

Effect of Amino Sugars on Catabolite Repression in *Escherichia coli*¹

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N-acetylglucosamine was found to be a good repressor source for catabolite repression of the β -galactosidase system in *Escherichia coli*. It was found capable of increasing the severity of repression by glucose or gluconate when included in the medium with either of these substrates. *N*-acetylglucosamine was shown to be assimilated under these conditions, but had no effect on culture growth rates. Its influence on catabolite repression was not altered by growth in the presence of inhibiting levels of penicillin. These findings indicated that catabolite repression may be associated with certain reactions of amino sugar metabolism. A working model has been formulated along these lines and will be used to explore this possible relationship further.

Previous reports from this laboratory indicated that glucose repression in *Escherichia coli* is closely coupled with the ability to oxidize efficiently the pyruvate that is produced endogenously during glucose dissimilation (5, 16). Repression was found to be turned on when metabolic conditions favored accelerated oxidative decarboxylation of pyruvate, and was found to be turned off when this oxidation was retarded. From these studies, and from the knowledge that a direct relationship exists between catabolite repression and cell growth rate, it was proposed that the energy-rich end products of pyruvate oxidation, namely, acetyl-coenzyme A (CoA) and adenosine triphosphate (ATP), are involved in some unknown manner as regulatory signals in catabolite repression.

It remained necessary, however, to show that acetyl-CoA and ATP are in fact involved in the repression, and to specify the nature of this involvement. In this connection, studies were undertaken to determine (i) if acetyl-CoA and ATP are directly involved in this repression, perhaps by directly acetylating or phosphorylating some protein repressor; or (ii) if acetyl-CoA and ATP are only indirectly involved, perhaps by acetylating or phosphorylating another metabolite that in turn functions as a co-repressor in this system. The findings presented in this report suggest that the second alternative may be the correct one. The data presented indicate that a

relationship may exist between catabolite repression and amino sugar metabolism in *E. coli*. *N*-acetylglucosamine was found to be a potent catabolite repressor source and was shown to be capable of augmenting repression by glucose or gluconate. Its assimilation is dependent on availability of acetyl-CoA and ATP.

MATERIALS AND METHODS

Chemicals. Isopropyl-thio- β -D-galactoside (IPTG), *O*-nitrophenyl- β -D-galactoside (ONPG), and *N*-acetylglucosamine were purchased from Mann Research Laboratories, New York, N.Y. Uniformly labeled leucine-¹⁴C and glucosamine-1-¹⁴C were obtained from New England Nuclear Corp., Boston, Mass. 2,5-Diphenylloxazole (PPO) and 1,4-bis-2-(5-phenyl-oxazolyl)-benzene (POPOP) were products of Packard Instrument Co., Inc., Downers Grove, Ill. *N*-acetylglucosamine-1-¹⁴C was prepared by acetylation of glucosamine-1-¹⁴C according to the procedure of Roseman and Ludoweig (20). Purity of the compound was checked by paper chromatography with *n*-butyl alcohol-pyridine-water (6:4:3). All other chemicals were of reagent grade and are readily available.

Culture and cultural conditions. *E. coli* ML 30 was used in all the experiments. The medium employed, the conditions of culture growth (aerobic and anaerobic), and induction of β -galactosidase with IPTG were as described elsewhere (5, 15). A unit of β -galactosidase activity was defined as that amount of enzyme that hydrolyzes 1 μ mole of ONPG per hr at 37 C in the presence of 2×10^{-3} M ONPG, 1.24×10^{-4} M reduced glutathione, and 0.05 M sodium phosphate buffer, pH 7.5. Rates of enzyme formation were determined by plotting units of enzyme activity per milliliter of culture against growth of the culture, in micrograms (dry weight) per milliliter of culture. The

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differential rate of enzyme formation (P value) was calculated from the slopes of these plots (4, 12).

Uniformly labeled leucine- ^{14}C and *N*-acetylglucosamine- ^{14}C assimilation was determined by scintillation counting of cells that had been collected and dried on membrane filters (Bac-T-Flex, type B-6, Schleicher and Schuell Co., Keene, N.H.) after extraction and washing with cold 5% trichloroacetic acid.

Chemical measurements. *N*-acetylglucosamine was determined colorimetrically according to the procedure of Levvy and McAllan (7) with the potassium tetraborate reagent (0.7 M) prepared as described by Reissig et al. (19). Dry-weight determinations were made turbidimetrically at 420 $m\mu$ by use of a Spectronic-20 colorimeter and a previously prepared standard curve relating absorbancy to dry weight.

RESULTS

Repression of β -galactosidase synthesis by glucose or gluconate is clearly demonstrated by the results shown in Fig. 1. Of particular interest in this experiment, however, was the finding that the amino sugar *N*-acetylglucosamine (AcGN) was almost as effective as glucose in promoting catabolite repression. Even more noteworthy was the observation that repression by glucose or gluconate was doubled when the cells were grown in medium containing AcGN and either of the other repressor sources. On the basis of these data, it was recognized that the effects of AcGN on catabolite repression could be a reflection of some relationship between the catabolite re-

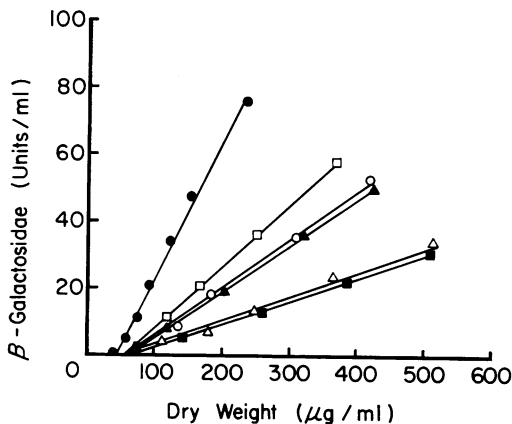


FIG. 1. Effect of *N*-acetylglucosamine on catabolite repression by glucose and gluconate. At a cell concentration of approximately 50 μg (dry weight) per ml, 2.5×10^{-3} M IPTG was added to aerobic cells growing exponentially in basal medium containing 0.25% casein hydrolysate and 0.02 M concentrations of the indicated substrates. *N*-acetylglucosamine additions (0.02 M) were made at the same time that inducer was added. (●) Glucosamine; (○) glucose; (△) glucose plus *N*-acetylglucosamine; (▲) gluconate; (■) gluconate plus *N*-acetylglucosamine; (□) *N*-acetylglucosamine.

pression and amino sugar metabolism. The following experiments were carried out in an effort to obtain further information along these lines.

Aerobic and anaerobic growth rates on glucose were unaffected by the addition of AcGN to the medium (Fig. 2a). Under both conditions, however, there was an enhanced severity of repression of enzyme synthesis owing to the added AcGN (Fig. 2b). Under aerobic conditions, the effect of AcGN was detected almost immediately after its addition to the medium. Temporary reversal of repression by anaerobic shock (4, 15) was observed as expected with the culture growing on glucose. In the anaerobic system containing glucose and AcGN, a short period of derepression was also detected. In this case, however, repression was resumed more rapidly than in the control system.

Of the amino sugars tested (Table 1), only AcGN was capable of producing significant repression. Also, it was the only amino sugar capable of enhancing repression by glucose. Glucose, AcGN, and *N*-acetylglucosamine (AcGalN) supported comparable rates of growth under these conditions. Glucosamine and galactosamine were able to support growth of the organism, but only at a low rate, presumably owing to difficulty in passage of the nonacetylated amino sugars across the cell membrane (1, 2).

The data presented in Fig. 3 show that AcGN is rapidly dissimilated when used as the major substrate, or when used in conjunction with a substrate such as glycerol that supports a low rate of growth. When combined with glucose in the culture medium, concomitant AcGN utilization still occurred, but at a rate diminished to 75 to 80% of that observed when AcGN was the major substrate.

The data listed in Table 2 provided an estimate as to the amount of AcGN that was assimilated during growth on the combined substrates. The total AcGN utilized was determined by colorimetric analysis of culture samples at 20-min intervals during exponential growth. The AcGN used in these experiments was labeled in the ^{14}C position, and the specific activity of the AcGN added to the cultures was calculated. The percentage of AcGN assimilated during growth was then calculated on the basis of the radioactivity remaining with the cells after extraction and washing with water or 5% trichloroacetic acid. From these experiments, it was concluded that 36 to 39% of the AcGN added was assimilated into the water-insoluble cell fraction and 26 to 32% of the AcGN added was assimilated into the trichloroacetic acid-insoluble cell fraction. The values obtained with

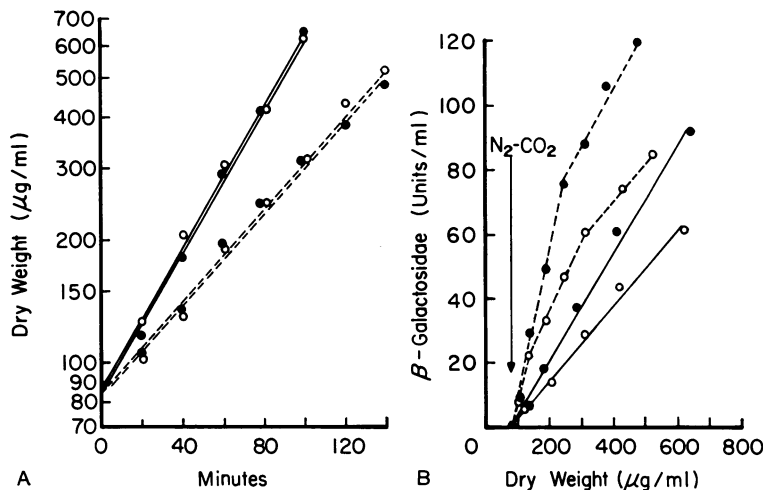


FIG. 2. Effect of *N*-acetylglucosamine on growth and anaerobic induction in *Escherichia coli*. (A) At zero-time, *N*-acetylglucosamine was added to cultures growing aerobically (solid line) or anaerobically (dashed line) on glucose. (●) Cultures containing only 0.02 M glucose; (○) cultures containing 0.02 M glucose plus 0.012 M *N*-acetylglucosamine. (B) At a cell concentration of approximately 70 µg (dry weight) per ml, 2.5×10^{-3} M IPTG was added to four aerobic cultures growing exponentially on 0.02 M glucose. At that time, two of the cultures were made anaerobic by bubbling with N_2 -CO₂ (see arrow), while the other two cultures were kept aerobic. (●) Cultures containing only 0.02 M glucose; (○) cultures containing 0.02 M glucose with 0.012 M *N*-acetylglucosamine added at the time of inducer addition.

TABLE 1. Effect of amino sugars on β -galactosidase formation in *Escherichia coli*

Additions ^a	Differential rates of β -galactosidase formation (<i>P</i> values)
None	45
Glucosamine	43
Galactosamine	47
<i>N</i> -acetylglucosamine	36
Glucose	15
Glucose + glucosamine	14
<i>N</i> -acetylglucosamine	19
<i>N</i> -acetylglucosamine + glucosamine	18
<i>N</i> -acetylglucosamine + <i>N</i> -acetylgalactosamine	18
Glucose + <i>N</i> -acetylglucosamine	8
Glucose + <i>N</i> -acetylglucosamine + glucosamine	8
Glucose + <i>N</i> -acetylglucosamine + <i>N</i> -acetylgalactosamine	9

^a Additions to the basal medium that included 0.25% casein hydrolysate. Glucose = 0.02 M; glucosamine = 0.02 M; galactosamine = 0.02 M; *N*-acetylglucosamine = 1.2×10^{-2} M; *N*-acetylgalactosamine = 1.2×10^{-2} M.

water washing could be considered high, since some pooled AcGN may not have been extracted. The values obtained after trichloroacetic acid extraction may be considered low, since some amino sugar containing polysaccharides were extracted into the trichloroacetic acid. In either case, one could conclude that one-quarter to one-third of the AcGN utilized by cultures growing on glucose was assimilated into the cells. More details in this regard are given in another report (6). The assimilation of large amounts of AcGN under these conditions suggests that its effect on catabolite repression may be related to its flow into biosynthetic pathways, rather than its dissimilation via catabolic reactions. This point is stressed further when one considers that all the requirements for growth of the organism are available from the glucose present in the medium (Fig. 2b). It is necessary, however, to obtain further information along this line before any sound conclusion can be made.

At this stage of the investigation, it was of interest to determine whether the effect of AcGN on β -galactosidase synthesis could be altered by penicillin, that is, by preventing its assimilation into the normal cell wall-cell membrane structures. The results of this study are presented in Fig. 4. The addition of penicillin to cultures

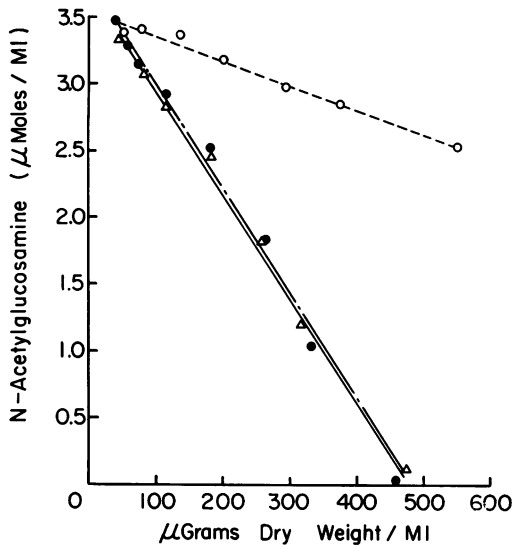


FIG. 3. Comparative rates of *N*-acetylglucosamine utilization in *Escherichia coli*. Three cultures were grown aerobically in basal medium containing 0.25% casein hydrolysate. During exponential growth at a cell mass of approximately 70 μ g (dry weight) per ml, *N*-acetylglucosamine (3.5×10^{-3} M) was added to all three cultures that otherwise contained: (●) no substrate; (Δ) 0.02 M glycerol; (\circ) 0.02 M glucose. Changes in *N*-acetylglucosamine concentration were then determined and plotted as a function of culture growth.

containing either glucose or glucose plus AcGN appeared to result in a complete reversal of catabolite repression (Fig. 4a). These data were obtained by plotting units of enzyme per milliliter of culture against the micrograms (dry weight) per milliliter of culture. It was subsequently found, however, that the dry weight determinations with the penicillin-treated cultures gave erroneous values. β -Galactosidase synthesis is plotted (Fig. 2b) as a function of protein increase as determined by the assimilation of 14 C-leucine. On the basis of these data, it was concluded that the effect of AcGN on catabolite repression occurred even when cell wall synthesis was inhibited by penicillin. This indicates that any effect on repression owing to assimilation or accumulation of amino sugar metabolites must occur prior to the penicillin-sensitive transpeptidation step involved in polymer formation.

DISCUSSION

No information is available concerning the identity of the low molecular-weight catabolite that is predicted to function as a co-repressor in catabolite repression of β -galactosidase in *E.*

TABLE 2. Utilization and incorporation of *N*-acetylglucosamine-1- 14 C by *Escherichia coli*^a

Incubation time	Trichloroacetic acid wash			Water wash		
	Utilized	Incorporated	Incorporated	Utilized	Incorporated	Incorporated
min	μ moles	μ moles	%	μ moles	μ moles	%
0	0	0	—	0	—	—
20	0	0.14	99	0	0.36	99
40	1.1	0.44	40	1.4	0.93	66
60	3.9	1.03	27	5.7	2.10	37
80	7.4	1.96	27	10.0	3.48	35
110	13.1	3.46	26	16.1	5.84	36
120	19.3	5.30	28	18.7	7.34	39
140	22.2	7.02	32	—	—	—

^a Cultures were grown in medium containing 0.02 M glucose and 1×10^{-3} M *N*-acetylglucosamine-1- 14 C (20,500 counts per min per μ mole). *N*-acetylglucosamine levels determined at 20-min intervals during growth. At the same time, 14 C which remained in the cells after trichloroacetic acid or water washing was determined. Percentage of incorporation of *N*-acetylglucosamine is calculated on the basis of micromoles incorporated per micromole utilized times 100.

coli. It is known not to be a specific end product of the *lac* operon such as galactose, galactose-1-phosphate or uridine diphosphate (UDP)-glucose (3, 17). On the basis of indirect evidence, it is suspected to be a high-energy metabolite, such as acetyl-CoA or ATP, or a metabolite whose formation is directly dependent on the availability of these high-energy compounds (11, 13, 15).

Components of the oxidative hexosemonophosphate pathway have also been implicated as having an important role in catabolite repression (8, 18). In this report, it was shown that AcGN is a potent source of this catabolite repressor, and that normal repression by glucose or gluconate was greatly enhanced when these substrates were metabolized concomitantly with AcGN. These findings pointed to a hitherto unexplored possibility that the catabolite regulator substance is located in the pathway leading to the synthesis of amino sugar polymers. In this connection, a working hypothesis has been formulated and will be considered at this time. Listed in Fig. 5 are the pathways involved in the dissimilation of the repressor sources: glucose, gluconate, and *N*-acetylglucosamine. Also shown is the relationship between these pathways and the reactions involved in amino sugar synthesis. In the early steps of glucose or gluconate metabolism, a biosynthetic divergence occurs, and leads to the formation of glucosamine-6-P

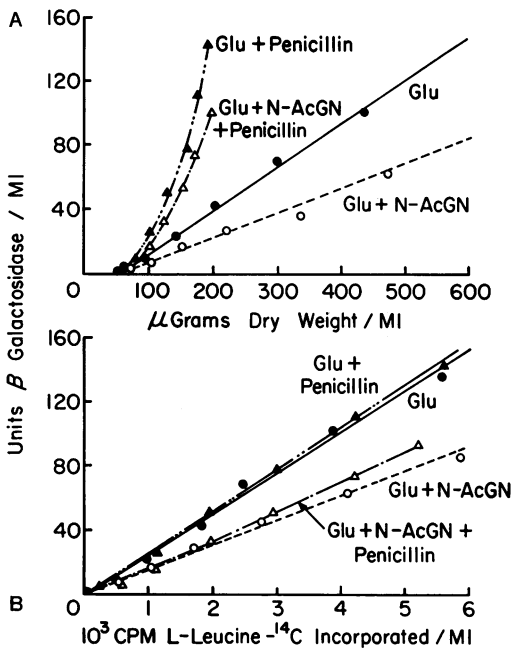


FIG. 4. Effect of penicillin on enhancement of catabolite repression by *N*-acetylglucosamine. Cultures were grown as described (Fig. 1) with 0.02 *M* glucose or 0.02 *M* glucose plus 4.5×10^{-3} *M* *N*-acetylglucosamine as substrates. Penicillin was added at the same time that isopropyl-thio- β -*D*-galactoside was added (45 μ g, dry weight, per ml) to initiate β -galactosidase synthesis. (A) β -Galactosidase formation was plotted as a function of mass increase as determined turbidimetrically. In (B), the same β -galactosidase levels were plotted as a function of the increase in protein synthesis as indicated by the incorporation of uniformly labeled *L*-leucine- 14 C into the trichloroacetic acid-insoluble cell fraction. The cultures contained: (●) 0.02 *M* glucose; (▲) 0.02 *M* glucose plus 3 μ g of penicillin per ml; (○) 0.02 *M* glucose plus 4.5×10^{-3} *M* *N*-acetylglucosamine; (△) 0.02 *M* glucose plus 4.5×10^{-3} *M* *N*-acetylglucosamine plus 3 μ g of penicillin per ml.

(GN6P) from fructose-6-P (F6P) and glutamine as catalyzed by the enzyme *L*-glutamine-*D*-fructose-6-P transamidase. During AcGN dissimilation, GN6P is formed after a phosphorylation of AcGN to AcGN6P and deacetylation to GN6P (21). A portion of the GN6P thus formed is diverted into the biosynthetic pathway; the remainder is deaminated, resulting in the formation of F6P, NH_4^+ and entry of the hexose chain into the glycolytic pool. In every case, the GN6P that is to be polymerized is acetylated with acetyl-CoA and then converted to the nucleotide derivative with the uridine triphosphate (UTP) made available by ATP production. An end product of these conversions, UDP-*N*-AcGN, is a key metabolite utilized in a variety of bio-

synthetic reactions in cell wall and cell membrane biosynthesis. It is possible that UDP-*N*-AcGN, or a closely related metabolite, such as UDP-*N*-acetylmuramic acid, may function as the co-repressor in catabolite repression. These compounds are *a priori* well suited to function in this capacity. UDP-*N*-AcGN is a nucleotide and thus capable of promoting allosteric changes in the catabolite repressor (CR) protein assumed to be a product of the CR locus (8). In addition, its structure should give ample chemical testimony to the cells that energy and carbon are abundantly available—a situation in which catabolite repression is maximal (15). UDP-*N*-AcGN is derived from a hexose skeleton and hexoses are the most potent suppliers of repression (10). It contains the amide group of glutamine and is thus indicative of availability of the glutamate family of amino acids, NH_4^+ , and ATP. Its structure is also derived from three high-energy metabolites—one molecule of acetyl-CoA and two molecules of ATP. One ATP is used for the addition of the amino group and the other ATP is used for the formation of the

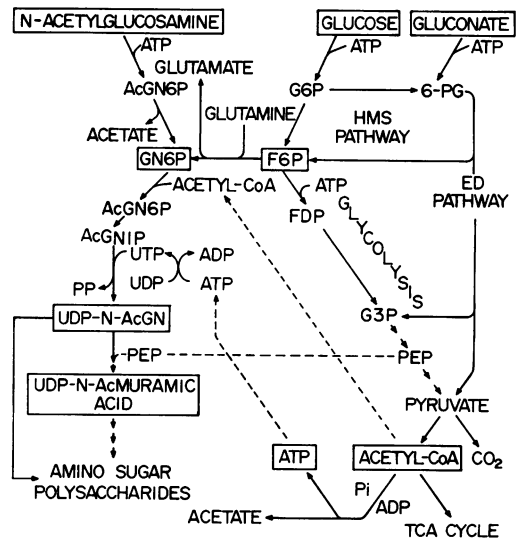


FIG. 5. Model of corepressor formation in catabolite repression. ATP = adenosine triphosphate; GN6P = glucosamine-6-phosphate; AcGN6P = *N*-acetylglucosamine-6-phosphate; G6P = glucose-6-phosphate; F6P = fructose-6-phosphate; FDP = fructose diphosphate; G3P = glyceraldehyde-3-phosphate; PEP = phosphoenolpyruvate; AcGNIP = *N*-acetylglucosamine-1-phosphate; UTP = uridine triphosphate; UDP = uridine diphosphate; UDP-*N*-AcGN = uridinediphospho-*N*-acetylglucosamine; UDP-*N*-AcMuramic acid = uridinediphospho-*N*-acetylmuramic acid; Pi = inorganic phosphate; PP = pyrophosphate; acetyl-CoA = acetyl coenzyme A.

UTP required for production of the amino sugar nucleotide. UDP-*N*-acetylmuramic acid contains an additional ATP and a C₃ unit through addition of phosphoenolpyruvate (22).

Catabolite repression is known to be associated with the ability of cells to grow rapidly (14). In fact, there is an inverse relationship between growth rate and the differential rate of β -galactosidase synthesis (15) in wild-type strains growing on various carbon sources. It is reasonable to assume that the growth of a cell in terms of protein and nucleic acid synthesis must be commensurate with the synthesis of the amino sugar polymers that form the basic structures of the cell wall and cell membrane. In this light, it is tempting to consider that metabolites like UDP-*N*-AcGN or UDP-*N*-acetylmuramic acid would be ideally suited to regulate certain dissimilatory and biosynthetic functions—a general role that in fact defines the phenomenon of catabolite repression.

Another point to be considered is that F6P is the metabolic site at which the major repressor substrates find a common point of convergence (Fig. 5). It has been suggested that catabolite repression and the Pasteur effect may be related processes, in that they serve similar purposes and are turned off and on under identical conditions (16). It is known that the Pasteur effect involves control over phosphofructokinase activity (9, 23, 24). Thus, when catabolite repression and the Pasteur effect are functioning, metabolic conditions favor accumulation of intermediates such as F6P and G6P. With these points in mind, one can visualize that conditions that promote these regulations also simultaneously promote amino sugar formation by favoring F6P accumulation, which, in turn, could favor GN6P formation. The enhancement of catabolite repression by addition of AcGN to cultures growing on glucose or gluconate (Fig. 1) could be explained on the basis of excess GN6P availability owing to AcGN metabolism. This availability is predicted to be prerequisite to establishing the catabolite repression response.

It is unlikely, however, that GN6P itself would function as the regulatory signal, since (i) AcGN used as the sole substrate gives good repression, but gives a better repression when coupled with another repressor source; and (ii) enhanced repression by AcGN under anaerobic conditions is observed only after a significant lag period. Both of these facts point to the likelihood that GN6P must be present at some excess level and must then be further modified before it is able to function as a regulator. This modification is predicted to involve acetyl-CoA and ATP as has already been described.

The data obtained from the penicillin studies (Fig. 4) indicated that, if a co-repressor activity is a part of the overall amino sugar pathway, it must be a substance located between GN6P and the penicillin-sensitive transpeptidation step involving the muramyl peptide compounds. Again, UDP-*N*-AcGN or UDP-*N*-acetylmuramic acid would be the most likely candidates for this function. Additional data concerning the possible relationship between catabolite repression and amino sugar metabolism are presented in an accompanying report (6).

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