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Amino Sugar Sensitivity in *Escherichia coli* Mutants Unable to Grow on *N*-Acetylglucosamine[1]

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Studies were conducted on two mutants of *Escherichia coli* that lack either glucosamine-6-phosphate deaminase or *N*-acetylglucosamine-6-phosphate deacetylase and which accumulate glucosamine-6-phosphate or *N*-acetylglucosamine-6-phosphate, respectively, when grown in the presence of *N*-acetylglucosamine. The addition of 10^{-4} to 10^{-5} M *N*-acetylglucosamine to these mutant strains caused a rapid and complete inhibition of growth on substrates that enter the catabolic pathways at or below the level of fructose-6-phosphate. Growth on glucose was inhibited to a lesser degree, whereas only minor inhibition occurred when the pentoses were used as substrates. Growth on gluconate was found to be totally unaffected by these levels of *N*-acetylglucosamine. The objective of this investigation was to determine the nature of this "amino sugar sensitivity" phenomenon and the conditions under which it could be overcome. It was found that this amino sugar sensitivity was abolished when an exogenous source of pentose such as uridine was included in the culture medium. Experiments are described indicating that the accumulated amino sugar phosphate metabolites interfere with an early step in hexose metabolism of both mutants, resulting in a pentose deficiency and consequent inhibition of growth on certain substrates.

White (10) and White and Pasternak (11) isolated mutants of *Escherichia coli* K-12 that were unable to grow on *N*-acetylglucosamine (AcGN) as an energy source. Two classes of mutants were obtained: one type lacked the enzyme glucosamine-6-phosphate (GN6P) deaminase (2-amino-2-deoxy-D-glucose-6-phosphate ketol-isomerase deaminating, EC 5.3.1.10), whereas the other type lacked the enzyme *N*-acetyl-glucosamine-6-phosphate deacetylase [proposed designation, 2-acetamido-2-deoxy-0-glucose-6-phosphate amido-hydrolyase, EC 3.5.1 (11)].

These mutants have been used to study the regulation of amino sugar metabolism in *E. coli* (10), and to determine the role played by amino sugar compounds in catabolite repression of the *lac* operon in this organism (1). A variety of other studies could be undertaken in which the unique features of these mutants could be put to good advantage. At this time, however, the use of these mutants for such studies is hampered by the fact that bacteriostasis or bacterolysis often occurs when an exogenous supply of AcGN is included in the culture medium. Inhibition of growth is the result of accumulation of GN6P or acetylglucosamine-6-phosphate (AcGN6P) in the deaminaseless and deacetylaseless cells, respectively (10). This inhibition which can be termed "amino sugar sensitivity" appears to be analogous to the well-known "sugar sensitivity" observed when galactose-negative mutants are exposed to galactose (4, 13) or when rhamnose is added to cells unable to metabolize this substrate completely (3). The purpose of this report is to describe some of the metabolic events that appear to be responsible for this "amino sugar sensitivity," and the conditions under which these mutant cells can be rendered immune to this sensitivity.

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MATERIALS AND METHODS

Chemicals. 2,5-Diphenyloxazole (PPO) and 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (dimethyl POPOP) were products of Packard Instrument Co., Inc., Downers Grove, Ill. The following radiochemicals were purchased from the New England Nuclear Corp., Boston, Mass.: glucose-*1*-^{14}C, glucose-*3,4*-^{14}C, glucose-*6*-^{14}C, gluconate-*1*-^{14}C, gluconate-*6*-^{14}C, pyruvate-*1*-^{14}C, succinate-*1,4*-^{14}C, and ^{14}C-leucine (U). Glucosamine (GN) and AcGN were purchased from Mann Research Laboratories, New York, N.Y. All other chemicals were of reagent grade and are readily available.

Cultures and cultural conditions. The mutant strains used in this study were derived from the parental *E.*

coli strain K-12-700 and were made available through the courtesy of R. J. White (University of Oxford, Oxford, England). A complete description of the cultures including the methods used for isolation of the mutants is described elsewhere (1, 10). *E. coli* strain K-12-1-1 lacks the enzyme GN6P deaminase. *E. coli* strain K-12-2-1 lacks the enzyme AcGN6P deacetylase. These mutants will hereafter be referred to as the deaminaseless and deacetylaseless strains, respectively.

All cultures were grown aerobically by rapid reciprocal shaking at 37 C in a mineral salts medium with or without 0.25% casein hydrolysate (acid-hydrolyzed, vitamin-free; Nutritional Biochemicals Corp., Cleveland, Ohio) and substrates as indicated. The medium employed and the conditions used for culture growth in this work were previously described (6).

Growth measurements. Growth was determined by following the change in absorbance of the cultures at 420 nm with a Spectronic 20 colorimeter (Bausch & Lomb, Inc., Rochester, N.Y.). Bacterial mass was expressed in micrograms (dry weight) per milliliter of culture by using a previously prepared standard curve relating absorbancy to cell dry weight. Values for relative growth are recorded in some experiments. This is the mass increase that occurred during a given time period (usually 120 min) of exponential growth relative to the initial cell concentration (which was taken to be 1.0). In other experiments growth was estimated by the rate of assimilation of ^{14}C-labeled L-leucine into the 5% trichloroacetic acid-insoluble cell fraction.

Radiorespirometric procedures: $^{14}CO_2$ measurements. To determine the effect of AcGN on the rate of substrate dissimilation, the radiorespirometric procedure described by Wang et al. (9) as modified by Okinaka and Dobrogosz (6) was employed. Nonproliferating cultures were used in these experiments. Cultures grown overnight on appropriate substrates were harvested by centrifugation at 4,500 × *g* and washed twice with cold 0.05 M sodium phosphate buffer (*p*H 7.5). The washed cells were diluted with the same buffer to a standard absorbancy of 1.0 at 420 nm by using a 1-cm light path cuvette and a model 2000 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). At the beginning of each experiment, 9.0 ml of this standard cell suspension [0.42 mg (dry weight) of cell/ml] was added to each radiorespirometer flask. Cold carrier substrate (20 μmoles) and the specifically labeled substrate (approximately 1 μc) were added to the flask sidearms. The final volume of the liquid was adjusted to 10.0 ml with water. In each experiment control cultures were run in triplicate and compared to another triplicate set containing 10 mM AcGN. The vessels were allowed to equilibrate for 10 min at 37 C in a reciprocating water bath shaker prior to mixing of cells and substrate. At zero time the labeled substrate was added from the sidearm, and $^{14}CO_2$ collection was begun as described elsewhere (6). Measurement of radioactivity was conducted with a scintillation fluid composed of toluene (666 ml), Triton X-100 (Packard Instrument Co., LaGrange, Ill.) (333 ml), PPO (5.5 g), and dimethyl POPOP (150 mg). All counting was done in a liquid scintillation counter (Packard Instrument Co., LaGrange, Ill.). ^{14}C recoveries were determined at the end of each set of radiorespirometric experiments. ^{14}C appearing in the cells was determined by collecting a sample of cells on membrane filters (Bact-T-Flex, type B-6, Schleicher & Schuell Co., Keene, New Hampshire). Radioisotope recoveries were calculated with the use of the following formula: [(total ^{14}C assimilated into cell fraction + total ^{14}C released as $^{14}CO_2$ + total ^{14}C remaining in soluble fraction)/total ^{14}C added to each reaction vessel] × 100 = percentage of ^{14}C recovered.

RESULTS

The general characteristics of growth of the parental and amino sugar mutant strains of *E. coli* K-12 are shown in Table 1. With the casein hydrolysate supplemented medium used in this study, all strains grew equally well on glucose. The parental strain grew as well on AcGN and the combination of AcGN plus glucose as it did on glucose itself, whereas this latter combination inhibited growth of both mutant strains. As originally described by White (10) the deaminaseless strain was unable to grow on GN or AcGN, whereas the deacetylaseless strain was able to grow on GN but not AcGN. Although AcGN caused an inhibition of growth on glucose in both mutants, the kinetics involved were different in each case (Fig. 1). With both mutants, inhibition was observed almost immediately after the addition of AcGN. In the case of the deaminaseless strain this inhibition increased in intensity progressively with continued incubation.

We felt that clues could be obtained concerning the nature of this amino sugar sensitivity by growing these mutants on a wide variety of substrates with and without AcGN. The results of such a survey are shown in Table 2. These experi-

TABLE 1. *Growth of parental and mutant strains on glucose and amino sugars*

Substrate[a] (0.02 M)	Relative growth[b]		
	Parental strain	Deaminase-less strain	Deacetyl-aseless strain
Glucose	4.8	4.8	4.2
AcGN	4.8	1.1	1.0
GN	3.5	1.0	2.1
AcGN + glucose	4.9	3.1	2.3

[a] All cultures were grown in mineral salts medium containing 0.25% casein hydrolysate and the indicated substrate.

[b] Values represent the relative increase in culture mass dry weight during 160 min of growth. The cell mass at the start of the experiment equals 1.

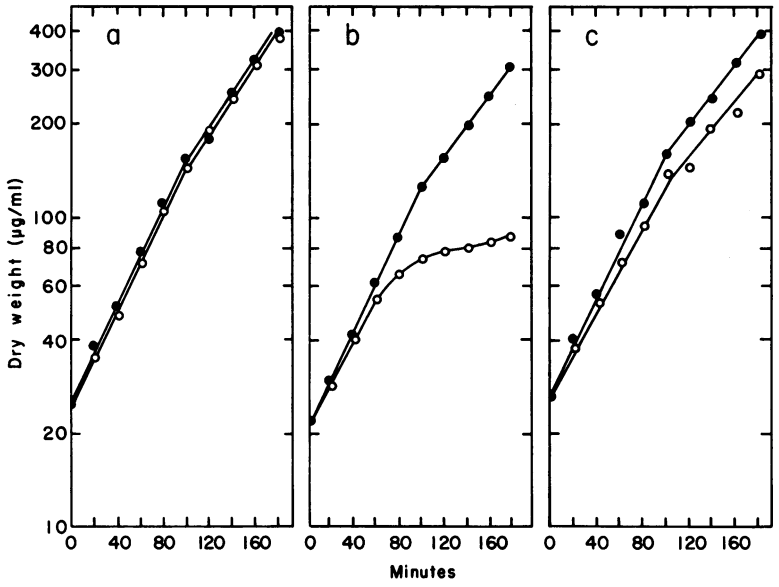

FIG. 1. *Effect of AcGN on growth of the parental and mutant strains on glucose. Cultures grown overnight on glucose were harvested, washed, and resuspended in fresh media containing 0.02 M glucose and 0.25% casein hydrolysate. They were preincubated for 120 min to insure exponential growth. At zero time 0.01 M AcGN was added to one culture (○), whereas the other culture (●) served as the control. (a) Parental strain; (b) the deaminaseless strain; (c) the deacetylaseless strain.*

ments were useful for a number of reasons. First, it was learned that the addition of AcGN had no effect on growth of either the parental or mutant strains when gluconate was the growth substrate. Second, it was clear that the growth inhibition caused by AcGN occurred only in the mutant strains. AcGN in fact enhanced the growth of the parental strain when included in the medium with normally poor substrates such as pyruvate, succinate, or the pentoses. Third, inhibition of the mutants by AcGN occurred in a specific pattern with respect to the nature of the growth substrate used. Growth on gluconate, as already mentioned, was unaffected. Growth on glucose and the pentoses exhibited some sensitivity, whereas growth was completely inhibited on all the substrates that are known to enter the catabolic pathways at a level equal to or below that of fructose-6-phosphate (F6P).

In the experiments described thus far 0.01 to 0.02 M concentrations of AcGN were used. These concentrations represented a considerable excess (Fig. 2.). Inhibition of the deaminaseless strain strain (graph a, Fig. 2) was detected with as little as 1.25×10^{-5} M AcGN. At this level and up to 1.25×10^{-4} M, the inhibition was eventually overcome and normal growth resumed in each case. At the latter concentration, inhibition was maintained for the entire experimental period. With the deacetylaseless mutant (graph b, Fig. 2), 1.25×10^{-5} M AcGN caused complete inhibition for the entire period. The differences between these strains can be attributed to the fact that the GN6P which accumulates in the deaminaseless strain is gradually assimilated into these cells, whereas the AcGN6P that accumulates in the deacetylaseless strain cannot be further metabolized in a normal manner. The inhibition produced by AcGN was rapid and occurred regardless of the time at which it was added to the culture (Fig. 3).

To determine the metabolic site(s) involved in this amino sugar sensitivity, a radiorespirometric analysis was undertaken in which the ability of untreated and AcGN-treated cells to convert specifically labeled ^{14}C substrates to $^{14}CO_2$ was measured. Non-proliferating cell suspensions were used, and a ^{14}C recovery was determined in each experiment to be certain that all the isotope could be accounted for. These inventories yielded 90 to 105% of added radioisotope with an average of 99%.

AcGN inhibited formation of $^{14}CO_2$ from glucose-*3,4*-^{14}C in both mutants but not in the parental strain (Fig. 4). It also inhibited $^{14}CO_2$ formation from glucose-*1*-^{14}C (not shown) by the deacetylaseless strain but not by the deaminaseless strain. In identical experiments with all three strains, AcGN had no effect on the dissimilation of gluconate-*1*-^{14}C, gluconate-*6*-^{14}C, glucose-*6*-^{14}C, pyruvate-*1*-^{14}C, or succinate-*1,4*-^{14}C.

TABLE 2. *Effect of acetylglucosamine (AcGN) on growth of the parental and mutant strains on various substrates*

Substrate (0.02 M)[a]	Relative growth[b]					
	Parental strain		Deaminaseless strain		Deacetylaseless strain	
	Control	+AcGN	Control	+AcGN	Control	+AcGN
Gluconate	3.6	4.2	3.8	3.7	3.6	3.6
Glucose	4.1	4.0	3.8	3.1	4.2	3.6
Ribose	2.8	3.6	2.8	2.6	2.8	2.6
Xylose	3.6	4.1	3.0	2.6	3.0	2.4
Arabinose	3.1	4.2	3.2	2.9	3.1	2.9
Mannitol	3.7	3.6	4.0	1.9	3.5	1.1
Fructose	3.5	3.8	3.6	1.1	3.0	1.0
Glycerol	3.7	4.0	3.8	1.0	3.7	1.1
Pyruvate	3.3	4.3	2.5	1.1	2.2	1.2
Succinate	2.4	3.4	2.8	1.1	2.6	1.1
None[c]	2.8	4.0	2.8	1.1	2.0	1.1

[a] All cultures were grown overnight in mineral salts medium containing 0.02 M levels of their respective substrates. The cultures were harvested and inoculated into fresh media containing 0.02 M substrate and 0.25% casein hydrolysate. They were preincubated for 90 min to insure exponential growth and had a cell mass at the start of the assay period of 70–130 μg (dry weight)/ml. Subsequent growth was measured after 120 min. In each set there was a control culture and a culture containing 10 μmoles/ml of AcGN.

[b] Given as the mass increase that occurred during 120 min of exponential growth relative to the initial cell concentration, which was set to equal 1.0.

[c] Cells grown only on the 0.25% casein hydrolysate present in the medium.

Thus, AcGN inhibited growth of the mutant strains on substrates such as pyruvate or succinate, but had no effect on their dissimilation.

A possible explanation for this inhibition which is consistent with both the growth data (Table 2) and radiorespirometric data is the following: AcGN6P or GN6P accumulate in abnormally high levels when the mutant cells are grown in the presence of AcGN (10). These amino sugars may then inhibit one or both of the reactions involved in the interconversion of glucose-6-phosphate (G6P) to 6-phosphogluconate or F6P. Neither of these reactions is involved in the metabolism of gluconate which occurs via an inducible Entner-Doudoroff pathway (2, 14) or in the metabolism of pentoses which occurs via the transaldolase-transketolase sequence of reactions (8). On the other hand these reactions are believed to play a role in pentose biosynthesis during aerobic metabolism of substrate such as

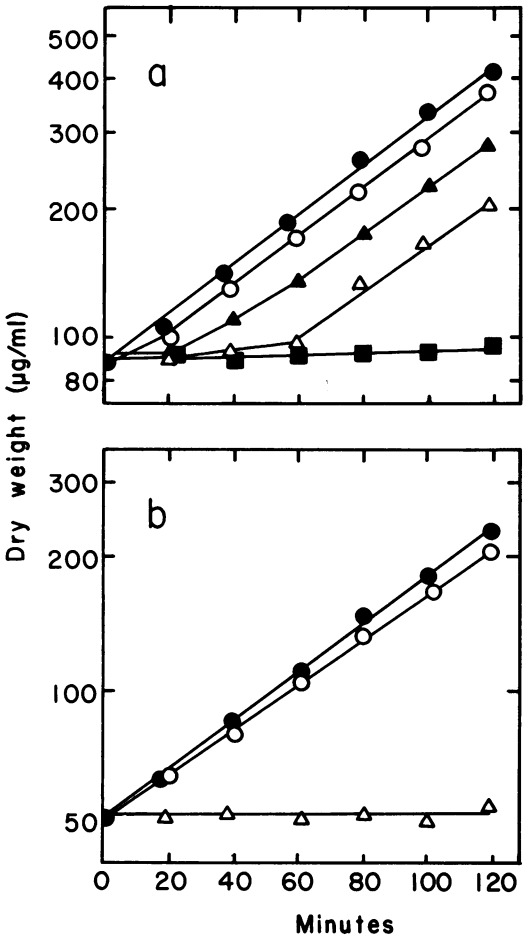

FIG. 2. *Effect of AcGN concentration on growth of the mutant strains. Cultures grown overnight in medium containing glycerol were harvested, suspended in 0.05 M phosphate buffer (pH 7.5), and inoculated into fresh medium containing 0.02 M glycerol and 0.25% casein hydrolysate. After 120 min of preincubation to insure exponential growth the indicated concentrations of AcGN were added, and growth of the cultures was measured. (a) Deaminaseless strain with the following concentrations of AcGN: ●, none; ○, 1.25 × 10^{-5} M; ▲, 2.5 × 10^{-5} M; △, 5 × 10^{-5} M; ■, 1.25 × 10^{-4} M. (b) Deacetylaseless strain, with the following concentrations of AcGN: ●, none; ○, 0.5 × 10^{-5} M; △, 1.25 × 10^{-5} M.*

fructose, mannitol, glycerol, pyruvate, succinate, etc. Any interference in the isomerization of F6P to G6P or the oxidation of G6P to 6-phosphogluconate during growth in glycerol, or both, for example, could conceivably result in growth inhibition due to an inadequate supply of pentose for nucleic acid biosynthesis. In other words, amino sugar sensitivity may be the result of pentose starvation.

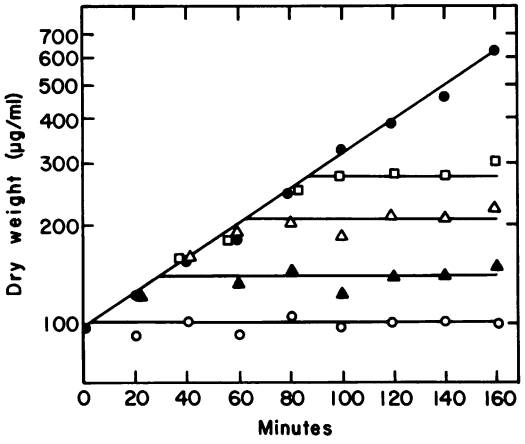

FIG. 3. *Effect of AcGN addition at various times during growth of the deacetylaseless mutant on glycerol. The cultures were grown in glycerol medium exactly as described in Fig. 2. AcGN at a final concentration of* 2.5×10^{-4} M *was added at 0 (○), 30 (▲), 60 (△), and 90 min (□). A control culture (●) without AcGN was included. Essentially identical results were obtained when the deaminaseless strain was used.*

If this were the case, it could be predicted that this sensitivity would be overcome when the inhibited cells were given an exogenous supply of pentose. This prediction was tested experimentally (Fig. 5). In this experiment the deacetylaseless strain was grown in a succinate-casein hydrolysate medium in the presence and absence of AcGN. Growth was estimated on the basis of the rate of assimilation of ^{14}C-leucine. All additions to these cultures, including the AcGN were made 40 min after the start of the experiment. It should be noted that whereas growth as measured by absorbance was inhibited immediately after the addition of AcGN, the incorporation of ^{14}C-leucine into the cells continued for some time thereafter. The inhibition of growth by AcGN was overcome by the addition of 10^{-3} M uridine to the culture medium (Fig. 5). Uracil itself had no effect on the inhibition, proving that it was the ribose moiety of uridine and not the uracil portion that was responsible for the reversal. In other experiments it was shown that the addition of uridine abolished amino sugar sensitivity during growth on all the substrates listed in Table 2. This was found to be the case with both mutant strains. The addition of ribose resulted in some reversal of repression as would be expected. This reversal was usually small and quite variable from experiment to experiment perhaps because of an incomplete uptake and metabolism of ribose in competition with the other substrates present in the medium (7).

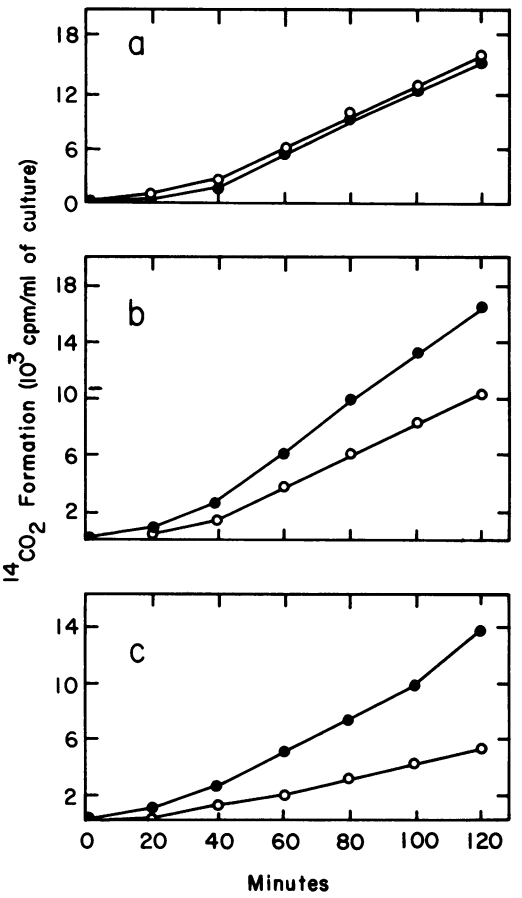

FIG. 4. *Effect of AcGN on the formation of* $^{14}CO_2$ *from glucose-3,4-*^{14}*C. Non-proliferating cultures were prepared as previously described (Materials and Methods). Each radiorespirometric reaction vessel contained 0.02* M *glucose including 1* μc *of glucose-3,4-*^{14}*X and the standardized cell suspension. The values plotted are the accumulated counts per minute at successive sampling periods. These values are plotted against incubation time. Each value represents the average of triplicate experiments. Each set of cultures was incubated with (○) or without (●) 0.01* M *AcGN. (a) Parental strain; (b) deaminaseless strain; (c) deacetylaseless strain.*

It could be argued that the addition of uridine to cultures inhibited by AcGN did not reverse the inhibition per se but merely served as a growth substrate. The results shown in Fig. 6 indicated that this was not true. Succinate-adapted cells of the deacetylaseless strain were transferred into fresh media containing 0.04 M succinate and various levels of uridine (graph a, Fig. 6) or transferred into fresh media lacking the succinate but containing the corresponding levels of uridine

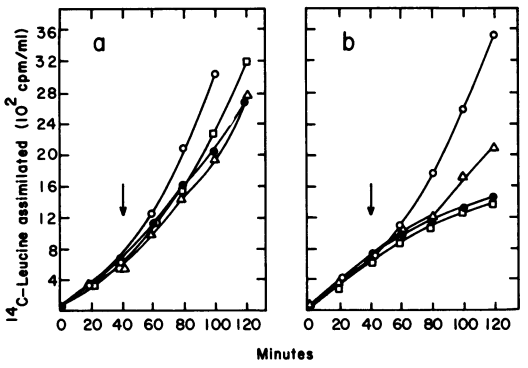

FIG. 5. *Reversal of AcGN inhibition of growth by uridine. A culture of the deacetylaseless strain grown overnight in succinate medium was harvested, washed, and resuspended in fresh medium containing 0.04 M succinate and 0.25% casein hydrolysate. These cultures were preincubated for 120 min after which ^{14}C-leucine was added (0.05 μc/ml of culture). At 40 min later the following additions were made: ●, none; ○, 10^{-3} M uridine; △, 10^{-3} M ribose; □, 10^{-3} M uracil. (a) Cultures incubated in the absence of AcGN; (b) cultures incubated in the presence of 5 × 10^{-3} M AcGN added at zero time. Growth was measured by the rate of assimilation of the ^{14}C-leucine into the 5% trichloroacetic acid-insoluble cell fraction.*

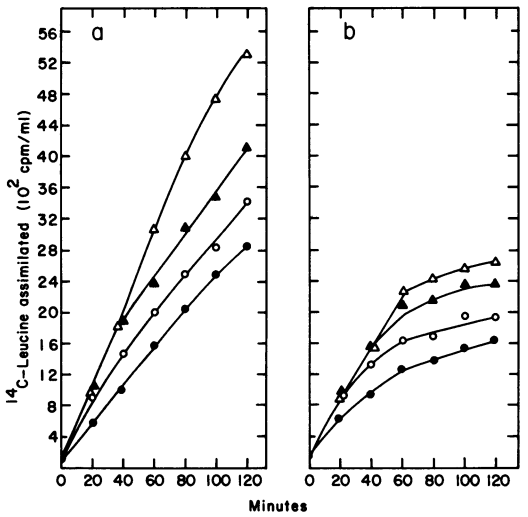

FIG. 6. *Effect of uridine on growth of the deacetylaseless mutant. A culture of the deacetylaseless strain grown overnight in succinate medium was harvested, washed, and resuspended in eight flasks of fresh medium containing 0.04 M succinate and 3 × 10^{-4} M L-leucine. After 120 min of preincubation, all eight cultures were centrifuged for 5 min at 12,000 × g. Four of the cultures were resuspended in fresh medium containing 0.04 M succinate, 3 × 10^{-4} M L-leucine, 1 μc ^{14}C-L-leucine (U) and 5 × 10^{-3} M AcGN (a). The other four cultures were resuspended in the same medium (graph b, Fig. 6). It can be seen that the concentrations of uridine used to overcome AcGN inhibition of growth could not in themselves support any comparable degree of cell growth. The same results were obtained when the deaminaseless strain was tested in this manner.*

DISCUSSION

Mutants of *Salmonella* and *E. coli* selected for an inability to grow on certain carbohydrates often exhibit a phenomenon of growth inhibition known as "sugar sensitivity" when they are subsequently exposed to this carbohydrate in a medium otherwise suitable for growth. The presence of the particular substrate that cannot be completely metabolized, because of the mutational lesion, can result in an intracellular accumulation of normal metabolites in abnormally high levels. The resultant metabolic imbalance can cause interference with other essential reactions resulting in bacteriostasis or bacteriolysis. This phenomenon was observed in certain galactose-sensitive mutants in *Salmonella* that lacked uridine diphosphate (UDP)-galactose-4-epimerase and which were lysed in the presence of galactose (4). Growth was a prerequisite for galactose-induced lysis, and it was proposed that the high concentration of UDP-galactose and galactose-1-phosphate which accumulated in the mutant in the presence of galactose was related to the initiation of lysis. Another example of this "sugar sensitivity" phenomenon was observed with galactose-negative mutants of *E. coli* (13) unable to use galactose as the sole source of carbon because of the absence of the enzymes galactose-1-phosphate uridyl transferase or UDP-galactose-4-epimerase. These mutants growing in a minimal medium containing glycerol underwent pronounced growth inhibition when 0.01 M galactose was added. This inhibition could be reversed by the later addition of glucose. Growth inhibition was also demonstrated in *Salmonella typhosa* strains unable to use rhamnose as a sole carbon and energy source. The lack of a rhamnose isomerase and rhamnulokinase with concomitant accumulation of rhamnulose or rhamnulose-1-phosphate, or both, appeared to cause the inhibition (3).

A preliminary examination of the amino sugar mutants of *Escherichia coli* used in this study indicated that a similar "sugar sensitivity" phenomenon was occurring in these organisms. In

minus the succinate (b). Growth was measured by the rate of assimilation of ^{14}C-L-leucine into the 5% trichloroacetic acid-insoluble cell fraction in the presence of the following concentrations of uridine: ●, none; ○, 2.5 × 10^{-4} M; ▲, 5.0 × 10^{-4} M; △, 10.0 × 10^{-4} M.

this case, however, it could be referred to as "amino sugar sensitivity." Clues were obtained concerning the nature of this inhibition by comparing the growth of the parental and mutant strains on a variety of substrates in the presence and in the absence of AcGN. From these studies alone, the following tentative conclusions were made. First, that the target reaction(s) for this inhibition is not one of the steps in the metabolism of gluconate via the Entner-Doudoroff system or the accompanying glycolytic, tricarboxylic acid cycle and electron transport reactions. Included in this are the reactions catalyzed by gluconokinase, 6-phosphogluconate dehydrase, and 2-keto-3-deoxy-6-phosphogluconate aldolase and all of the reactions common to glycolysis between glyceraldehyde-3-phosphate and pyruvate oxidation. This conclusion was based on the fact that AcGN produced no inhibition of growth when the parental or mutant strains were grown on gluconate, and that inhibition of growth on other substrates caused by AcGN could be reversed by adding gluconate to the culture medium. Lack of inhibition on gluconate would also suggest that pentose production from gluconate via gluconokinase and 6-phosphogluconate dehydrogenase was unaffected by *N*-AcGN. Secondly, that the target reaction(s) for inhibition was probably not included among the reactions responsible for the production of F6P from the pentoses via the transaldolase-transketolase reactions or for its subsequent utilization via glycolysis. This conclusion was based on the observation that AcGN had only limited ability to inhibit growth on D-ribose, D-xylose, or L-arabinose. Thirdly, AcGN caused essentially total inhibition of growth with all substrates that enter the glycolytic and tricarboxylic acid cycle at a level equal to or below that of F6P and which are not directly or closely associated with the production of pentose.

Reasoning from these conclusions and considering the interconnections of the various pathways involved, the following hypothesis has been formulated. The metabolites AcGN6P and GN6P accumulate in high concentrations when the deacetylaseless and deaminaseless strains respectively are exposed to exogenous AcGN (10). These compounds impede the flow of carbon at either one or both of two sites—the reactions catalyzed by phosphohexoisomerase and G6P dehydrogenase. Inhibition of one or both of these reactions during growth of the mutants on fructose, mannitol, glycerol, pyruvate, succinate, or amino acids produces a pentose starvation resulting in cessation of growth. The fact that AcGN does not inhibit growth on substrates from which ribose-5-phosphate can be directly synthesized (i.e., gluconate, D-ribose, D-xylose, and L-arabinose), and the fact that growth inhibition on all other substrates appears to be reversed by the addition of uridine, but not uracil, to the culture medium gives some credence to this hypothesis. The possibility cannot be ignored, however, that some hexose starvation is also produced under these conditions. There is no experimental evidence along this line at the present time.

Further evidence favoring this hypothesis was obtained by the use of a radiorespirometric technique. In these experiments non-proliferating cultures were used so that the inhibitory effect of AcGN on growth of the mutants would not be a factor in interpreting the results. These cells were given specifically labeled ^{14}C-substrates and the effect of added AcGN on the production of $^{14}CO_2$ was measured. These studies clearly demonstrated that AcGN had no effect on the formation of $^{14}CO_2$ arising from the metabolism of gluconate-*1*-^{14}C, gluconate-*6*-^{14}C, pyruvate-*1*-^{14}C, glucose-*6*-^{14}C or succinate-*1,4*-^{14}C, although it did inhibit the formation of $^{14}CO_2$ from glucose-*3,4*-^{14}C with the two mutants but not with the parental strain, and it inhibited $^{14}CO_2$ formation from glucose-*1*-^{14}C with the deacetylaseless strain but not with the others.

This general pattern of AcGN inhibition is consistent with the hypothesis discussed above, namely, that the derivatives of AcGN are capable of interfering with phosphohexoseisomerase and G6P dehydrogenase. Inhibition of phosphohexoisomerase activity in both mutants would lead to a decrease in the rate of pyruvate formation which, in turn, would account for the decreased rate of $^{14}CO_2$ formation from glucose-*3,4*-^{14}C. The fact that AcGN does not effect $^{14}CO_2$ formation from pyruvate-*1*-^{14}C under the same conditions indicates that the formation of pyruvate is inhibited and not its oxidation. The fact that $^{14}CO_2$ formation from glucose-*1*-^{14}C but not from glucose-*6*-^{14}C is inhibited in the deacetylaseless strain suggests that AcGN6P interferes with G6P dehydrogenase. That it is this step in the hexosemonophosphate shunt pathway, and not the 6-phosphogluconate dehydrogenase step, that is sensitive to the amino sugar is indicated by the fact that AcGN does not inhibit growth of either mutant on gluconate nor does it alter the rate of production of $^{14}CO_2$ from gluconate-*1*-^{14}C. An inhibition in each case would likely have occurred if the 6-phosphogluconate dehydrogenase reaction was sensitive to the accumulated amino sugars. Consistent with these interpretations is the report that GN6P is a potent inhibitor of phosphohexoisomerase in cell-free extracts (12), and that GN6P competes effectively with G6P in the G6P dehydrogenase reaction (5).

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