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# Nitrogen Repression of the Allantoin Degradative Enzymes in *Saccharomyces cerevisiae*

JUNE BOSSINGER, ROBERT P. LAWTHER, AND TERRANCE G. COOPER

*Department of Biochemistry, Faculty of Arts and Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15261*

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*Saccharomyces cerevisiae* can utilize allantoin as a sole nitrogen source by degrading it to ammonia, "$CO_2$," and glyoxylate. We have previously shown that synthesis of the allantoin degradative enzymes is contingent upon the presence of allophanate, the last intermediate in the pathway. The reported repression of arginase by ammonia prompted us to ascertain whether or not the allantoin degradative system would respond in a similar manner. We observed that the differential rates of allantoinase and allophanate hydrolase synthesis were not decreased appreciably when comparing cultures grown on urea to those grown on urea plus ammonia. These experiments were also performed using the strain and conditions previously reported by Dubois, Grenson, and Wiame. We found allophanate hydrolase production to be twofold repressed by ammonia when that strain was grown on glucose-urea plus ammonia medium. If, however, serine or a number of other readily metabolized amino acids were provided in place of ammonia, production of the allantoin degradative enzymes was quickly (within 20 min) and severely repressed in both strains. We conclude that repression previously attributed to ammonia may result from its metabolism to amino acids and other metabolites.

Growth of *Saccharomyces cerevisiae* using allantoin or urea as sole nitrogen source is predicated upon its possession of five enzymes catalyzing the degradation of allantoin as shown in Figure 1. We have shown that synthesis of at least four of the five pathway enzymes is contingent upon the presence of allophanic acid, the last pathway intermediate (1, 2, 19). More recently, data have been obtained indicating that response of allophanate hydrolase to addition and removal of inducer is strikingly rapid (6). Many of these experiments, however, were performed in excess ammonia (0.1%) and as such must be reconciled with numerous reports indicating that allantoin and arginine degradation is sensitive to "ammonia repression." Van de Poll and his collaborators (12, 13, 16, 18) reported an extensive series of experiments characterizing the abilities of various carbon and nitrogen sources to repress and derepress allantoinase in *S. carlsbergenesis*. These data, however, are complicated and compromised by the fact that varying concentrations of Casamino Acids were added to the growth medium as a stimulatory agent for allantoinase. In view of the demonstration that allophanate, an eventual degradation product of arginine, is the inducer of allantoinase in *S. cerevisiae*, it is likely that most of their experiments monitored some combination of induction and repression of allantoinase synthesis. These authors did, however, make the interesting observation that the specific activity of allantoinase was decreased when cultures were grown in the presence of certain amino acids.

Repression of arginase synthesis has been extensively examined by both Middlehoven (7, 8) and Wiame (3, 20). The latter authors (3) report that cells growing in the presence of ammonia plus arginine or urea exhibit an 8- and 13-fold decrease in the specific activities of arginase and urea amidolyase, respectively, as compared to cells grown on arginine or urea alone.

In view of these results, we initiated a thorough study to define the physiological conditions necessary for repression of the allantoin degradative pathway. Our studies were greatly aided by the availability of oxaluric acid, a non-metabolizable inducer of the allantoin system (11). Use of this gratuitous inducer permitted us to divorce induction of the system from its metabolism and hence to monitor the effects of single nitrogen sources upon the differential

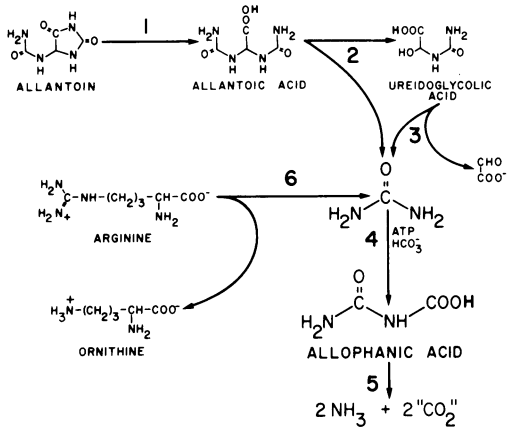


FIG. 1. *The reactions involved in the degradation of allantoin and arginine. The enzymes which catalyze these reactions are: allantoinase, allantoicase, ureidoglycolate hydrolase, urea carboxylase, allophanate hydrolase, and arginase; they are indicated in the figure by the numbers 1 to 6, respectively.*

rates of enzyme synthesis. Data yielded from these studies demonstrate that our wild-type strain, M-25, is somewhat more resistant to ammonia repression than the one used by Dubois et al. (3). However, we also demonstrate that a significant degree of the repression they reported may have been the result of: (i) addition of citrate to their growth medium and (ii) the expression of their data as specific activities instead of differential rates of synthesis. The major result of this report is the finding that several readily metabolized amino acids repress the rate of allantoinase synthesis by up to 100-fold and that the onset of this repression must occur in a matter of minutes after the repressing agent is added.

## MATERIALS AND METHODS

**Strains and culture conditions.** Strain M-25 is a prototrophic diploid prepared by mating the haploid strains S-162 (*α, lys 1, his 6, ura 1*) and S-185 (*α, ade 6, leu 1*), and ϵ1278b is a prototrophic haploid of the *α* mating type. The culture medium and assay procedures employed here were identical to those reported by Cooper and Lawther (2). Wiame's medium was employed in the experiment depicted in Fig. 2 and was prepared as described earlier (5). Cell density measurements were made turbidimetrically as described by Sumrada and Cooper (11).

**Isolation and identification of the soluble amino acids.** Procedures for determination of amino acid pools are similar to those used by Moat et al. (9). Strain M-25 was grown in medium containing 0.6% glucose and 0.1% of the nitrogen source except in the following cases: (i) lactate medium contained 2.0% lactate and ergosterol (6 mg dissolved in 16.7 ml of Tween 80 and 3.3 ml of ethanol per liter); (ii) urea was used at a final concentration of $10^{-2}$ M; (iii) nitrogen-starved cells were grown on 0.6% glucose and 0.1% ammonia for 24 h, filtered, washed with water and nitrogen-free medium, and then resuspended in prewarmed, preaerated nitrogen-free medium for 12 h; and (iv) Wiame's medium (5) contained 2.0% glucose as a carbon source.

At a cell density of 60 Klett units, cycloheximide was added (1 mg/100 ml of culture), and the cells were chilled on ice. The chilled cells were harvested by centrifugation and washed three times with cold water. The final pellet was resuspended in 8 ml of 70% methanol and the solution was heated to 70 C for 1 h. The precipitate was removed by centrifugation, and the resulting supernatant was evaporated to dryness. The resulting residue was suspended in water, deproteinized by membrane filtration and subjected to amino acid analysis of the basic amino acid components using a 5.5-cm column of Beckman type PA-35 resin.

## RESULTS

**Ammonia repression of the allantoin degradative enzymes.** Dubois et al. (3) recently reported that allantoinase and urea amidolyase (urea carboxylase and allophanate hydrolase acting in concert) are strongly repressed by ammonia. Since many of our experiments concerning induction of the allantoin degradative system (1, 2, 19) were performed in the presence of excess ammonia (0.1%), it was necessary to determine whether or not our strains were also

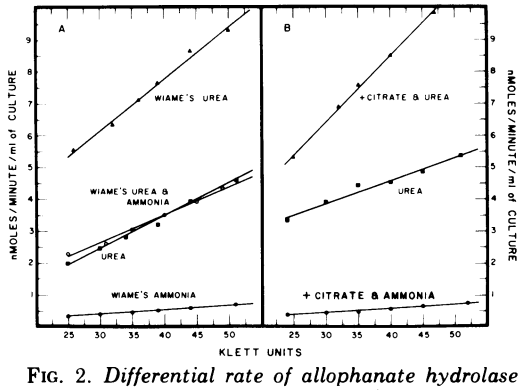


FIG. 2. *Differential rate of allophanate hydrolase synthesis in a wild-type strain of Saccharomyces cerevisiae (M-25) growing in various media. Media compositions are those used by Cooper and Lawther (2) or Wiame (5). (A) The media and nitrogen sources were: our minimal medium + $10^{-2}$ M urea (■), LaCroute minimal medium + $10^{-2}$ M urea (▲), LaCroute minimal medium + $10^{-2}$ M urea + 0.1% ammonia (○), and LaCroute minimal medium + 0.1% ammonia (●). (B) The media and nitrogen sources were: our minimal medium + 1.0% citrate + $10^{-2}$ M urea (▲), our minimal medium + $10^{-2}$ M urea (■), and our minimal medium + 1.0% citrate + 0.1% ammonia (●).*

subject to "ammonia repression." Differential rates of allantoinase and allophanate hydrolase synthesis were, therefore, measured in cells grown on medium containing either allantoin, urea, or arginine alone as sole nitrogen source, or in combination with ammonia. As shown in Table 1 the differential rates of allantoinase and allophanate hydrolase synthesis did not decrease at all when ammonia was added to urea-containing medium, and decreased only 30 to 60% when ammonia was added to either allantoin or arginine-containing medium. There are conceivably two reasons for the striking difference between the results reported here and those obtained by Dubois et al. (3)—an inherent difference in the sensitivities of the two different strains being compared, or differences in the medium upon which they were grown. The contribution made by each of these possibilities was determined by comparing the differential rates of allophanate hydrolase synthesis observed for the two strains grown in the two types of media. Our wild-type strain, M-25, was grown on our standard minimal medium using urea as sole nitrogen source and on the medium reported by Dubois using ammonia, ammonia plus urea, or urea as sole nitrogen source. As shown in Fig. 2A much higher amounts of enzyme were observed in cells grown on the latter medium. To interpret the data from this type of experiment appropriately, the terms specific activity and differential rate of synthesis must be clearly distinguished. The specific activity of an enzyme may be defined as the units of enzyme activity observed per milligram of solubilized protein. Differential rate of synthesis, on the other hand, may be defined as the fraction of total protein synthesis devoted to production of a given enzyme and is usually reported as the amount of enzyme activity produced during a given amount of balanced growth or a given amount of amino acid incorporation into hot trichloroacetic acid-precipitable material. In Fig. 2A the slopes of the lines are the differential rates of allophanate hydrolase synthesis while the amount of activity at any of the points divided by the amount of protein in that sample is the specific activity. The point to be emphasized is that the specific activity of an enzyme is not always an accurate reflection of its differential rate of synthesis. This is exemplified by considering the amounts of allophanate hydrolase observed in cells grown on the medium used by Wiame's laboratory and our medium with urea as sole nitrogen source (Fig. 2). The ratio of differential rates of allophanate hydrolase synthesis observed in these media is 1.61. However, the ratio of the specific activities of this enzyme at 25 Klett units is 2.85. Growth of cells in the medium of Dubois et al. clearly results in a much higher amount of enzyme per cell, but not in a greatly increased differential rate of synthesis. Inspection of the components which made up these two media reveals that the major difference in them was the inclusion of a large quantity of citrate in the medium used by Dubois et al. To ascertain whether or not it was citrate that was responsible for the differences we observed, the experiment described above was repeated in our medium in the presence and absence of citrate. As shown in Figure 2B citrate clearly accounts for the observed differences. The means by which citrate elicits this response, however, remains obscure. Differences in sensitivity to "ammonia repression" were determined by growing the haploid wild-type strain, $\epsilon$1278b, of Dubois et al. and our strain M-25 on medium containing ammonia, urea, or ammonia plus urea as nitrogen source. The differential rate of allophanate hydrolase synthesis is decreased by twofold in strain $\epsilon$1278b when ammonia is included along with urea as the nitrogen source, whereas no appreciable decrease is observed with strain M-25 (Table 2). A second difference between the strains is the uninduced rate of synthesis, which is higher in strain M25-12b than in $\epsilon$1278b.

TABLE 1. *Sensitivity of strain M-25 to ammonia repression*[a]

| Nitrogen source | Increase in enzyme activity[b] per 10 Klett units of growth | |
|---|---|---|
| | Allantoinase | Allophanate hydrolase |
| Allantoin | 0.94 | 1.58 |
| Allantoin + ammonia | 0.63 | 0.86 |
| Urea | 0.69 | 1.63 |
| Urea + ammonia | 0.69 | 1.82 |
| Arginine | 0.50 | 0.96 |
| Arginine + ammonia | 0.19 | 0.53 |
| Ammonia | 0.06 | 0.05 |

[a] The final concentration of all the nitrogen sources was 0.1% with the exception of urea which was $10^{-2}$ M. The enzyme activities were assayed as described previously beginning at a cell density of 25 Klett units and taking samples at 5 Klett unit intervals for one generation.

[b] Activities are expressed in nanomoles of product produced per minute per milliliter of culture. The values are the slopes of curves similar to those shown in Fig. 2, 3, and 4.

**Repression of the allantoin degradative enzymes by amino acids.** Since *S. cerevisiae* is capable of metabolizing a variety of amino

TABLE 2. *Sensitivity of strains M-25, M25-12b, and ε1278b to ammonia repression*[a]

| Nitrogen source | Strain | Enzyme level at 25 Klett units | Increase in enzyme activity[b] per 10 Klett units of growth |
|---|---|---|---|
| Urea | ε1278b | 1.29 | 0.79 |
| Urea + ammonia | ε1278b | 0.38 | 0.34 |
| Ammonia | ε1278b | 0.14 | 0.01 |
| Urea | M25-12b | 2.44 | 1.15 |
| Urea | M-25 | 4.03 | 1.35 |
| Urea + ammonia | M-25 | 3.03 | 1.40 |
| Ammonia | M-25 | 0.23 | 0.10 |

[a] Allophanate hydrolase was measured at 5 Klett unit intervals as described previously. The nitrogen sources were in final concentrations of 0.1% ammonia and $10^{-2}$ M urea.

[b] Activities are expressed as nanomoles of product produced per minute per milliliter of culture. The values are the slopes of curves similar to those shown in Fig. 2, 3, and 4.

acids, it is reasonable to ask whether or not any of these compounds will bring about repression of the allantoin degradative system. To determine the extent of repression elicited by each amino acid, two cultures were prepared; one growing on ammonia and the other on the amino acid as sole nitrogen source. At a cell density of 25 Klett units, oxaluric acid, a non-metabolizable inducer of the allantoin degradative system (11) was added and the culture was sampled at 5 Klett unit intervals of growth. Allantoinase and allophanate hydrolase synthesis are totally repressed by asparagine (Fig. 3). The asparagine-grown cells contained barely measureable amounts of activity which could not be increased by addition of inducer. However, a wide range of repression is observed if several amino acids are surveyed (Tables 3 and 4). Included in these tables are the amounts of activity found at the time of inducer addition and the differential rate of enzyme synthesis in its presence and absence. Note that the uninduced level of these enzymes is also greatly influenced by the degree of repression exerted upon the cells.

To demonstrate that an amino acid must be able to serve as a nitrogen source if it is to bring about repression, the effects of D- and L-asparagine upon allophanate hydrolase synthesis were followed. D-Asparagine, which alone will not support cell growth, has little effect upon the rate of allophanate hydrolase synthesis, whereas the L isomer of the same amino acid totally repressed synthesis (Fig. 4). The growth rates of the three cultures used in this experiment were 120, 125, and 120 min for ammonia, ammonia plus D-asparagine, and ammonia plus L-asparagine, respectively. It is possible that the decreased rate of enzyme production in the presence of D-asparagine is the result of a general inhibition of protein synthesis, because the growth rate of that culture was slightly depressed. The data in Fig. 5 demonstrate that the extent of repression is related to the amount of amino acid present, albeit in a complex manner. Van de Poll et al. (17) have reported that glucose concentration exerts a profound effect upon the repression of allantoinase in *S. carlsbergenesis*. Middlehoven, on the other hand, did not observe an effect of carbon source on the specific activity of arginase. To ascertain whether or not similar effects could be observed in strain M-25, cultures were grown on lactate or glucose under conditions of increasing repression. The degree of repression observed in the presence of glucose is quite similar to that observed in the presence of lactate (Table 5). Cells grew much more slowly on ammonia-lactate medium than on ammonia-glucose medium (doubling times of 210 min and 120 min, respec-

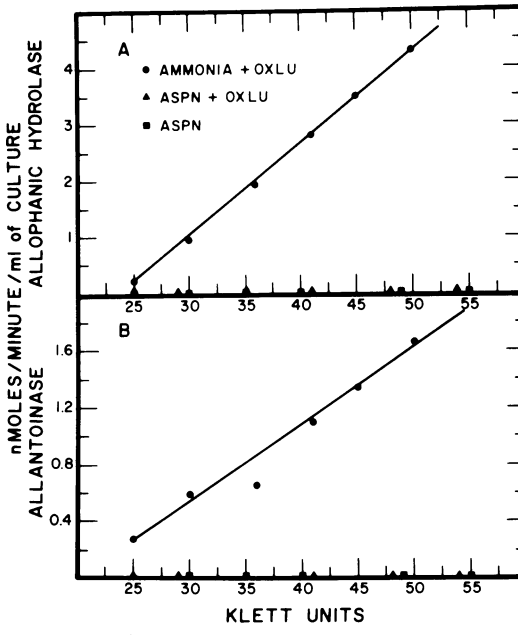


FIG. 3. *Nitrogen repression of (A) allophanate hydrolase and (B) allantoinase synthesis in a wild-type strain of Saccharomyces cerevisiae (M-25). The culture and enzyme assay procedures were similar to those described earlier (2). Sources of nitrogen in both (A) and (B) were 0.1% ammonia (●) and 0.1% asparagine (■, ▲). Oxaluric acid, a non-metabolizable inducer of the allantoin degradative system was added (0.25 mM final concentration) at a cell density of 25 Klett units to the ammonia culture (●) and one of the asparagine cultures (▲).*

TABLE 3. *Repression of allantoinase synthesis by various amino acids*[a]

| Nitrogen source | Doubling time[b] (min) | Enzyme level at 25 Klett units | Increase in enzyme activity[c] per 10 Klett units of growth | |
|---|---|---|---|---|
| | | | Minus OXLU | Plus OXLU |
| Leucine | 184 | 0.25 | 0.18 | 0.61 |
| Ornithine | 139 | 0.41 | 0.09 | 0.41 |
| Ammonia | 115 | 0.20 | 0.08 | 0.39 |
| Arginine | 130 | 1.25 | 0.25 | 0.38 |
| Citrulline | 282 | 1.04 | 0.23 | 0.33 |
| Proline | 280 | 0.28 | 0.07 | 0.31 |
| Glutamate | 175 | 0.23 | 0.00 | 0.29 |
| Aspartate | 141 | 0.33 | 0.14 | 0.28 |
| Alanine | 119 | 0.09 | 0.13 | 0.18 |
| Serine | 120 | 0.04 | 0.00 | 0.01 |
| Glutamine | 110 | 0.05 | 0.00 | 0.00 |
| Asparagine | 124 | 0.00 | 0.00 | 0.00 |

[a] Allantoinase was assayed as described previously (2). For each amino acid, two cultures with 0.1% of the amino acid as a nitrogen source and one culture with 0.1% ammonia as a nitrogen source were grown to a cell density of 25 Klett units. Oxaluric acid (OXLU) (0.25 mM final concentration) was added to the ammonia culture, and one of the amino acid cultures and samples of all three cultures were taken at 5 Klett unit intervals for one generation.

[b] Normalized to ammonia at 115 min.

[c] Activities are expressed as nanomoles of product produced per minute per milliliter of culture. The values are the slopes of curves similar to those shown in Fig. 2, 3, and 4.

TABLE 4. *Repression of allophanate hydrolase synthesis by various amino acids*[a]

| Nitrogen source | Doubling time[b] (min) | Enzyme level at 25 Klett units | Increase in enzyme activity[c] per 10 Klett units of growth | |
|---|---|---|---|---|
| | | | Minus OXLU | Plus OXLU |
| Leucine | 184 | 0.96 | 0.38 | 3.02 |
| Proline | 280 | 0.19 | 0.26 | 2.20 |
| Ornithine | 139 | 0.62 | 0.22 | 1.71 |
| Arginine | 130 | 2.73 | 0.77 | 1.65 |
| Citrulline | 282 | 1.17 | 0.38 | 1.58 |
| Ammonia | 115 | 0.28 | 0.13 | 1.46 |
| Glutamate | 175 | 1.57 | 0.12 | 1.13 |
| Aspartate | 141 | 1.10 | 0.16 | 0.73 |
| Alanine | 119 | 0.46 | 0.15 | 0.53 |
| Glutamine | 110 | 0.02 | 0.00 | 0.25 |
| Serine | 120 | 0.06 | 0.00 | 0.07 |
| Asparagine | 124 | 0.02 | 0.00 | 0.01 |

[a] The allophanate hydrolase assay and experimental procedures were explained in Materials and Methods and Table 3, respectively.

[b] Normalized to ammonia at 115 min.

[c] Activities are expressed as nanomoles of product produced per minute per milliliter of culture. The values are the slopes of curves similar to those shown in Fig. 2, 3, and 4.

tively). This may correlate with the finding that there is about a twofold greater differential rate of synthesis in cells grown on glucose-urea medium compared to lactate-urea medium.

If the extent of repression depends upon the amount of transaminatable nitrogen within the cell, growth of cultures on two nitrogen sources, entering by separate permeases, would be expected to produce a synergistic heightening of

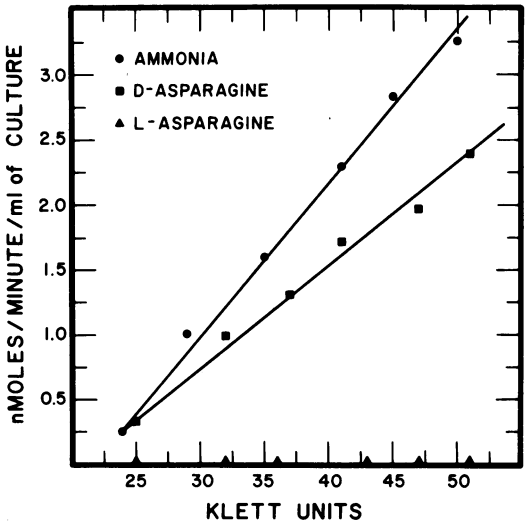


FIG. 4. *Effect of* D- *and* L-*asparagine on the differential rate of allophanate hydrolase synthesis in a wild-type strain of Saccharomyces cerevisiae. Oxaluric acid was added (0.25 mM final concentration) to all three cultures at a cell density of 25 Klett units. The nitrogen sources were 0.1% ammonia (●), 0.05% ammonia + 0.05% D-asparagine (■), and 0.05% ammonia + 0.05% L-asparagine (▲).*

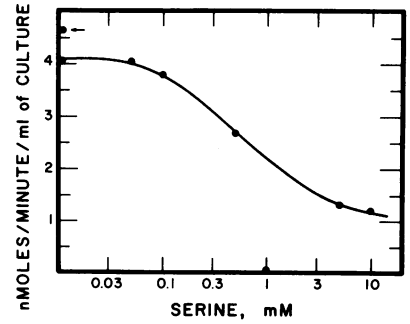


FIG. 5. *Differential rate of allophanate hydrolase synthesis in a wild-type strain of Saccharomyces cerevisiae (M-25) as a function of the serine concentration in the medium. At a cell density of 15 Klett units, serine was added to each culture producing the final concentrations indicated. The point indicated by an arrow had no serine added. Urea was added ($10^{-2}$ M final concentration) to all of the cultures at a cell density of 30 Klett units, and the increase in enzyme activity in one subsequent generation (30 to 60 Klett units) was measured.*

TABLE 5. *Effect of carbon source on differential rate of allophanate hydrolase synthesis in S. cerevisiae*[a]

| Nitrogen source | Enzyme level at 25 Klett units | Increase in enzyme activity per 10 Klett units of growth |
|---|---|---|
| Lactate-urea | 2.49 | 0.67 |
| Lactate-urea-ammonia | 1.44 | 0.47 |
| Lactate-ammonia | 0.62 | 0.19 |
| Glucose-urea | 4.13 | 1.35 |
| Glucose-ammonia-urea | 3.05 | 1.65 |
| Glucose-ammonia | 0.23 | 0.08 |

[a] Allophanate hydrolase was measured at 5 Klett unit intervals beginning at a cell density of 25 Klett units as described in previous experiments. The nitrogen sources in the medium were at the following final concentrations: 0.1% ammonia; $10^{-2}$ M urea + 0.1% ammonia; and $10^{-2}$ M urea. The final concentrations of the carbon sources were 0.6% glucose or 2% sodium lactate. Ergosterol (6.0 mg ergosterol dissolved in 16.7 ml of Tween 80 and 3.3 ml of ethanol per liter of media) was added to the lactate containing medium to enable growth.

repression. The differential rate of allophanate hydrolase synthesis in cells growing on ammonia plus alanine is much lower than would be expected from the rates observed in cells grown on either alanine or ammonia alone (Fig. 6). To ascertain whether or not these synergistic effects were the result of expanding the intracellular pool of ammonia, the quantities of basic amino acids in cultures of strain M-25, grown on various nitrogen sources, were determined. No correlation can be drawn between the size of the intracellular pool of ammonia and the degree of repression exerted by a given compound (Table 6). The basic amino acids arginine, lysine, and ornithine vary greatly depending upon the nitrogen source provided, whereas ammonia varies only slightly and in an unpredictable manner, i.e., there is more ammonia in cells grown on allantoin and urea than there is in cells grown on serine and asparagine.

**Time course of nitrogen repression.** Studies directed toward elucidation of the mechanism underlying repression of the allantoin degradative system must begin with an analysis of the time course of its onset. To this end a culture was grown to a cell density of 15 Klett units. At that time the culture was divided into three equal portions, two of which received urea. The uninduced culture and one of the induced cultures were sampled at 5 Klett unit intervals for a period of two generations. The third culture was sampled in like manner, but glutamine was added at a cell density of 25 Klett units. The full effects of repression were exerted before the next point was taken, both in the case of allantoinase and allophanate hydrolase (Fig. 7). Note that allophanate hydrolase continues to increase at a slow rate, whereas the increase in allantoinase is halted immediately. It is not clear at the moment whether this difference is real or an artifact resulting from the limited resolution of our current allantoinase assay.

**Repression of arginase by amino acids.** Dubois et al. (3) reported that arginase is sensitive to ammonia repression. To see whether or not arginase was following the same general response to ammonia and amino acids as the allantoin degradative system, arginase was measured in cells grown under a variety of physiological conditions. The differential rate of arginase synthesis is decreased only moderately by addition of ammonia to minimal glucose-arginine medium in strain M-25 (Table 7). In a parallel experiment, however, it can be shown that addition of serine brings about a severe decrease in the differential rate of arginase

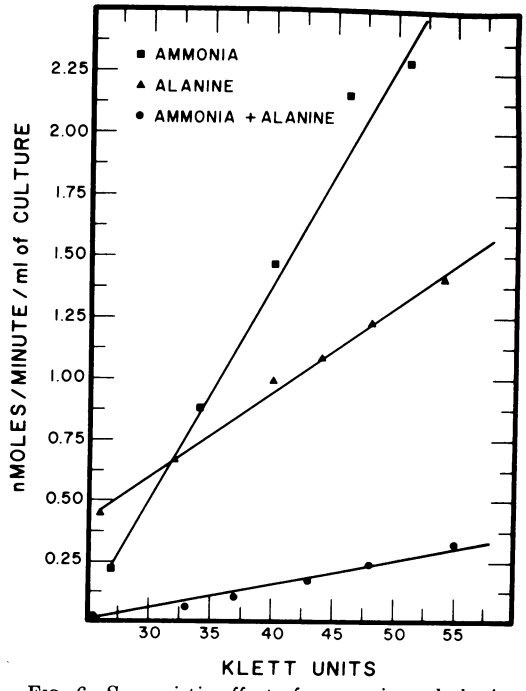


FIG. 6. *Synergistic effect of ammonia and alanine on the differential rate of allophanate hydrolase synthesis in a wild-type strain of Saccharomyces cerevisiae (M-25). The culture and assay conditions were as described previously (2). Oxaluric acid was added to all three cultures (0.25 mM final concentration) at a cell density of 25 Klett units. The nitrogen sources were 0.1% ammonia (■), 0.1% alanine (▲), 0.05% ammonia + 0.05% alanine (●).*

TABLE 6. *Intracellular pools of basic amino acids in cultures of strain M-25 grown on various nitrogen sources*[a]

| Nitrogen source | Amino acid (nmol/mg of dried cells) | | | |
|---|---|---|---|---|
| | Lysine | Histidine | Ammonia[b] | Arginine |
| Yep-D | 66.8 | 14.9 | 6.7 | 32.0 |
| Asparagine | 30.5 | 4.1 | 8.9 | 50.2 |
| Glutamine | 58.8 | 6.2 | 6.5 | 31.0 |
| Aspartate + ammonia | 81.3 | 5.6 | 6.2 | 74.8 |
| Serine + ammonia | 36.6 | 3.9 | 6.8 | 18.1 |
| Serine | 33.1 | 3.1 | 7.3 | 20.6 |
| Aspartate | 77.4 | 5.8 | 18.6 | 62.4 |
| Alanine | 89.2 | 3.6 | 10.2 | 20.3 |
| Ammonia | 34.6 | 4.7 | 7.4 | 55.1 |
| Urea | 57.8 | 5.6 | 16.8 | 32.2 |
| Allantoin | 41.9 | 3.8 | 16.6 | 84.3 |
| Proline | 60.6 | 4.3 | 6.9 | 21.5 |
| Ornithine | —[c] | 2.9 | 12.5 | 13.3 |
| Lactate[c] + ammonia | 33.4 | 5.7 | 9.2 | 19.5 |
| Nitrogen starved cells[d] | 5.4 | 0.6 | 4.7 | 2.6 |
| Proline in Wiame's medium[e] | 25.6 | 5.0 | 6.4 | 18.8 |
| Ammonia in Wiame's medium[f] | 14.1 | 6.2 | 7.6 | 60.8 |

[a] Samples were prepared as described in Materials and Methods. All values were normalized to a cell density of 60 Klett units.

[b] The ammonia value observed for a buffer blank was 1.97. This value, however, has not been subtracted from any of the figures listed.

[c] This value could not be determined accurately, because ornithine cochromatographs with lysine. This peak was off scale due to ornithine pool expansion.

[d] Lactate was used in place of glucose as carbon source.

[e] Medium was deficient in nitrogen only.

[f] This is the medium reported by Dubois et al. (3).

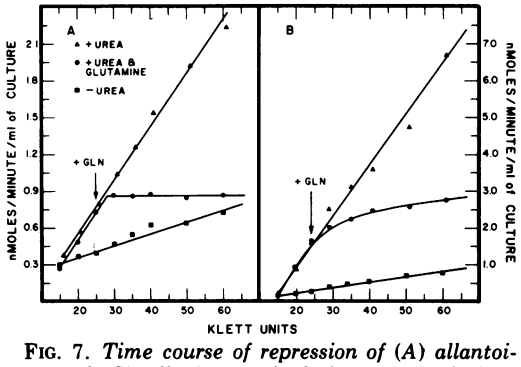


FIG. 7. *Time course of repression of (A) allantoinase and (B) allophanate hydrolase synthesis in a wild-type strain, (M-25). The culture was grown on minimal glucose ammonia (0.1%) medium. At a cell density of 15 Klett units, urea ($10^{-2}$ M final concentration) was added to two of the cultures (▲) and (●). Glutamine (0.1% final concentration) was then added to one of these cultures (●) at a cell density of 25 Klett units as indicated by the arrow. No additions were made to the third culture (■).*

production. It may be significant that regulation of arginine degradation is subject to the same influences exerted upon the allantoin degradative pathway. In our earlier work (2) it was suggested that induction of arginase and its subsequent action upon arginine could bring about induction of the allantoin degradative system. The data shown in Fig. 8 measure the extent to which this may be accomplished. It is clear that, when cells are growing on arginine as sole nitrogen source, the allantoin degradative enzymes are not fully induced. This is shown by

TABLE 7. *Sensitivity of arginase synthesis to repression by ammonia and serine*[a]

| Nitrogen source | Arginase activity[b] |
|---|---|
| Arginine | 3.90 |
| Arginine + ammonia | 2.60 |
| Arginine + serine | 0.65 |
| Ammonia | 0.35 |

[a] Arginase was assayed radiochemically as described by Whitney and Magasanik (19). Samples were taken at a cell density of 25 Klett units and at 5 Klett unit intervals thereafter. The nitrogen sources and various combinations were all at a final concentration of 0.1%.

[b] Increase in enzyme activity per 10 Klett units of growth. Activities are expressed in nanomoles of product produced per minute per milliliter of culture. The values are the slopes of curves similar to those shown in Fig. 2, 3, and 4.

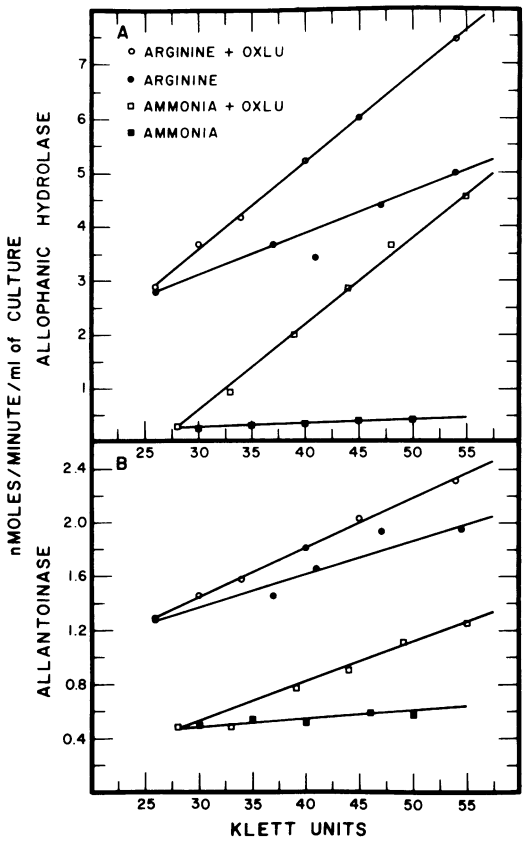


FIG. 8. *Differential rate of (A) allophanate hydrolase and (B) allantoinase synthesis in a wild-type strain of Saccharomyces cerevisiae (M-25) growing on arginine or ammonia as sole nitrogen source. The culture and assay conditions are similar to those used by Cooper and Lawther (2). The nitrogen sources in both (A) and (B) were 0.1% arginine (●) and (○) and 0.1% ammonia (■) and (□). At a cell density of 25 Klett units, oxaluric acid, a nonmetabolizable inducer of the allantoin degradative system was added (0.25 mM final concentration) to one arginine (○) and one ammonia (□) culture.*

the significant increase in the differential rates of allantoinase and allophanate hydrolase synthesis observed if oxaluric acid is added to the culture. Operation of the allantoin degradative system at less than maximum capacity heightens its responsiveness to repression, because the rate of enzyme production is decreased both by repression of the allantoin system itself and by a decreased supply of inducer which results from repression of arginase.

## DISCUSSION

We have demonstrated that sensitivity to ammonia repression varies somewhat from strain to strain. However, the extent of repression exerted by ammonia is minor when compared to that seen with various readily utilized amino acids. The experiments presented argue that the extent of repression cannot be correlated with the ammonia content of a culture. This suggests that repression attributable to ammonia is likely the result of its being metabolized to transaminatable amino acids within the cell. This is in close agreement with a preliminary report from Van de Poll (14, 15) indicating that a strain of *S. carlsbergenesis* lacking nicotinamide adenine dinucleotide phosphate (NADP)-specific glutamic dehydrogenase was not subject to ammonia repression, but was strongly repressed by asparagin. In view of these facts it is reasonable to suggest that ammonia per se is not the molecule that brings about repression of various degradative systems and may, therefore, be more appropriate to term the phenomenon nitrogen repression. Roon and Even (10) reported that asparagine and glutamine addition to cultures growing on ammonia decreased the specific activity of NADP-specific glutamic dehydrogenase. These observations may also be related to the phenomenon of nitrogen repression.

Although this investigation has defined the physiological conditions of repression, we are merely at the threshold of studies directed toward elucidation of its mechanism. A step toward this goal was made with the observation that the onset of repression occurs very soon after the addition of an appropriate amino acid. Such a swift response may be particularly meaningful in view of the experiments reported by Lawther and Cooper (6). They demonstrated that 3 min elapse between addition of inducer to a culture and the appearance of active allophanate hydrolase. Also reported was the observation that removal of inducer resulted in a rapid loss (half life of 3.25 min) of the capacity to produce hydrolase. It may be significant that addition of glutamine to a culture (Fig. 8) results in nearly complete loss of the capacity to produce enzyme within approximately 20 min. This is consistent with a synthetic capacity half-life in the vicinity of 3 min.

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