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Nucleotide Activation of Liver Microsomal Glucuronidation*

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The classical studies of Dutton and Storey (1) have shown that liver microsomes catalyze the transfer of glucuronic acid from uridine diphosphate glucuronic acid to phenols. In an attempt to detect a reaction between uridine diphosphate glucuronic acid and uridine diphosphate *N*-acetylglucosamine, the effect of the latter on phenol glucuronidation was tested. It was expected that a reaction between the two nucleotides should decrease glucuronidation. Contrary to the expectation, increased formation of phenyl glucuronide was obtained on adding uridine diphosphate *N*-acetylglucosamine. The largest effect was produced with rat liver microsomes and was further increased by addition of adenosine triphosphate. Inasmuch as sugar nucleotides such as uridine diphosphate glucose or guanine diphosphate mannose could not replace uridine diphosphate *N*-acetylglucosamine, it was considered of interest to study the phenomenon in more detail.

EXPERIMENTAL PROCEDURE

Methods and Materials—*o*-Aminophenol was purified by sublimation. *p*-Nitrophenol was recrystallized from ethanol-water. Crystalline barium ATP, UDP-glucuronic acid (NH_4^+, 90% purity), and the uridine phosphates were obtained from the Sigma Chemical Company. UDP-*N*-acetylglucosamine, UDP-glucose and GDP-mannose were isolated from yeast by the procedure of Pontis *et al.* (2). Potassium glucuronic acid-1-P was prepared by chemical oxidation of glucose-1-P (3). *N*-Acetylglucosamine-1-P was prepared chemically (4).

The solvents used in chromatography were: Solvent A: ethanol-1 M ammonium acetate (75:30); Solvent B: ethanol-1 M ammonium acetate, pH 3.8 (75:30) (5).

Guinea pig microsomes were prepared by homogenizing the exsanguinated liver in 4 volumes of 0.154 M KCl, discarding the precipitate obtained after 10 minutes of centrifugation at 2,000 × *g*, and then collecting the precipitate after 90 minutes of centrifugation at 18,000 × *g*. The precipitate was washed twice in an equal volume of KCl with 60 minutes of centrifugation each time, and finally resuspended in the same volume of KCl. All operations were performed at 0–4°.

Rat liver microsomes were prepared similarly with the following modifications. Homogenization was in 3 volumes of KCl, the washings were usually with water with 20 to 30 minutes of centrifugation, and the final precipitate was resuspended in 0.2 to 0.3 of the original homogenate volume with water. These preparations were not stable for storage when frozen.

UDP-transglucuronylase was followed in most of the experiments with guinea pig liver by measurement of *o*-aminophenol conjugation, with the final incubation volumes reduced to 0.2 ml (1). Saturation of the enzyme with *o*-aminophenol was found at 0.5 mM, and near saturation with UDP-glucuronic acid at 4.0 mM. A modification of the method of Isselbacher (6), which measures *p*-nitrophenol disappearance, was used for most of the rat microsome studies. The system contained 0.03 ml of 0.7 mM *p*-nitrophenol in 0.5 M phosphate buffer (pH 7.5), 0.03 ml of 1.1 mM UDP-glucuronic acid, and 0.04 ml of rehomogenized microsomes, final volume, 0.1 to 0.15 ml. The tubes were incubated at 37° with shaking. The reaction was stopped by the addition of 0.4 ml of 0.2 N trichloroacetic acid. After centrifugation and addition of 0.02 ml of 10 N KOH to the supernatant fluid, the color was read at 400 mμ. Suitable controls were always included, and all experiments were run in duplicate.

Preparation of Labeled UDP-glucuronic Acid—UDP-glucuronic acid dehydrogenase was partially purified by carrying a calf liver acetone powder extract through the first ammonium sulfate precipitation as described by Strominger *et al.* (7), dissolving the precipitate in a small volume of water and dialyzing overnight against 0.02 M sodium acetate (pH 5.9), centrifuging the dialyzed material and discarding any precipitate formed.

A mixture of 0.86 μmole of unlabeled UDP-glucose, 0.21 μmole of C^{14}-hexose-labeled UDP-glucose (200,000 c.p.m.) (8), 4 μmoles of DPN (pH 7), 50 μmoles of sodium glycinate (pH 8.7), 90 μmoles of EDTA[1] (pH 7.7), and a large excess of UDP-glucose dehydrogenase in a final volume of 9.2 ml was adjusted to pH 8.6 to 8.8 with dilute ammonia and incubated at room temperature. The reaction was followed by reading the absorbancy of an aliquot at 340 mμ against an appropriate blank with no UDP-glucose added. After there was no further increase in absorbancy (30 minutes), the reaction was stopped by adjusting to pH 5 with acetic acid and placing the mixture in boiling water for 1 minute with constant stirring. The mixture was cooled, the denatured protein removed by centrifugation, and the precipitate washed with 2 ml of water plus a drop of 1 N acetic acid. Nucleotides were isolated by adsorption and elution of the combined supernatants from a column prepared with 3 ml of a 5% Norit A and 5% Celite suspension (2). The column was then washed successively with 5 ml of water, 3 ml of 0.01 M EDTA (pH 7), and 3 ml more of water. The nucleotides were eluted with 18 ml of 50% ethanol, and this solution was adjusted to pH 6.7 to 6.9 followed by evaporation to dryness in a vacuum at 30–40°. The UDP-glucuronic acid was obtained in pure form by paper electrophoresis and chromatography as described by Strominger and Mapson (9). Separation of the UDP-glucuronic

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[1] The abbreviation used is: EDTA, ethylenediaminetetraacetate.

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acid from DPN, DPN side products, and most of the UDP-glucose was achieved by paper electrophoresis for 2 hours at 14 volts per cm in 0.1 M ammonium acetate buffer (pH 5.2) containing 0.001 M EDTA. The apparatus described by Markham and Smith (10) was used for this step. Further descending chromatography of the UDP-glucuronic acid eluate for 45 hours in Solvent A removed any remaining traces of UDP-glucose. The area of paper corresponding to UDP-glucuronic acid was washed for 2 hours in 95% ethanol and then eluted with water.

The purity of the radioactive UDP-glucuronic acid was confirmed by chromatography in Solvent B. Only one radioactive spot was found, identical with a UDP-glucuronic acid standard. If EDTA was omitted during incubation with the crude UDP-glucose dehydrogenase, no UDP-glucuronic acid was formed and only non-nucleotide glucuronic acid was found. This was probably due to pyrophosphatase activity in the crude calf liver extract.

RESULTS

Studies with Rat Liver Microsomes—Addition of UDP-*N*-acetylglucosamine to rabbit liver microsomes produced a 60% increase in UDP-transglucuronylase activity, and serum albumin addition caused no further increase. Still larger increases were obtained with rat liver microsomes. Since UDP-transglucuronylase activity in rat liver is rather low, measurement of *p*-nitrophenol disappearance, which is a more sensitive method, was used. All of the experiments were done with freshly prepared microsomes, because rat liver microsomes lose most of their activity upon storage at −10°.

An experiment showing the effects of different UDP-*N*-acetylglucosamine concentrations on rat UDP-transglucuronylase is shown in Fig. 1. Saturation was reached at about 0.3 mM. The relative specificity of this activation was confirmed by showing that chromatographically pure UDP-glucose, GDP-mannose, and uridine produced no activation. An increase in the rate of glucuronidation was produced by ATP when added alone or in the presence of excess UDP-*N*-acetylglucosamine. Very large activations were observed when both ATP and UDP-*N*-acetylgucosamine were added together (as high as 25-fold in some experiments). Some typical experiments are shown in Table I. It should be noted the combined effects of both nucleotides was more than additive in some cases. Small activations were produced by AMP and UTP, and these two nucleotides also increased the effect of UDP-*N*-acetylglucosamine, but the increases were smaller than with ATP. Control experiments

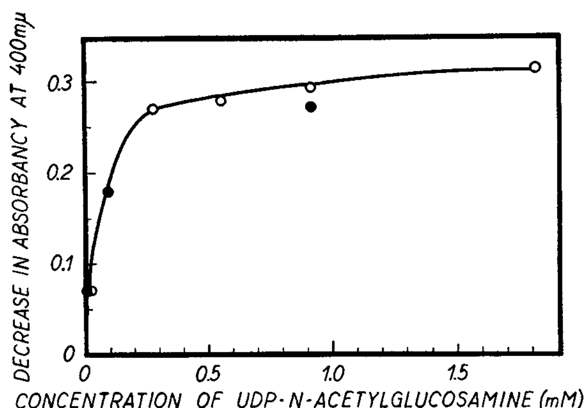

FIG. 1. Effect of UDP-*N*-acetylglucosamine on *p*-nitrophenol glucuronidation by rat liver microsomes. Analyses as described in *Methods and Materials*. ○——○, incubation for 60 minutes at 37° with shaking; ●——●, same microsomes frozen, thawed, and washed in isotonic KCl, incubation for 90 minutes at 37° with 3 times as much enzyme.

TABLE I

Effect of nucleotide additions on rat liver microsomal UDP-transglucuronylase

Analyses were with *p*-nitrophenol as described in *Methods and Materials*.

Experiment	Additions	Incubation time	Decrease in absorbancy at 400 mμ	Activation factor
		min		
1	None	60	0.016	
	UDP-*N*-acetylglucosamine (1 mM)	60	0.052	3.2
	ATP (2 mM)	60	0.081	5.1
	UDP-*N*-acetylglucosamine (1 mM) + ATP (2 mM)	37	0.421*	26.3
	AMP (2 mM)	60	0.057	3.6
	UDP-*N*-acetylglucosamine (1 mM) + AMP (2 mM)	60	0.168	10.5
2	None	60	0.051	
	UDP-*N*-acetylglucosamine (0.9 mM)	60	0.125	2.5
	ATP (1.8 mM)	60	0.124	2.4
	UDP-*N*-acetylglucosamine (0.9 mM) + ATP (1.8 mM)	60	0.263	5.2
	ATP (9 mM)	60	0.139	2.7
	AMP (1.8 mM)	60	0.079	1.5
3†	None	20	0.018	
	UDP-*N*-acetylglucosamine (1 mM) + ATP (5 mM)	20	0.301 (0.435)	17 (24)
4	None	30	0.054	
	UDP-*N*-acetylglucosamine (1.4 mM)	30	0.158	2.9
	ATP (2 mM)	30	0.142	2.6
	UDP-*N*-acetylglucosamine (1.4 mM) + ATP (2 mM)	30	0.330 (0.489)	6.1 (9.0)
	UTP (2 mM)	30	0.075	1.4
	UDP-*N*-acetylglucosamine (1.4 mM) + UTP (2 mM)	30	0.266	4.9

* Calculated for 60 minutes.

† No buffer added, all solution at pH 7.

without UDP-glucuronic acid showed no p-nitrophenol conjugation with ATP or UDP-N-acetylglucosamine added individually or at the same time.

EDTA produced an activation of rat liver UDP-transglucuronylase, rather than the inhibition observed with guinea pig (Fig. 2). The activation by UDP-N-acetylglucosamine and ATP was decreased from 3.6-fold to 2-fold with EDTA present at 10 mM.

Preincubation experiments with rat liver microsomes showed that UDP-N-acetylglucosamine and ATP prevented the disappearance of UDP-glucuronic acid as measured by subsequent incubation with fresh rat or guinea pig microsomes and p-nitrophenol. This indicated that the activation in rat liver might be due to a competitive substrate effect, the added nucleotides preventing UDP-glucuronic acid breakdown. If this were the case, then in the presence of excess UDP-glucuronic acid, there should be no further activation. The results of experiments to test this are shown in Fig. 3. It may be seen from the data of Experiments A and B that, even at high concentrations of UDP-glucuronic acid, absolute saturation of the enzyme with substrate was not obtained. However, at concentrations of UDP-glucuronic acid above 6.0 mM, at which there was near saturation with substrate, there was still a 1.6- to 2.3-fold increase in rates of glucuronidation produced by the addition of UDP-N-acetylglucosamine and ATP. Furthermore, this increase was produced by low levels of these nucleotides. The amounts of activation observed in these experiments were much lower than those found in the experiments in which the UDP-glucuronic acid concentration was 0.17 to 0.34 mM (see Table I). Thus, it appeared that part but not all of the activation could be explained by prevention of UDP-glucuronic acid breakdown.

Studies with Guinea Pig Liver Microsomes—Guinea pig liver microsomes have a higher UDP-transglucuronylase activity than those of rat. The microsomes prepared in isotonic KCl could be stored at −10° for several weeks with little loss of activity. One preparation when tested with rethawing each time had 86% of the original activity after 3 days, 95% after 5 days, and 81%

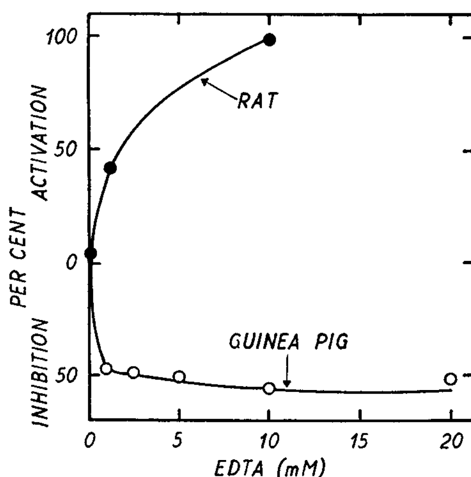

FIG. 2. Effect of EDTA on UDP-transglucuronylase in guinea pig and rat liver microsomes. Incubation for 30 minutes with shaking at 37°. *Guinea pig*—o-Aminophenol (0.75 mM), UDP-glucuronic acid (1.3 mM), UDP-N-acetylglucosamine (1.4 mM), liver microsomes, and EDTA incubated in 0.05 M potassium phosphate (pH 7.4). Analyses as described in *Methods and Materials*. *Rat*—analyses with p-nitrophenol as described in *Methods and Materials*. UDP-glucuronic acid concentration was 0.28 mM.

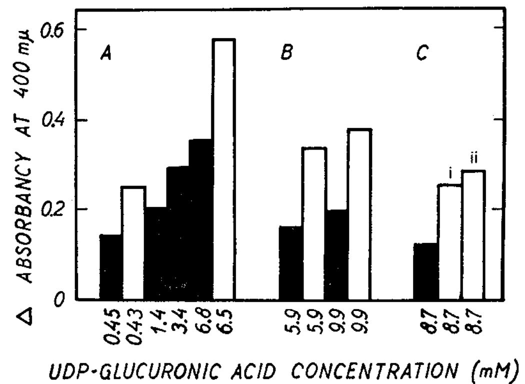

FIG. 3. Activation of rat liver microsomal UDP-transglucuronylase. Shaded bars without, empty bars with the addition of ATP and UDP-N-acetylglucosamine. The ATP and UDP-N-acetylglucosamine concentrations were, respectively, 2.0 and 1.4 mM in *A*, 1.8 and 1.2 mM in *B*, 1.5 and 1.1 mM in *C* (i), and 15 and 11 mM in *C* (ii). Analyses were with p-nitrophenol as described in *Methods and Materials*. Incubation time of 8 minutes in *A* and *C*, 5 minutes in *B*.

after 6 days. Activation by UDP-N-acetylglucosamine was not very great, but was increased by further addition of the dialyzed 18,000 × g supernatant. The largest increase in UDP-transglucuronylase found with guinea pig microsomes was 86% in the presence of excess UDP-N-acetylglucosamine and supernatant. Crystalline bovine serum albumin could replace the dialyzed supernatant, and this protein activation could be more clearly demonstrated with digitonin-solubilized microsomes (see next section).

No evidence for the accumulation of any intermediates was found by preincubation with various combinations of UDP-glucuronic acid, UDP-N-acetylglucosamine, microsomes, and dialyzed supernatant. When UDP-glucuronic acid, microsomes, and supernatant were incubated, the reaction mixtures boiled, and fresh microsomes, supernatant, and o-aminophenol added, there was no decrease in the amount of glucuronidation. This was found to be the case both in the presence and absence of UDP-N-acetylglucosamine. Thus, in the case of the guinea pig, the UDP-N-acetylglucosamine was not serving to protect the UDP-glucuronic acid from other enzymatic degradations. Addition of ATP had no effect on the guinea pig UDP-transglucuronylase with or without added UDP-N-acetylglucosamine.

EDTA over the concentration range from 1 to 20 mM produced a 50% inhibition of guinea pig o-aminophenol conjugation (Fig. 2). Similar inhibition of p-nitrophenol conjugation without UDP-N-acetylglucosamine or ATP addition also was observed. UDP was inhibitory (70% at 2.7 mM). However, UDP accumulation probably did not have any effect on the UDP-transglucuronylase, since the guinea pig microsomes contained an active UDP-phosphatase. No activation was produced by UDP-glucose (1.5 mM). Guinea pig microsomes lost only 20% of the original activity after 3 hours of incubation alone at 37°, and this decrease could be overcome by addition of UDP-N-acetylglucosamine and serum albumin.

Solubilization of UDP-Transglucuronylase with Digitonin—The guinea pig enzyme could be "solubilized" by treatment of the microsomes with digitonin. Optimal solubilization was obtained by treatment of 1 part of resuspended microsomes with 1.5 volumes of a 1% solution of digitonin (Merck, crystalline). After standing for 30 minutes with stirring in an ice bath, this

Table II

Effect of bovine serum albumin on digitonin-solubilized UDP-transglucuronylase

Analyses were by measurement of p-nitrophenol disappearance. Digitonin-solubilized enzyme was dialyzed overnight.

Experiment	Incubation time	Absorbancy decrease		Activation factor
		Additions		
		None	Serum albumin	
	min			
1	50	0.017	0.089 (0.46%)*	5.2
			0.117 (0.62%)	6.9
2	60	0.029	0.112 (0.15%)	3.9
3	90	0.047	0.118 (0.17%)	2.5

* Figures in parentheses are the final serum albumin concentrations in grams per 100 ml.

mixture was centrifuged for 10 minutes at 10,000 × g. About 40% of the original activity was found in the perfectly clear supernatant fluid, in contrast to the untreated UDP-transglucuronylase, which sedimented even without centrifugation. More activity could be extracted by a second digitonin treatment, but turbid supernatants were obtained after centrifugation at 10,000 × g. Such preparations could be stored at −10°, although there was some loss of activity. UDP-N-acetylglucosamine had little or no effect on the solubilized fractions, but a large activation was produced by serum albumin (Table II).

Studies with Radioactive UDP-Glucuronic Acid—Experiments were extended with labeled UDP-glucuronic acid in order to establish more definitively which reactions were occurring in the microsomes. The general procedure was to incubate UDP-glucuronic acid, rat liver microsomes, and other compounds at 37°. All the solutions were adjusted to pH 7 with dilute NH_3, and no buffer was added. The reactions were stopped by adding 3 volumes of 95% ethanol followed by acidification with acetic acid. The supernatant liquid plus four washings of the residue were evaporated to dryness at 30–40° after adjusting to pH 7 with ammonia. The samples were then chromatographed in Solvent B. The results of three such experiments are shown in Fig. 4. In Experiment A, radioactive UDP-glucuronic acid and microsomes alone were incubated for 30 minutes. Most of the UDP-glucuronic acid disappeared and two new spots corresponding to glucuronic acid-1-P and glucuronic acid appeared. The only visible ultraviolet spot corresponded to free uridine. In B, an experiment run at the same time is shown. It was identical, except that UDP-N-acetylglucosamine and ATP were also added. The same new spots appeared and there was more free glucuronic acid and less glucuronic acid-1-P radioactivity. The glucuronic acid radioactive spot both with and without the other nucleotide additions was eluted and found identical with glucuronic acid standards upon rechromatography in Solvent A. In Experiment C, a longer incubation experiment (60 minutes) with added UDP-N-acetylglucosamine and ATP is shown. There was no ultraviolet spot visible corresponding to UDP-glucuronic acid and all the radioactivity was in spots corresponding to glucuronic acid-1-P and glucuronic acid. The glucuronic acid spot was found to move the same as a glucuronic acid standard in

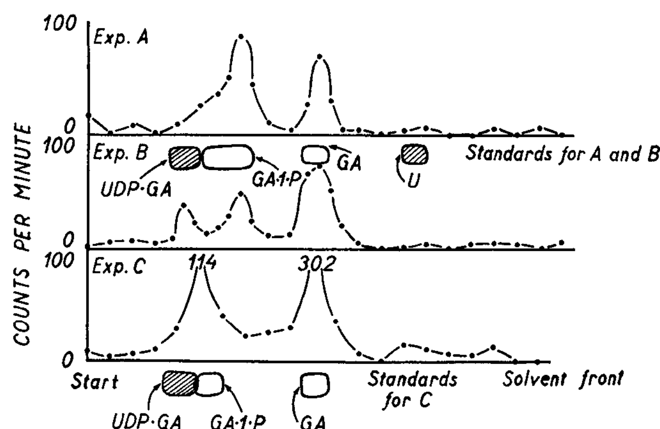

Fig. 4. Distribution of radioactivity in chromatograms in Solvent B. Incubation mixtures as described in text. Chromatograms were run on Whatman No. 1 paper cut as described by Matthias (11). Radioactivity was determined by cutting each strip into 1.8-sq. cm. pieces, and these were counted individually in a windowless flow counter. Pieces containing significant radioactivity were cut in half and each part recounted. In Experiment C, the numbers, *114* and *302*, refer to the radioactivity in counts per minute found at those positions on the chromatographic strip. UDP-glucuronic acid standards were located with an ultraviolet lamp and the sugars by alkaline $AgNO_3$ (12) with 1.5 N KOH in ethanol in place of 0.5 N and with heating of the paper over a steam bath in order to reveal glucuronic acid-1-P. The abbreviations used are: GA-1-P, glucuronic acid-1-phosphate; GA, glucuronic acid; UDP-GA, uridine diphosphate glucuronic acid; U, uridine.

tert-amyl alcohol-formic acid-water (4:1:1.5) (13), which separates glucuronic acid from iduronic acid.

The addition of p-nitrophenol to an incubation mixture of radioactive UDP-glucuronic acid, microsomes, UDP-N-acetylglucosamine, and ATP produced a large increase in the radioactive spot corresponding to glucuronic acid. In this experiment, there was hardly any radioactivity corresponding to glucuronic acid in the mixture incubated without the addition of the phenol. The formation of free glucuronic acid could have been caused by β-glucuronidase in the microsomal preparations, and the presence of this enzyme was in fact shown by measurement of phenolphthalein β-glucuronide hydrolysis at pH 7.1 by the method of Fishman *et al.* (14). DeDuve *et al.* (15) previously had shown that a portion of rat liver β-glucuronidase is present in the microsomal fraction. UDP-N-acetylglucosamine and ATP had no effect on this hydrolytic action.

Measurement of Phosphatase Activities—More evidence on the nature of the reactions being studied was obtained by measuring the liberation of inorganic phosphate. This data is summarized in Table III. At pH 7.6 in Tris-maleate buffer, inorganic phosphate was released by washed rat liver microsomes from UDP-glucuronic acid, ATP, UDP-glucose, UDP-N-acetylglucosamine, UMP, UDP and UTP, whereas N-acetylglucosamine-1-P and glucuronic acid-1-P were not hydrolyzed. However, with no buffer added, the microsomes hydrolyzed glucuronic acid-1-P slowly at pH 7.

DISCUSSION

The enzymatic reactions shown to occur in rat liver microsomes in the present investigation are summarized in diagram D 1.

TABLE III

Liberation of inorganic phosphate by action of rat liver microsomes

Substrates plus microsomes were incubated in a final volume of 0.1 ml in 0.1 M Tris-maleate buffer (pH 7.6) at 37° with shaking. Reactions were stopped by addition of 0.1 ml of 1 N trichloroacetic acid and inorganic phosphate determined by the method of Fiske and SubbaRow (16) with final volumes reduced to 0.5 ml.

Substrates	Amount added	Incubation time	Inorganic phosphate formed
	μmoles	*hrs*	*μmoles*
UDP-glucuronic acid	0.23	1	0.20
UDP-glucuronic acid	0.11	1	0.09
UDP-glucuronic acid	0.11	2	0.11
UDP-glucuronic acid	0.11	3	0.10
UDP-*N*-acetylglucosamine	0.28	1	0.14
UDP-glucose	0.25	1	0.37
UTP	0.20	1	>0.5
UDP	0.27	1	>0.5
UMP	0.26	1	0.14
ATP	0.20	1	0.45
N-Acetylglucosamine-1-P	0.34	1	<0.01
Glucuronic acid-1-P	0.31	1	0.00
Glucuronic acid-1-P	0.31	3	<0.02
Glucuronic acid-1-P	0.28	1*†	0.00
Glucuronic acid-1-P	0.28	1†	0.00
Glucuronic acid-1-P	0.28	1‡	0.03

* Whole homogenate tested.

† Incubation in 0.05 M Tris-maleate (pH 7.1).

‡ Without buffer at pH 7.

UDP-glucuronic acid-pyrophosphatase, UMP-phosphatase, and glucuronic acid-1-P-phosphatase activities also have been found in rat kidney particulates (17) and in soluble extracts from rat skin (18). UDP-*N*-acetylglucosamine inhibited UDP-glucuronic acid breakdown in skin extracts.

A large part of the activation produced by UDP-*N*-acetylglucosamine and ATP on the rate of glucuronidation can be explained by inhibition of UDP-glucuronic acid breakdown by microsomal pyrophosphatase. Inasmuch as UDP-glucuronic acid, ATP, and UDP-*N*-acetylglucosamine are hydrolyzed by the microsomes, presumably there is a competition of each of the substrates for the pyrophosphatase site. The increased activation by ATP in the presence of excess UDP-*N*-acetylglucosamine and lack of activation by other nucleotides may indicate the presence of more than one enzyme with this hydrolytic activity. However, the facts that (*a*) an activation still existed in rat liver microsomes in the presence of near excess UDP-glucuronic acid, (*b*) there was no measurable UDP-glucuronic acid disappearance in preincubation experiments with guinea pig liver microsomes, and (*c*) there was a specificity of the nucleotides involved, point to an additional activation of another sort.

The inhibition by EDTA of glucuronidation in guinea pig and activation in rat liver microsomes may be explained by EDTA inhibition of both pathways of UDP-glucuronic acid metabolism. When guinea pig liver microsomes and UDP-glucuronic acid were incubated together, no destruction of UDP-glucuronic acid was found. Presumably, there was little or no pyrophosphatase activity in these microsomal preparations. Therefore, the EDTA inhibited the UDP-transglucuronylase and decreased the amount of phenol conjugated. However, in the rat, where there is an active pyrophosphatase, the major effect of EDTA may be an inhibition of this enzyme with resultant inhibition of UDP-glucuronic acid disappearance and hence apparent activation of glucuronidation. The decreased activation by UDP-*N*-acetylglucosamine and ATP in the presence of EDTA and decreased breakdown of radioactive UDP-glucuronic acid in the presence of EDTA support this explanation. It is interesting that even high levels of EDTA did not cause complete inhibition of guinea pig liver microsomal UDP-transglucuronylase.

The increased radioactivity in glucuronic acid and decreased amount in glucuronic acid-1-P found in the presence of UDP-*N*-acetylglucosamine and ATP may be explained by formation of glucuronic acid by the combined actions of UDP-transglucuronylase and β-glucuronidase. The added nucleotides would inhibit the pyrophosphatase and allow more UDP-glucuronic acid to be metabolized through this alternate route. This assumes the presence of endogenous phenol acceptors in the microsomes.

The hydrolysis of UDP-glucuronic acid by microsomes probably represents a major source of glucuronic acid for ascorbic acid formation in liver. Preliminary evidence for glucuronic acid-1-P formation from UDP-glucuronic acid by microsomes also was reported recently by Evans *et al.* (19). It is of interest that with one exception all of the enzymes necessary for the conversion of UDP-glucuronic acid to ascorbate by the sequence of reactions shown below have been found to be present in rat liver microsomes (20–25). UDP-glucuronic acid → glucuronic acid → D-glucuronolactone → L-gulonolactone $\xrightarrow{O_2}$ ascorbate.

The only enzyme not present is that which catalyzes the reduction of glucuronic acid and its lactone by TPNH to L-gulo-

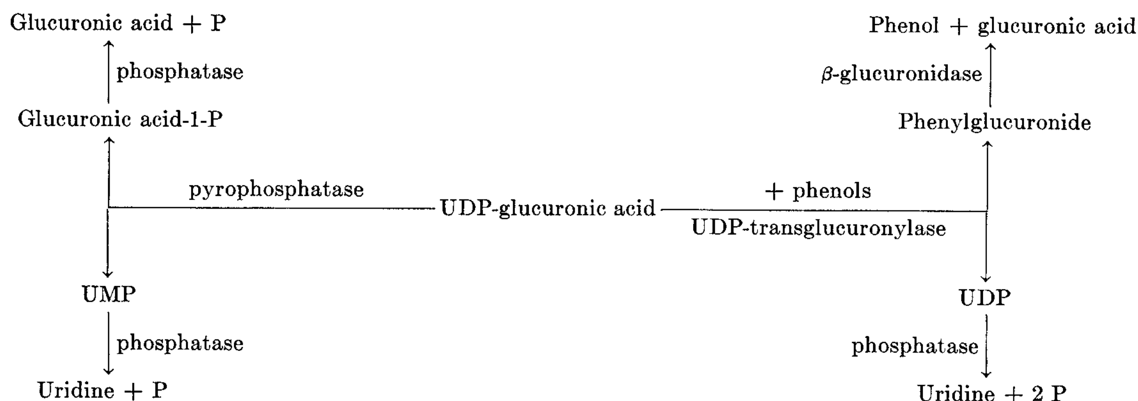

DIAGRAM 1

nate and its lactone, which was found in the soluble fraction (20, 24). There is one report, however, by Chatterjee *et al.* (26) of the direct conversion of D-glucuronolactone to ascorbate by goat liver microsomes. This reaction occurred only in the presence of cyanide. It is therefore conceivable that a direct pathway may exist *in vivo* in liver microsomes for the conversion of UDP-glucuronic acid to ascorbate.

SUMMARY

1. Addition of uridine diphosphate *N*-acetylglucosamine and adenosine triphosphate was found to produce a large increase of uridine diphosphate-transglucuronylase activity in rat liver microsomes. Part of the activation was caused by inhibition of uridine diphosphate glucuronic acid breakdown to glucuronic acid-1-phosphate, glucuronic acid, and uridine, but there was an additional, as yet unexplained, increase. Other sugar nucleotides could not replace uridine diphosphate *N*-acetylglucosamine, and uridine triphosphate or adenosine monophosphate were less effective than adenosine triphosphate.

2. Guinea pig liver microsomal uridine diphosphate-transglucuronylase was activated, but to a lesser extent, by uridine diphosphate *N*-acetylglucosamine, by dialyzed liver supernatant fluid, and by bovine serum albumin. The enzyme could be solubilized by treatment with digitonin.

3. Washed rat liver microsomes were shown to contain hydrolytic activity toward a large number of nucleotides and also β-glucuronidase activity.

4. Ethylenediaminetetraacetate inhibited glucuronidation by guinea pig liver microsomes, but activated the system in rat liver.

5. The nature of these reactions is discussed.

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