HEPATIC UDP-GLUCURONYLTRANSFERASE IN WISTAR AND GUNN RATS - IN VITRO ACTIVATION BY DIETHYLNITROSAMINE

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Dutton (1966) has previously pointed out that it is difficult to assess the absolute UDP-glucuronyltransferase activity in different tissues, there being little known of the in vitro factors regulating the activity of this enzyme. Investigations are further complicated by lack of studies on solubilised transferase and by the presence, in many tissues, of UDPGA-destroying pyrophosphatases. The results in this paper demonstrate, nevertheless, that hepatic UDP-glucuronyltransferase from Wistar and Gunn rats may be activated in vitro by the carcinogen diethylnitrosamine (DEN). The stimulatory effect appears to be confined to rat liver and is particularly marked with Gunn rat liver preparations - to the extent that the reported transferase deficiency of this tissue (Schmid et al, 1958; Lathe and Walker, 1958) is no longer apparent when DEN is included in the assay system.

DEN-activation occurs with both microsomal and solubilised transferase and may be demonstrated using either o-aminophenol or paracetamol as substrate. Kinetic analysis of the activation indicates that DEN does not alter the affinity of the enzyme for substrate.

Materials and Methods

Male adult Wistar or Gunn rats were used throughout, animals being killed for liver enzyme studies by stunning and cervical dislocation. Following

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rapid excision of the liver, homogenates were prepared in alkaline isotonic KCl. UDPGA and digitonin were purchased from the Sigma Chemical Co. and ^{14}C-UDPGA from the Radiochemical Centre, Amersham.

For preparation of microsomes, whole homogenates were first spun at 4,000 g for 30 min. Microsomes, sedimented from the resultant supernatant by spinning for 60 min at 100,000 g, were resuspended in KCl in one-fifth of the original volume. Solubilisation of glucuronyltransferase was achieved by the method of Pogell and Leloir (1961), the microsomal suspension being stirred with an equal volume of 1% digitonin for 30 min at 0°. The supernatant after centrifugation at 100,000 g for 60 min was used as solubilised transferase.

UDP-glucuronyltransferase activity using o-aminophenol as substrate was determined by the method of Dutton and Storey (1962) using 4.9×10^{-4}M UDPGA and spectrophotometric estimation of the product at 565 mμ. The total incubation volume of 0.6 ml contained either 0.1 ml of 10% whole homogenate, 0.1 ml of microsomal suspension or 0.2 ml of solubilised enzyme.

Results

Activation of rat liver UDP-glucuronyltransferase was found to be maximal around 1.6×10^{-2}M DEN. It is apparent from the results in Table 1 that, at this concentration of DEN, approximately 3-fold activation of Wistar rat liver transferase occurred, the degree of activation being the same with either homogenate, microsomal or solubilised preparations. With Gunn rat liver homogenates more than 20-fold activation was obtained. No stimulation was observed with liver preparations from species other than the rat. The activation did not result from reduced UDPGA breakdown, for DEN was not found to affect UDPGA pyrophosphatase and the activation of transferase was demonstrable in UDPGA-saturated systems.

DEN-stimulated synthesis of o-aminophenylglucuronide as estimated colorimetrically in the Dutton and Storey procedure was confirmed by another method utilising ^{14}C-UDPGA (Fig. 1). As is evident from comparison of Chromatograms A and B, synthesis of ^{14}C-o-aminophenylglucuronide in the

TABLE 1

In vitro effect of DEN on rat liver UDP-glucuronyltransferase

Enzyme preparation	glucuronide formed*		B/A
	(A) No DEN	(B) DEN (1.62×10^{-2}M)	
1. Wistar rat liver			
Homogenate	0.49	1.44	2.9
Microsomes	0.052	0.168	3.2
Digitonin-solubilised enzyme	0.053	0.165	3.1
2. Gunn rat liver			
Homogenate	0.060	1.33	22.2

*Results are expressed as μmoles o-aminophenyl glucuronide formed/preparation from 1 g wet wt liver/30 min. Each value quoted represents the mean value of separate determinations on preparations from at least five animals.

presence of DEN was more than twice that when the carcinogen was excluded. The product formed in the presence of DEN was almost completely hydrolysed by subsequent incubation with β-glucuronidase (Chromatogram C). No DEN-activation of UDP-glucuronyltransferase could be detected using p-nitrophenol, phenolphthalein or menthol as substrates but, with 4 mM paracetamol as substrate, approximately 4-fold activation of Wistar rat liver transferase was observed using the above radioactive procedure.

Results from kinetic studies on the solubilised transferase are shown in Fig. 2. It is evident that addition of DEN does not change the K_m for o-aminophenol (4.4×10^{-4}M). Similar experiments with varying UDPGA

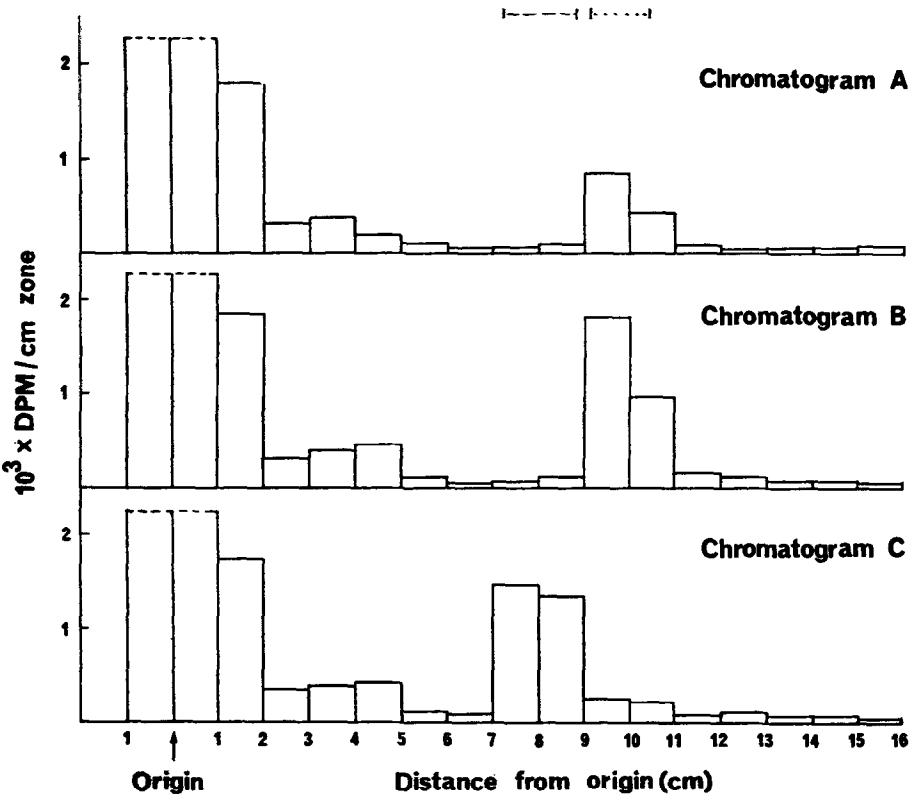

Figure 1. DEN-activation of UDP-glucuronyltransferase - confirmation using labelled UDPGA.

Incubations contained o-aminophenol and buffer as used by Dutton and Storey (1962) together with 10µl of 10% Wistar rat liver homogenate in a total volume of 50µl. The donor nucleotide was 4.9×10^{-4}M ^{14}C-UDPGA (24.5mc/mM). The 30 min incubations at 37° were terminated by immersion of the incubation tubes in boiling water for 1 min. Following addition of 0.2ml water and centrifugation, 10µl of the incubate was spotted in a 1cm-wide band on a thin layer (250µ) silica gel plate. After development over 16cm in a methanol: H_2O (9:1) solvent system, 1cm zones were scraped off into vials containing 5 ml dioxane-naphthalene scintillator and counted in a Nuclear Chicago Scintillation Counter using the Channels Ratio Method of Quench Correction.

Chromatograms A and B were from incubates containing No DEN and 1.62×10^{-2}M DEN respectively. Chromatogram C was from an incubate containing DEN and which had been subsequently incubated with β-glucuronidase. The value for the two left-hand columns in each chromatogram was 2×10^4. Standard o-aminophenyl glucuronide (I.....I) and glucuronic acid (I----I) ran as indicated.

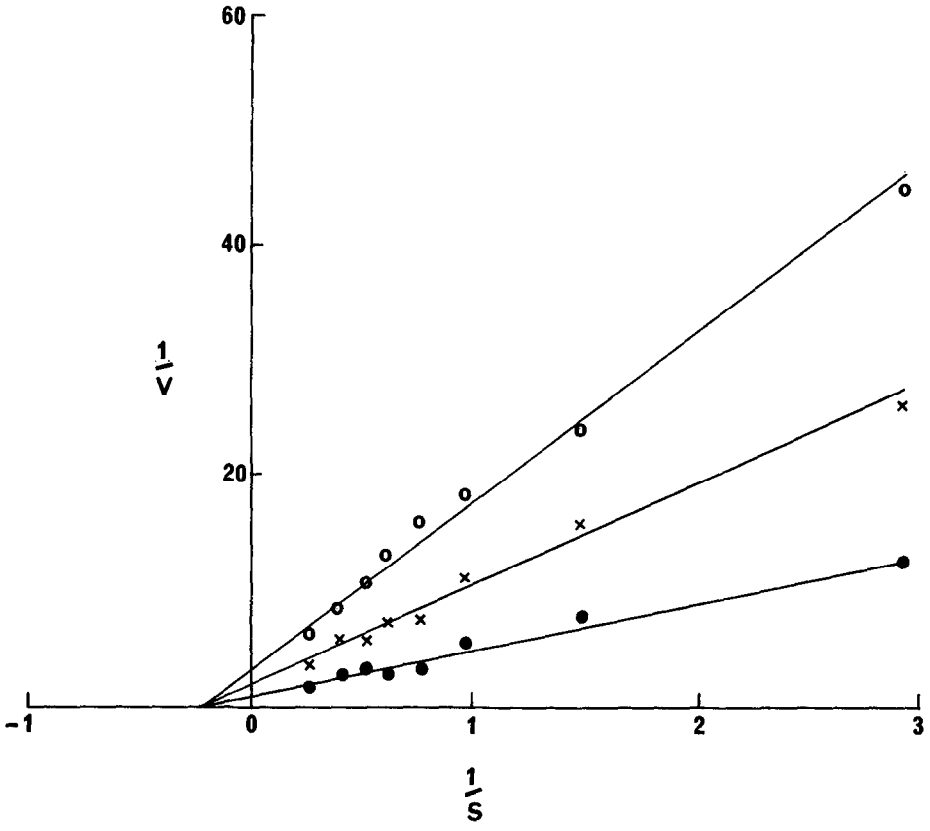

Figure 2. Kinetics of the DEN-activation of solubilised Wistar rat liver UDP-glucuronyltransferase.

Incubations were carried out for 10 min in the presence of o——o No DEN, x——x 8.0 x 10^{-3}M DEN and ●——● 1.6 x 10^{-2}M DEN. Conditions of assay are as described in the text with the exception that the o-aminophenol concentration was varied as indicated and that the final UDPGA concentration used was 4.9 x 10^{-3}M.

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concentrations suggest that the K_m for the nucleotide is also unaffected by DEN.

Discussion

Several agents are known to stimulate glucuronide synthesis in vitro. The stimulation by ATP and UDP-N-acetylglucosamine described by Pogell and

Leloir results from reduced UDPGA destruction, while the effect of EDTA (Halac and Reff, 1967) may be related to its ability to alter microsomal structure. On the other hand, activation of solubilised transferase by albumin (Pogell and Leloir, 1961) seems to involve a direct effect on the UDP-glucuronyl-transferase protein. It is likely, from the above results, that this latter mechanism also operates in DEN-activation of transferase, although there is no measurable change in the affinity of transferase for substrate. A DEN effect resulting from activation of otherwise non-functional enzyme or from interference with an endogenous system controlling transferase activity is also possible.

The specific effect of DEN on rat liver transferase is interesting, in particular the very marked activation of the Gunn enzyme. In the presence of DEN the transferase activities of Wistar and Gunn rat livers are comparable, suggesting that no transferase deficiency exists in Gunn liver. In support of this, there are previous reports of apparently normal glucuronide synthesis in Gunn rats (Arias, 1961; Javitt, 1966).

Clarification of the mechanism of DEN-activation and of the precise lesion in Gunn rat liver must await further studies on the in vitro factors regulating UDP-glucuronyltransferase activity. DEN in vitro does not activate microsomal drug-metabolising enzymes in general. It has recently been shown, for example, to inhibit the microsomal enzyme involved in hexobarbitone metabolism (Stevenson and Greenwood, 1968).

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