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THE FEEDBACK CONTROL OF SUGAR NUCLEOTIDE BIOSYNTHESIS IN LIVER

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Feedback inhibition—the inhibition of the first enzymatic reaction unique to a pathway by the end product of that pathway—plays an important role in regulating various biosynthetic sequences. In bacteria and also in higher organisms this mechanism serves to control the formation of purines, pyrimidines, and amino acids.[1] Data presented in this communication demonstrate that the biosynthesis of certain sugar nucleotides in rat liver is likewise subject to feedback inhibition.

The pathways leading to the formation of UDP-N-acetyl-D-glucosamine (UDPAG)[2] and CMP-N-acetylneuraminic acid (CMP-NAN) are outlined in Figure 1.[3] Both sugar nucleotides represent the activated form to which the respective sugars must be converted prior to incorporation into glycoproteins and other macromolecules. UDPAG and CMP-NAN may therefore be considered the end products of the multistep pathways leading to their formation. Each of these end products inhibits the first enzyme of the pathway which is unique to its biosynthesis.

Materials and Methods.—Glycerol-1,3-C^{14} and D-glucosamine-1-C^{14} were purchased from New England Nuclear Corp. Tritiated D-glucosamine was obtained by hydrolyzing UDPAG which had been tritiated by the Wilzbach method; it was purified by chromatography on Dowex 50-H^+ (ref. 4) and on paper, in solvent IV. C^{14}-labeled UDPAG was made from radioactive N-acetyl-

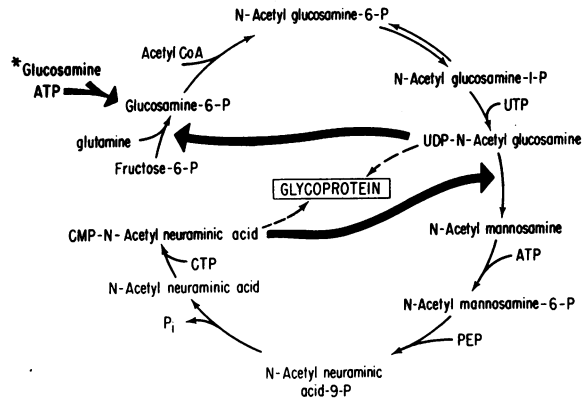

FIG. 1.—Pathway of biosynthesis of UDP-N-acetylglucosamine and CMP-N-acetylneuraminic acid in liver. The short dark arrow denotes the site of entry of injected glucosamine. The long dark arrows indicate sites of feedback inhibition.

α-D-glucosamine-1-P[5] and UTP in the presence of a crude yeast extract, and was isolated by paper chromatography in solvent II. dTDP-N-acetyl-D-glucosamine was prepared from N-acetyl-D-glucosamine-1-P and dTMP-morpholidate.[6] CMP-NAN was prepared enzymatically by the method of Warren and Blacklow,[7] using a crude extract of *Neisseria meningitides* which was kindly provided by Dr. Mergenhagen. The ratio of cytidine to N-acetylneuraminic acid was unity after purification in solvent I. GDP-D-mannose and GDP-L-fucose were gifts of Dr. V. Ginsburg. CDP-D-glucose and CDP-paratose were gifts of Dr. R. Mayer. Other nucleotides and sugar nucleotides were purchased from commercial sources. D-Glucosamine-6-P was a gift of Dr. L. Glaser. The crystalline barium salt of D-fructose-6-P was obtained from Boehringer and Sons. Puromycin and the aminonucleoside of puromycin were purchased from Nutritional Biochemicals Corp.

The following chromatographic solvents were used: I, ethanol–1 *M* ammonium acetate, pH 7.5 (75:30); II, ethanol–1 *M* ammonium acetate, pH 3.8 (75:30); III, isobutyric acid–1 *M* NH_4OH (10:6); IV, isoamyl acetate–acetic acid–water (3:3:1).

UDP-N-acetylhexosamine was isolated from rat livers by ethanol extraction, adsorption to and elution from charcoal, and paper chromatography in solvents I and II.[8] The method was modified in the experiment described in Figure 2 by substituting electrophoresis at pH 3.7 for the charcoal adsorption step, and by chromatography of the product in solvents I, II, and III. Radioactivity of the purified sugar nucleotides was determined in a liquid scintillation spectrometer, in a system containing 0.5 ml of water and 8.0 ml of Bray's solution.[9]

Preparation and assay of enzymes from rat liver: L-Glutamine-D-fructose-6-P transaminase was prepared by the method of Pogell and Gryder.[10] The ammonium sulfate fraction (purified fourfold over the 18,000 × *g* supernatant solution) was subjected to filtration through Sephadex G-25 prior to use. The enzyme was assayed in a reaction mixture consisting of 30 μmoles sodium phosphate buffer of pH 7.6, 12 μmoles L-glutamine, 5 μmoles D-fructose-6-P, and the enzyme, in a total volume of 1 ml. Incubation was carried out for 30 min at 37° and terminated by heating for 1 min at 100°. The suspension was then centrifuged and an aliquot of the supernatant solution assayed for hexosamine by the method of Ghosh *et al.*[11]

The enzyme which converts UDPAG to N-acetyl-D-mannosamine (UDPAG 2-epimerase) was prepared by a slight modification of the method of Cardini and Leloir.[12] The assay was carried out in a reaction mixture which contained 30 μmoles Tris buffer of pH 7.5, 15 μmoles $MgSO_4$, 5 μmoles cysteine, 0.5 μmole C^{14}-labeled UDPAG (2400 cpm), and the enzyme, in a final volume of 0.2 ml. After a 15-min incubation the reaction mixture was heated for 1 min at 100°. One milliliter of a suspension of Dowex 1-X8 formate, containing enough resin (130 mg dry weight) to adsorb all the remaining C^{14}-UDPAG, was added. An aliquot of the supernatant fluid, which contained the neutral N-acetylmannosamine, was plated and counted in an end-window gas flow counter. N-acetylmannosamine was shown to be the only neutral product by paper electrophoresis in 0.05 *M* sodium tetraborate. The rate of N-acetylmannosamine liberation was linear for at least 30 min and was proportional to enzyme concentration.

Glucosamine-6-P transacetylase was made by fractionating the supernatant solution from 100,000 × *g* centrifugation with solid $(NH_4)_2SO_4$ between 40% and 70% saturation. The reaction mixture contained 40 μmoles of Tris buffer, pH 8.0, 4 μmoles $MgCl_2$, 0.3 μmole EDTA, 1.0 μmole CoA, 4.5 μmoles ATP, 4 μmoles Na acetate, 3 μmoles cysteine, 3 μmoles D-glucosamine-6-P, and

the enzyme, in a total volume of 0.7 ml. After 30 min at 37° the reaction was stopped by heating at 100° and an aliquot was assayed for the appearance of acetylhexosamine by the method of Levvy and McAllan.[13]

Although only partially purified, the glutamine–fructose-6-P transaminase and the glucosamine-6-P transacetylase preparations did not catalyze the breakdown of UDPAG. Similarly, CMP-NAN was stable when incubated with the enzyme forming acetylmannosamine.

Results and Discussion.—Demonstration of feedback control in vivo: The original experiments of this series were carried out as part of a study of glycoprotein biosynthesis in the liver. Other investigators have shown that D-glucosamine-C^{14}, administered to rats, is efficiently incorporated by the liver into protein-bound N-acetylglucosamine and N-acetylneuraminic acid.[14, 15] The completed glycoproteins are then secreted into the blood stream.[16]

In order to study the sugar nucleotide precursors of glycoproteins, rats were simultaneously treated with puromycin to stop protein synthesis[17] and with glucosamine-C^{14} to label the nucleotide-bound N-acetylhexosamine pool. It was reasoned that if the synthesis of protein molecules were inhibited, the sugar nucleotide precursors of the carbohydrate chains of the glycoproteins should accumulate. The results of this experiment are shown in Figure 2. In the puromycin-treated and in the control rats, UDP-N-acetylhexosamine [UDP(AG)] accounts for most of the ethanol-soluble radioactivity at the three points selected for study.[18] In the control animals, both total activity and specific activity of the UDP(AG) pool were high 15 min after injection and declined to one tenth this value in 2 hr. In the puromycin-treated animals, incorporation of labeled glucosamine into sugar nucleotide occurred normally, but during the 2-hr experimental period there was no loss of counts from the UDP(AG) pool—no utilization of that pool had occurred. Furthermore, it appeared that the synthesis of UDP(AG) had also been impaired, since its specific activity did not fall in 2 hr, during which time the entire pool of UDP(AG) had turned over at least once in the control animal.[19] If synthesis had proceeded normally in the absence of utilization, the specific activity of UDP(AG) would have fallen to one third to one half its original value.

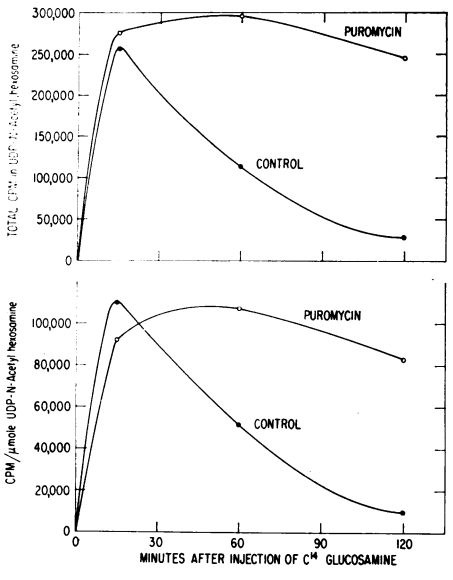

FIG. 2.—Incorporation of glucosamine-C^{14} into UDP-N-acetylhexosamine of liver in control and puromycin-treated rats. Three rats (male Sprague-Dawley, 210 gm) were injected intraperitoneally at zero time with 1 ml of saline, and three with 1 ml of saline containing 15 mg of puromycin. A second dose was administered at 1 hr to the two animals which were kept for the 2-hr period. Glucosamine-C^{14} (2.5 μc, 0.25 μmole, in 0.5 ml of saline) was injected into the tail vein at zero time. All injections were performed under light ether anesthesia. At the times indicated, the animals were sacrificed, and the UDP-N-acetylhexosamine was isolated from the liver and counted as described in *Methods*.

The cessation of UDP(AG) synthesis in the absence of utilization could have been due to depletion of available UTP when the normal cycle of UTP regeneration was interrupted, or to a feedback inhibition by the UDP(AG) on some enzymatic step of

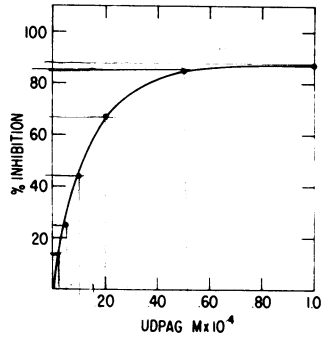

FIG. 3.—Inhibition of L-glutamine–D-fructose-6-P transaminase by UDP-N-acetylglucosamine. UDPAG was added to the standard reaction mixture (see *Methods*) to the indicated concentration.

its biosynthetic pathway. In a preliminary trial, glucosamine-C^{14} was injected 30 min after the administration of puromycin; it was incorporated into UDP(AG) in a normal fashion, indicating that UTP was still available and that the activity of the enzymes converting glucosamine-6-P to UDPAG (see Fig. 1) had not been affected. The inhibited step was postulated to be the one between fructose-6-P and glucosamine-6-P.

This hypothesis was verified by administering simultaneously glycerol-C^{14} and glucosamine-H^3 to rats which had been pretreated with puromycin. Fifteen minutes after administration of the isotopes, the animals were sacrificed, and the incorporation of C^{14} and H^3 into the UDP(AG) pool of the liver was determined. The results are shown in Table 1. In the animals pretreated with puromycin the incorporation of glucosamine-H^3 into UDP(AG) was comparable to that in control animals, but the incorporation of glycerol-C^{14} into nucleotide-linked acetylhexosamine[20a] was markedly depressed. The animal pretreated with the aminonucleoside of puromycin, which does not inhibit protein synthesis or prevent the normal turnover of UDP(AG), gave a result similar to controls.[21]

The results of the experiments described above may best be explained by postulating that a mechanism of feedback inhibition is operating to control the biosynthesis of UDP(AG) in the liver. When UDP(AG) utilization stops because glycoprotein synthesis is impaired by puromycin, UDPAG prevents further synthesis of itself by inhibiting the first enzyme unique to its pathway, i.e., glutamine–fructose-6-P transaminase. As a result, the incorporation of glycerol-C^{14} into UDP(AG) is inhibited in rats pretreated with puromycin, since glycerol *must* be converted to fructose-6-P before it can be incorporated into the hexosamine position of UDP(AG). The entry of glucosamine, on the other hand, bypasses this control step; it is therefore not affected by the feedback inhibition and its incorporation into UDPAG proceeds at a normal rate.

Inhibition of L-*glutamine*–D-*fructose-6-P transaminase:* The transaminase isolated from rat liver was found to be markedly inhibited by UDPAG (Fig. 3).

TABLE 1

INCORPORATION OF GLUCOSAMINE-H^3 AND GLYCEROL-C^{14} INTO UDP-N-ACETYLHEXOSAMINE OF RAT LIVER

Pretreatment	UDP(AG), recovered, μmoles	H^3, cpm/μmole	C^{14}, cpm/μmole	Ratio C^{14}/H^3
Saline	2.4	1010	1280	1.26
Saline	3.2	630	920	1.46
Aminonucleoside of puromycin	2.3	580	780	1.36
Puromycin	2.0	1280	450	0.35
Puromycin	2.5	740	360	0.49

Male Sprague-Dawley rats (220 gm) were injected intraperitoneally with either 1 ml of saline, 1 ml of saline containing 30 μmoles aminonucleoside, or 1 ml of saline containing 30 μmoles of puromycin. Forty-five minutes later 0.5 ml of saline containing 2.7 μmoles of glycerol-C^{14} (6.5×10^6 cpm) and 0.2 μmole of glucosamine-H^3 (28,000 cpm) were injected into the tail vein. After 15 min, the rats were sacrificed, the livers removed, and UDP-N-acetylhexosamine was isolated and counted (*Methods*). Counts of H^3 and C^{14} were computed by the discriminator ratio method of Okita *et al.*[20]

The kinetics of this inhibition are of the competitive type (Fig. 4), i.e., UDPAG increases the K_m for fructose-6-P without changing the maximal velocity. From the data in Figure 4, the K_m for fructose-6-P was calculated to be 8×10^{-4} M, and the K_i for UDPAG, 5×10^{-6} M.[22] Since the concentration of UDP(AG) in a 10-gm liver is approximately 2–3 μmoles, the K_i is certainly within a physiological range.

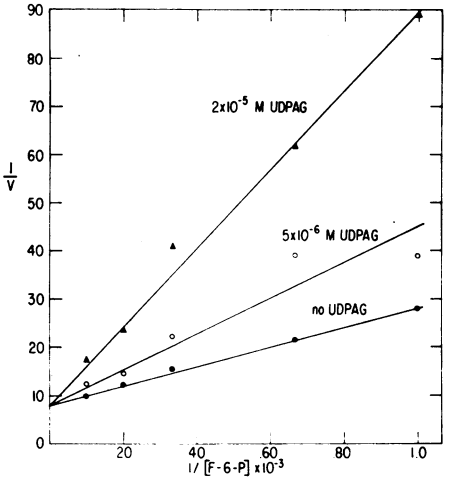

FIG. 4.—Effect of UDP-N-acetylglucosamine on the K_m for fructose-6-P of glutamine–fructose-6-P transaminase. The reaction mixtures contained 35 μmoles phosphate buffer, pH 7.6, 20 μmoles L-glutamine, and enzyme, in 1 ml. Fructose-6-P and UDP-N-acetylglucosamine were added at the indicated concentrations. The velocity, V, is expressed as μmoles of glucosamine-6-P formed in 30 min.

The inhibition of the enzyme by UDPAG is quite specific, as shown in Table 2. The specificity for the nucleotide portion of the molecule seems to be more stringent than that for the sugar residue, since UDP-D-glucose at 1×10^{-3} M inhibits as well as UDPAG at 2×10^{-5} M, while 1×10^{-3} M dTDP-N-acetylglucosamine fails to inhibit at all. N-acetylglucosamine-1-P, a precursor of UDPAG, does not inhibit at a concentration of 0.5×10^{-3} M, providing evidence that this is truly an end-product inhibition. Puromycin at 0.6×10^{-3} M had no effect on the enzyme.

UDPAG at 7×10^{-4} M had no inhibitory effect on glucosamine-6-P transacetylase, the second enzyme on the pathway to UDP-acetylhexosamine formation.

These findings are in agreement with the results of the experiments performed *in vivo*. The transaminase, which is the first enzyme of the pathway, is inhibited by the end product, UDPAG, while the subsequent steps are not affected. This situation is similar to that observed in other systems of feedback inhibition in which only the first enzyme in the pathway is affected by the inhibitor.[1]

TABLE 2

EFFECT OF VARIOUS SUGAR NUCLEOTIDES AND RELATED COMPOUNDS ON L-GLUTAMINE-D-FRUCTOSE-6-P TRANSAMINASE

Compound	Concentration, M	Inhibition, %
UDP-N-acetyl-D-glucosamine	2×10^{-5}	66
UDP-D-glucose	5×10^{-4}	50
UDP-D-glucose	1×10^{-3}	70
dTDP-N-acetyl-D-glucosamine	1×10^{-3}	0
N-acetyl-α-D-glucosamine-1-P	5×10^{-4}	0
ADP-D-glucose	1.5×10^{-3}	0
dTDP-D-glucose	1×10^{-3}	0
5-Fluoro UDP-D-glucose	1×10^{-3}	0
UDP-D-glucuronic acid	5×10^{-4}	31
GDP-D-mannose	6×10^{-4}	10
GDP-L-fucose	6×10^{-4}	28
UDP	1.6×10^{-3}	18
N-acetylneuraminic acid	1×10^{-3}	0
CMP-N-acetylneuraminic acid	5×10^{-4}	0

The compounds were added to the standard reaction mixture (*Methods*) to give the indicated concentrations.

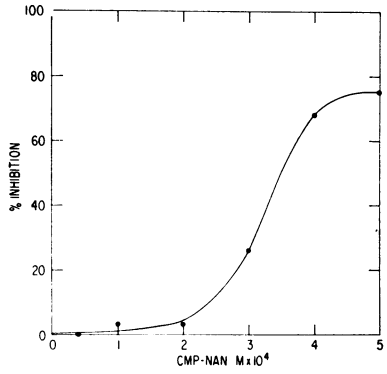

FIG. 5.—Inhibition of UDP - N-acetylglucosamine 2 - epimerase by CMP-N-acetylneuraminic acid. CMP-NAN was added to the standard assay mixture (see *Methods*) to the indicated concentration.

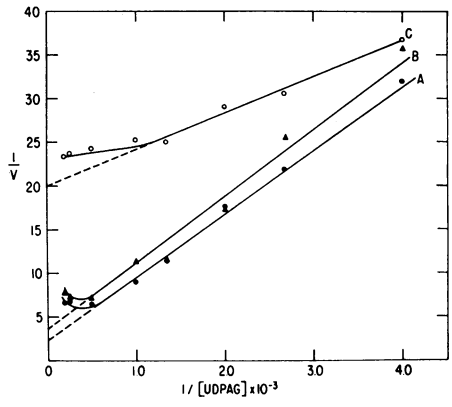

FIG. 6.—Effect of CMP-N-acetylneuraminic acid on V_{max} and K_m of the UDP-N-acetylglucosamine 2-epimerase. The standard reaction mixture was used except that UDPAG was varied as indicated. Curves *A*, *B*, and *C* represent the presence of 0, 2.3×10^{-4} *M* and 4.6×10^{-4} *M* CMP-NAN, respectively. The velocity, *V*, is expressed as μmoles of N-acetylmannosamine formed in 20 min.

Inhibition of UDP-N-acetylglucosamine 2-epimerase by CMP-NAN: It was of interest to determine whether the pathway leading to the synthesis of CMP-N-acetylneuraminic acid was subject to a similar kind of feedback inhibition. One would expect *a priori* that the organism has some mechanism for controlling this entirely irreversible pathway (see Fig. 1), which would otherwise be a constant drain on the supply of UDPAG. The flow of substrate through this pathway must be quantitatively significant, since the N-acetylneuraminic acid and N-acetylglucosamine residues which occur in plasma glycoproteins in a ratio of, on the average, 1 to 2,[23] are labeled at comparable rates.[14, 15] If that flow continued at its usual rate in the presence of puromycin, the label from the injected glucosamine would have been recovered predominantly in CMP-NAN, rather than in UDP(AG), as had been shown.

UDPAG 2-epimerase isolated from the liver is inhibited by CMP-NAN as shown in Figure 5. First it should be noted that the concentration of CMP-NAN required for 50 per cent inhibition of the enzyme is 30 times greater than the concentration of UDPAG required for 50 per cent inhibition of the transaminase. Second, the sigmoidal shape of the inhibition curve suggests that the affinity of the enzyme for CMP-NAN increases as it becomes more saturated with the inhibitor. It has been proposed that such sigmoidal curves may be due to the interaction of subunits.[24, 25] Binding of CMP-NAN by UDPAG 2-epimerase appears not only to change the affinity of the enzyme for the inhibitor, but also its affinity for the substrate, UDPAG. As shown in Figure 6, CMP-NAN decreases both the K_m for UDPAG and the maximum velocity of the reaction. At low substrate concentration these effects tend to cancel one another and produce little or no inhibition, while at high substrate concentrations the K_m effect is minimized and the decrease in velocity is apparent. This is best illustrated when the concentration of CMP-

NAN is high (Fig. 6, curve *C*). The kinetics of this reaction are further complicated by substrate inhibition at high substrate concentrations.

The effect of various nucleotides and sugar nucleotides on the epimerase is shown in Table 3. This table illustrates the high specificity required of the inhibitor. No other compound tested, including N-acetylneuraminic acid and CMP, shows significant inhibition even when present in high concentrations.

TABLE 3

EFFECT OF VARIOUS SUGAR NUCLEOTIDES AND RELATED COMPOUNDS ON UDP-N-ACETYLGLUCOSAMINE 2-EPIMERASE

Compound	Concentration, *M*	Inhibition, %
CMP-N-acetylneuraminic acid	4×10^{-4}	68
CMP-N-acetylneuraminic acid (heated at 100° for 2 min in 0.01 *N* HCl)	4×10^{-4}	0
N-acetylneuraminic acid	5×10^{-3}	13
5'-CMP	2.5×10^{-3}	0
CDP	2.5×10^{-3}	10
CTP	2.5×10^{-3}	10
CDP-D-glucose	9×10^{-4}	0
CDP-paratose	9×10^{-4}	0
GDP-L-fucose	1.2×10^{-3}	14
UDP-D-glucose	1×10^{-3}	−9
N-acetylneuraminosyl lactose	2.5×10^{-3}	0

The compounds were added to the standard assay mixture (*Methods*) to give the concentrations indicated.

The concentration of CMP-NAN required to inhibit the UDPAG 2-epimerase *in vitro* raises an interesting problem. The intracellular concentration of this nucleotide probably does not approach the concentration needed for inhibition. How then does the control mechanism operate *in vivo?* In view of the extensive intracellular organization of mammalian cells, it may be that the concentration of the inhibitor is sufficiently high at the site of enzyme action to have a significant effect.

Possible physiological role of the feedback control of sugar nucleotide synthesis: One may postulate at least two benefits which the control of the synthesis of UDP-N-acetylhexosamine and CMP-N-acetylneuraminic acid bring to the animal. Control of glutamine–fructose-6-P transaminase renders the synthesis of UDPAG relatively independent of the level of hexose-6-P, which fluctuates widely in response to feeding[26] or changes in the rate of glycolysis.[27] Furthermore, when the rate of glycoprotein synthesis is altered, the animal is enabled to adjust rapidly the rate of synthesis of UDPAG and CMP-NAN to the requirement for these compounds. The level of the enzymes in the pathways to the sugar nucleotides may thus be kept high enough to cope with increased demand, without imposing on the animal the burden of continuous overproduction of sugar nucleotides.

Preliminary experiments point out some possible consequences of uncontrolled synthesis of UDPAG. A single injection of 50 μmoles of glucosamine increased the concentration of UDP(AG) in the liver by 3 μmoles in 15 min, thus doubling the size of the pool. One rat, infused with 5 per cent glucosamine for 2.5 hr, had 17 μmoles of UDP(AG) in the liver. Such an enormous expansion of the UDP(AG) pool may strain the ability of the liver to maintain a proper level of other uridine-containing nucleotides, with consequent aberrations not only in the metabolism of compounds containing galactose or glucuronic acid, but also in the synthesis of nucleic acid and protein.

There are conditions, such as exposure to penicillin, which will cause bacteria to accumulate large amounts of aminosugar nucleotides.[28] This reflects a less rigid control of the production of these compounds in bacteria than in rat liver. To date, we have not been able to demonstrate any effect of UDPAG on the glutamine–fructose-6-P transaminase isolated from *Salmonella paratyphi* A and *Escherichia coli* B. The possibility that bacteria regulate aminosugar nucleotide biosynthesis by repression-derepression or by feedback control of enzymes other than the transaminase has not been investigated.

Summary.— UDP-N-acetylglucosamine inhibits L-glutamine–D-fructose-6-phosphate transaminase, the first enzyme unique to its biosynthesis. Similarly, the formation of N-acetylmannosamine by UDP-N-acetylglucosamine 2-epimerase is inhibited by the end product, CMP-N-acetylneuraminic acid. Compounds closely related to these inhibitors have little or no effect.

The feedback inhibition of the L-glutamine–D-fructose-6-phosphate transaminase has been demonstrated *in vivo* by the administration of puromycin, which prevents the utilization of UDP-N-acetylglucosamine.

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[18] Ten minutes after injection of glucosamine-C^{14}, the radioactive UDP-N-acetylhexosamine consists of an equilibrium mixture of 2 parts of UDP-N-acetylglucosamine and 1 part UDP-N-acetylgalactosamine.[8]

[19] The half life of UDP(AG) estimated from the three points in Fig. 2 is 45 min. A better approximation based on a larger number of rats gave a value of 30 min.[8] These calculations are based on the reasonable but unproved assumption that the labeled UDP(AG) mixes randomly with the pre-existing pool(s).

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[21] It has been reported that both puromycin and its aminonucleoside stimulate glycogenolysis [Hofert J. F., and R. K. Boutwell, *Arch. Biochem. Biophys.*, **103**, 338 (1963)]. The possibility existed that in the animals treated with puromycin, the incorporation of glycerol into UDP(AG) was depressed because of dilution of glycolytic intermediates resulting from glycogen degradation. The similarity of the results obtained with the aminonucleoside-treated rat and the control rats shows that such dilution of the C^{14} label did not occur to a significant extent.

[22] As the transaminase preparation contained enough phosphoglucose isomerase to convert the

substrate, fructose-6-P, to an equilibrium mixture with glucose-6-P, it was necessary to apply a correction to the concentration of fructose-6-P added in order to obtain its *actual* concentration in the reaction mixture. The K_m thus obtained for fructose-6-P is in fairly good agreement with the value of 3.8×10^{-4} *M* reported by Ghosh *et al.*[11] for a purified preparation of rat liver enzyme which was free of isomerase.

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*DELAYED FLUORESCENCE IN DNA-ACRIDINE DYE COMPLEXES**

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The DNA-acridine dye complexes have been studied extensively by a variety of techniques. Such studies are of importance for a number of reasons. A principal biological interest centers around the attempt to understand the powerful mutagenic activity of many acridine dyes on T2 and T4 bacteriophages.

Two different types of complexes have been distinguished (Steiner and Beers, 1961; Bradley and Wolf, 1959; Bradley and Felsenfeld, 1959). The first, termed Complex I, occurs at high dye to nucleotide ratios. Another type of complex, Complex II, occurs at low dye to nucleotide ratios. This paper will deal exclusively with certain phosphorescence properties of Complex II. Lerman (1961, 1963) has presented evidence indicating that Complex II has a configuration in which a dye molecule is intercalated between the base pairs of DNA.

Work on the quenching of DNA phosphorescence by Mn^{++} ions has indicated that an excited triplet in DNA is delocalized (Bersohn and Isenberg, 1964). Such a delocalization suggests that a phosphorescence study of DNA-acridine dye complexes might be fruitful. While singlet transitions from one molecule to another may occur via long-range dipole-dipole coupling, triplet migration presumably occurs only via wave-function overlap. It might be expected, therefore, that an intercalated dye molecule would interfere with the migration of triplet excitation. Conversely, any interference or interaction that could be shown might serve as a verification of the intercalation model. As will be seen, not only was the expected interaction observed, but this took the unexpected form of an energy transfer from an excited triplet of DNA to an excited singlet of the dye. An analysis of the data shows that the observed energy transfers are reasonable on the assumption of an intercalation model and may be taken as a verification of this model.