 20 μM per 100 mg. Longer replenishment exposures do not increase the concentrations of glutamic acid above these levels and shorter treatments fail to achieve them.

Another method of varying the degree of replenishment is to maintain the period of exposure at a constant value and to vary the external concentration of the amino acid mixture supplied. The results of such experiments are summarized in figure 4. In these experiments 15 hr nitrogen starved cells suspended in the usual medium containing 2 per cent glucose were exposed for 15 min at 30 C with aeration to various concentrations of the amino acid mixture. Subsequent to the 15 min replenishing period, the cells were centrifuged, washed, resuspended in nitrogen-free synthetic medium, and their capacity to respond to 3 per cent maltose anaerobically measured.

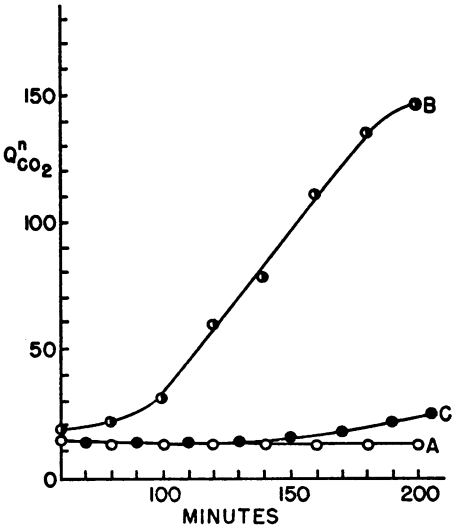

Figure 5. Effect of nitrogen starvation of replenished cells on the capacity to synthesize maltozymase.

Twelve hour nitrogen starved cells (curve A) were replenished by a 15 min exposure to 0.5 per cent casein hydrolyzate and 2 per cent glucose (curve B) and then subjected to a second 60 min starvation (curve C). The capacity to synthesize maltozymase anaerobically is described by the corresponding curves.

The enzymatic activities, in terms of $Q^N_{CO_2}$ on maltase, attained after 180 min of induction are

plotted in figure 4 against concentration of casein digest employed. Here again one finds that the minimal nitrogen requirements of a prolonged nitrogen starved cell for maximal restoration of enzyme synthesizing capacity are provided by 15 min aerobic exposure at 30 C to 0.5 per cent enzymatic digest of casein and 2 per cent glucose.

The results of the present section suggest that any method of limiting the degree of restoration of the free amino acid pool levels decreases correspondingly the restorative capacity of the replenishment procedure on the capacity of the treated cells to synthesize enzyme.

TABLE 4

The effect of starvation, replenishment, and a subsequent starvation on the glutamic acid content of the free amino acid pool

Suspensions of 12 hour N starved cells (A) were exposed to 0.5 per cent casein hydrolyzate and 2 per cent glucose aerobically for 15 minutes (B). The replenished cells were nitrogen starved for 60 minutes at 30 C in nitrogen-free synthetic medium containing 2 per cent glucose (C). The glutamic acid content of the free amino acid pools was analyzed by the decarboxylase method.

CELLS	μM OF GLUTAMIC ACID/100 MG DRY CELLS
A. 12 hr nitrogen starved	1.2
B. Replenished with amino acid mixture	16.2
C. 2nd 60 min nitrogen starvation	2.1

The effect of subsequent starvation on the capacity of nitrogen replenished cells to synthesize enzyme. It was of interest to push the analysis one step further and demonstrate that the restored enzyme synthesizing capacity of the nitrogen replenished cells was indeed a reflection of the increased content of free amino acids which has been shown to occur as a consequence of the replenishment incubation. If this were the case, it would be expected that depletion of the replenished pool by a short starvation should again result in the loss of the newly acquired capacity to synthesize enzyme. To test this, 12 hour nitrogen starved log phase cells were prepared in the usual manner and incubated aerobically for 15 min at 30 C in the synthetic medium containing 0.5 per cent of the amino acid mixture and 2 per cent glucose. Following washing and resuspension in nitrogen-free synthetic medium, a portion of the replenished cells was subjected to another nitrogen starvation for 60 minutes in the usual manner. Figure 5 summarizes the comparison of the resultant enzyme synthesizing capacities. Curve A describes the behavior of the 12 hour nitrogen starved log phase cells with which the experiment was begun. Curve B exhibits the behavior of these same cells subsequent to replenishment of the pool, and curve C, the behavior of the replenished cells after a nitrogen starvation of 60 minutes. It is clear that the nitrogen replenishment resulted as previously in a dramatic restoration of enzyme synthesizing capacity and that this is lost rapidly on an additional starvation. These behaviors clearly are paralleled by the levels of glutamic acid found in the free amino acid pools which are summarized in table 4. Thus, the 12 hour starved cells possessed 1.2 μM of glutamic acid per 100 mg of dry cells. Replenishment brought this level up to 16.2, thus restoring it to levels above those found in freshly harvested log phase cells. The 60 minute starvation period of such replenished cells brought the level down to 2.1 μM.

These results indicate that free amino acid pool levels and enzyme forming capacity invariably parallel each other in a cycle of nitrogen starvation and replenishments. It seems reasonable to conclude then that the effects of the starvation and replenishment procedures on enzyme synthesizing capacity can be referred to the effects of these processes on the availability of the free amino acids for protein synthesis.

DISCUSSION

Internal replenishment of the free amino acid pool. It is important, in interpreting experiments such as those reported here, to recognize that the "free amino acid pool" may not be an exhaustive measure of the available supply of amino acids for the synthesis of new protein molecules. In particular, there exists the possibility that preexisting proteins may be stimulated to break down to their constituent amino acids and thus replenish the pool by an internal device.

That some such mechanism functions is suggested strongly by the comparative behavior of unstarved cells which are replenished and then submitted to a second starvation. It will be noted from table 1 that fresh cells starved for 150 min suffered a decrease of their glutamic acid content

from 10.5 μM to 2.1 μM which was attended only by a 37 per cent decrease in enzyme synthesizing capacity. With these cells, the starvation had to be continued for 600 min to bring the enzyme synthesizing ability down to about 5 per cent of the control values. This situation is to be compared with what is observed (figure 5 and table 4) in the response to a second starvation of prolonged starved cells which have been replenished. Here we see that a 60 min starvation is sufficient to bring the glutamic acid content from 16.2 down to 2.1 μM per 100 mg and at the same time virtually abolish enzyme synthesizing ability.

This striking increase in sensitivity to the starvation procedure of both pool levels and enzyme forming ability in cells which previously have been exposed to an extended starvation and subsequent replenishment can be understood in terms of the effect of protracted nitrogen starvation on an internal replenishment mechanism. Thus, as the starvation process progresses, those proteins which tend to break down easily will do so and so contribute to the free amino acid pool. With prolongation of the starvation procedure the amino acids by such recycling from the proteins through the pool will tend to be trapped in the least labile proteins and in the ones which are therefore least capable of supplying free amino acids to the pool for the formation of new enzymes. One should end up thus with a cell almost completely dependent upon external replenishment of the free amino acid pool. Cells so treated would be expected as a consequence to be extremely sensitive to the effectiveness of the replenishment as well as to any subsequent manipulations which decrease the pool level. More information on this aspect of the problem has been accumulated in the course of examining the effect of induced enzyme synthesis on the pool level and is detailed in a separate publication (Halvorson and Spiegelman, 1952).

Implications for the precursor problem. It was the purpose of the present investigation to ascertain whether variations in the levels of the free amino acid pool would affect the capacity of yeast cells to synthesize enzyme. The data obtained indicate that ability to synthesize new enzyme molecules in terms of either rate or final yield is strongly dependent upon the availability of an adequate supply of free amino acids. These results agree then with the earlier investigation (Halvorson and Spiegelman, 1953) in which the utilizability of the internal free pool was modified by exposure to suitable analogues of the amino acids.

The findings reported in the present paper and the conclusions derivable from them are in complete accord with recent investigations with the β-galactosidase system in *E. coli*. Previous work by Cohn and Torriani (1951, 1952) had established the existence of an enzymatically inactive protein (Pz) present in noninduced cells which was related serologically to the β-galactosidase. The obvious structural relatedness between the two, coupled with the fact that Pz tends to fall in cells induced to synthesize β-galactosidase, suggested that Pz might be the precursor converted into active enzyme. Monod, Pappenheimer, and Cohen-Bazire (1952) undertook to test this possibility by employing a series of mutants of *E. coli* each one of which was deficient for a single amino acid. These mutants then were subjected to a "specific" starvation by being grown up in a medium in which the metabolite required was in limiting quantities, whereas all others were in excess. Immediately upon the cessation of growth which attended the exhaustion of the limiting metabolite an inductor of the β-galactosidase was introduced. It was found that little or no enzyme was synthesized by cells so treated, despite the fact they contain normal amounts of Pz. Such cells, however, did form enzymes immediately upon the addition of the amino acid they require.

In conclusion we may state that the data thus far available with both yeast and bacteria emphasize the importance of amino acids in enzyme synthesis and make unlikely any mechanism which invokes an amino acid independent transformation of a preexistent complex precursor into active enzyme.

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SUMMARY

A study has been made of the effect of variation of the levels of the free amino acid pools on

the capacity of yeast cells to synthesize maltozymase. Pool levels were modified by a series of nitrogen starvations and replenishments of differing degrees of effectiveness. A strong correlation was established between the enzyme synthesizing capacity and the availability of the internal amino acids for protein synthesis. The results support the conclusion that free amino acids constitute the quantitatively predominant source of nitrogen in the formation of new enzyme molecules. No evidence has been uncovered thus far which compels the postulation of an amino acid independent transformation of a preexisting complex protein precursor into active enzyme.

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