Effect of glucose on 7-hydroxycoumarin glucuronide production in periportal and pericentral regions of the liver lobule

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The effect of starvation and glucose addition on glucuronidation was assessed in sublobular regions of the lobule in perfused livers from phenobarbital-treated rats. Fibre-optic micro-light guides were placed on periportal and pericentral areas on the surface of livers to monitor the fluorescence (excitation 366 nm, emission 450 nm) of free 7-hydroxycoumarin from the tissue surface. After infusion of 7-hydroxycoumarin (80 μ M) under normoxic conditions, steady-state increases in fluorescence were reached in 6–8 min in both regions. Subsequently, the formation of non-fluorescent 7-hydroxycoumarin glucuronide was inhibited completely by perfusion with N₂-saturated perfusate containing 20 mM-ethanol. The difference in fluorescence between anoxic and normoxic perfusions was due to glucuronidation under these conditions. In livers from fed rats, rates of glucuronidation in periportal and pericentral regions of the liver lobule were 8 and 19 μ mol/h per g, respectively. In contrast, rates of glucuronidation were 3 and 9 μ mol/h per g, respectively, in periportal and pericentral regions of livers from starved rats. Infusion of glucose (20 mM) had no effect on rates of glucuronidation in livers from fed rats; however, glucose increased rates of glucuronidation rapidly (half-time, $t_{0.5}$ = 1.5 min) in periportal and pericentral regions to 7 and 17 μ mol/h per g, respectively in livers from starved rats. These results indicate that the rapid synthesis of the cofactor UDP-glucuronic acid derived from glucose is an important rate-determinant for glucuronidation of 7-hydroxycoumarin in both periportal and pericentral regions of livers from starved rats.

Glucuronidation is an important pathway in the hepatic metabolism of a large array of xenobiotics and endogenous substances (Dutton, 1980). Several factors, including substrate concentration, activities of a family of transferase enzymes, supply of the cofactor UDP-glucuronic acid (Moldéus *et al.*, 1979; Reinke *et al.*, 1981; Thurman *et al.*, 1981; Koster *et al.*, 1981; Pang & Terrell, 1981; Singh & Schwartz, 1981; Aw & Jones, 1982, 1984) and the activity of microsomal β -glucuronidase (Belinsky *et al.*, 1984; Sokolove *et al.*, 1984), may influence rates of production of glucuronides by the liver.

Several toxic compounds, such as acetaminophen and 2-acetylaminofluorene, damage specific regions of the liver lobule (De Baun *et al.*, 1970; Jollow *et al.*, 1974). In large part, these chemicals

are detoxified by the glucuronidation process (Price & Jollow, 1982; Mulder & Meerman, 1983). Therefore, information about regulation of glucuronidation in specific lobular zones may be important in understanding mechanisms of zone-specific hepatotoxicity.

A method was developed to measure rates of glucuronidation non-invasively in periportal and pericentral regions of the liver lobule, based on the fluorescence of 7-hydroxycoumarin measured with fibre-optic micro-light guides (Conway *et al.*, 1984). Local rates of glucuronide production were calculated from the concentration differences of free substrate entering and leaving each region of the liver lobule. In livers from fed phenobarbital-treated rats, pericentral areas formed glucuronides of 7-hydroxycoumarin at rates about 3 times faster than did periportal regions. Rates correlated with the activity of UDP-glucuronosyltransferase

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measured in microdissected samples of sublobular areas (Conway *et al.*, 1984).

Isolated hepatocytes and perfused livers from starved rats have lower rates of glucuronidation than do preparations from fed rats, most likely owing to limited rates of UDP-glucuronic acid synthesis (Reinke *et al.*, 1981). This hypothesis is supported by the observation that addition of carbohydrate stimulates glucuronidation markedly in livers from starved rats (Schwartz, 1980; Reinke *et al.*, 1981; Thurman *et al.*, 1981; Aw & Jones, 1982, 1984). The purpose of the present work was to evaluate the importance of UDP-glucuronic acid supply on glucuronidation in periportal and pericentral regions of the liver lobule of the perfused rat liver.

Methods

Animals

Female Sprague-Dawley rats weighing 250–400 g were pretreated with phenobarbital (1 g/litre) in drinking water (Marshall & McLean, 1969) for 15–30 days before perfusion experiments to enhance the colour contrast between periportal and pericentral regions of the liver lobule, which facilitates exact placement of micro-light guides. All rats were starved for 20–24 h before perfusion experiments.

Liver perfusion

Details of the non-recirculating perfusion technique have been described elsewhere (Scholz *et al.*, 1973). The perfusate was Krebs–Henseleit (1932) bicarbonate buffer, pH 7.4, saturated with O_2/CO_2 (19:1). Sulphate-free perfusate used in these experiments was prepared by replacing $MgSO_4$ with $MgCl_2$. Under these conditions, maximal rates of sulphation were less than $0.25 \mu\text{mol/h}$ per g. 7-Hydroxycoumarin (Sigma Chemical Co., St. Louis, MO, U.S.A.) was dissolved in dimethylformamide and added to the perfusate at final concentrations indicated in the Figures and Tables. The final dimethylformamide concentration infused into the liver (0.025%) had no effect on hepatic O_2 uptake or rates of glucuronidation. For perfusions in the anterograde direction, the perfusate was pumped into the portal vein at rates of 2.5–3.5 ml/min per g of liver. Effluent perfusate was collected via a cannula placed in the vena cava. In some experiments, the direction of flow was reversed (retrograde perfusions) to maximize substrate delivery to pericentral regions of the liver lobule. The O_2 concentration in the effluent perfusate was monitored continuously with a Teflon-shielded Clark-type platinum electrode. O_2 uptake was calculated as described by Scholz *et al.* (1973) to assess tissue viability.

Free 7-hydroxycoumarin in the effluent perfusate was measured fluorimetrically (excitation 366 nm, emission 450 nm) with a quartz light guide (tip diameter 2 mm) attached to a Johnson Foundation fluorimeter. Glucuronide conjugates of 7-hydroxycoumarin were hydrolysed enzymically with pure β -glucuronidase (Sigma; catalogue no. G-1883) (Reinke *et al.*, 1981) and measured as described elsewhere (Conway *et al.*, 1984). Fluorescence of unknown samples was compared with that of authentic 7-hydroxycoumarin standards incubated under identical conditions, and rates of conjugation were calculated from the concentration of glucuronide in the effluent perfusate, the flow rate and liver wet weight.

Micro-light guide

Light and dark spots visible on the surface of the haemoglobin-free perfused rat liver correspond to periportal and pericentral regions of the liver lobule. Micro-light guides, consisting of two strands of glass fibre, were constructed as described previously (Ji *et al.*, 1980). The 7-hydroxycoumarin fluorescence was monitored simultaneously in periportal and pericentral areas of the left lateral lobe of the perfused liver with micro-light guides placed on the surface with micromanipulators. One strand of the micro-light guide is connected to a 100 W mercury arc lamp and the other strand to a photomultiplier. The liver was illuminated with 366 nm light, and fluorescence of 7-hydroxycoumarin was measured at 450 nm (Ji *et al.*, 1981).

Results

Rates of production of 7-hydroxycoumarin glucuronide in perfused livers from fed and starved rats

Steady-state rates of production of non-fluorescent glucuronic acid conjugates were measured in livers from fed phenobarbital-treated rats perfused in the anterograde direction with 7-hydroxycoumarin (Conway *et al.*, 1984; Fig. 1). Double-reciprocal analysis of the data showed that rates of glucuronide production of about $21 \mu\text{mol/h}$ per g occurred; half-maximal rates were observed with 50–60 μM substrate. Infusion of 20 mM-glucose had no effect on rates in the fed state (results not shown). In livers from starved rats, however, glucuronide production was half-maximal with about 10 μM -7-hydroxycoumarin and maximal rates were only about $4 \mu\text{mol/h}$ per g (Fig. 1). The effect of glucose on steady-state rates of production of 7-hydroxycoumarin glucuronide was assessed in livers perfused in both the anterograde and retrograde directions with 80 μM -7-hydroxycoumarin. In perfusions in both directions, glucuronide production was 3.6–3.8 $\mu\text{mol/h}$ per g in livers from starved rats (Table 1). Infusion of glucose

stimulated the production of glucuronides by 120 and 160% in perfusions in the anterograde and retrograde directions, respectively. In the presence of glucose, rates of production of 7-hydroxycoumarin glucuronide were about 9 $\mu\text{mol/h}$ per g

(Table 1) irrespective of the direction of perfusion, values similar to rates observed in livers from fed rats perfused with 80 μM -7-hydroxycoumarin (Fig. 1).

Rates of production of 7-hydroxycoumarin glucuronide in periportal and pericentral regions of livers from starved phenobarbital-treated rats

Calibration of 7-hydroxycoumarin fluorescence from the liver surface. Measurement of sublobular rates of glucuronide production with micro-light guides has been described in detail elsewhere (Conway *et al.*, 1984). Briefly, micro-light guides are placed on periportal and pericentral regions of the liver lobule. 7-Hydroxycoumarin is then infused and its fluorescence monitored during normoxic perfusions. Fluorescence signals are then converted into concentrations of free 7-hydroxycoumarin in tissue, and local rates of production of non-fluorescent glucuronides are calculated from the concentration differences of free substrate entering and leaving each sublobular region.

After the addition of 7-hydroxycoumarin during normoxic perfusion, the fluorescence of 7-hydroxycoumarin was calibrated in each liver with a known concentration of 7-hydroxycoumarin in the tissue by infusing 20mM-ethanol in N_2 -saturated perfusate ($\text{N}_2 + \text{E}$; Figs. 2 and 3). Under these conditions, all the infused 7-hydroxycoumarin was recovered in the effluent unmetabolized (i.e. glucuronide production was inhibited completely). Because 7-hydroxycoumarin was not metabolized in the anoxic liver in the presence of ethanol, the fluorescence arising from the liver surface was assumed to be due to free 7-hydroxycoumarin in the tissue, after correction for the small increases in nicotinamide nucleotide fluorescence due to ethanol and N_2 alone. This assumption is supported by the observation that the fluorescence detected from the liver surface increased in a linear fashion when 7-hydroxycoumarin concentration in the tissue was varied (Conway *et al.*, 1984).

Fig. 1. Rate of production of 7-hydroxycoumarin glucuronide by perfused livers from fed ($\blacktriangle$) and starved ($\bullet$) phenobarbital-treated rats

Livers were perfused in the anterograde direction for 20min with Krebs–Henseleit buffer. In each liver from fed and starved rats, one concentration of 7-hydroxycoumarin dissolved in Krebs–Henseleit buffer was infused for 20–30min and the perfusate was collected and assayed for glucuronides (Conway *et al.*, 1984; see the Methods section). The average free 7-hydroxycoumarin concentration in the liver was calculated from (inflow + outflow concentrations of free 7-hydroxycoumarin)/2. Data from 4 to 14 livers were averaged for each concentration of 7-hydroxycoumarin (mean $\pm$ S.E.M.).

Table 1. Effect of glucose on rates of production of 7-hydroxycoumarin glucuronide in livers from starved phenobarbital-treated rats

7-Hydroxycoumarin (80 μM) was infused into livers from starved phenobarbital-treated rats for about 20min followed by glucose infusion for about 15min. Concentrations of 7-hydroxycoumarin glucuronide in the effluent perfusate were determined as described in the Methods section. Maximal rates of glucuronide formation were calculated before and during glucose infusion as described in the Methods section. Results are means $\pm$ S.E.M.: *** $P < 0.001$ comparing rates before and after glucose infusion with a matched-pairs t test. n : anterograde, 13; retrograde, 8.

Additions	Rate of glucuronide production ($\mu\text{mol/h per g}$)	
	Anterograde perfusions	Retrograde perfusions
None	3.8 $\pm$ 0.3	3.6 $\pm$ 0.2
20mM-glucose	8.5 $\pm$ 0.4***	9.3 $\pm$ 0.4***

Determination of 7-hydroxycoumarin concentration in the tissue during normoxia. Micro-light guides were placed on periportal and pericentral regions, and a stable fluorescence baseline was established (Fig. 2). When 7-hydroxycoumarin ($80\mu\text{M}$) was infused, fluorescence reached higher, steady-state values in both regions in about 8 min. After 15 min of 7-hydroxycoumarin infusion, 20 mM-glucose was infused, which decreased fluorescence rapidly to lower maximal steady-state values in both regions in 6–8 min (half-time, $t_{0.5}$, for the effect of glucose was 1–2 min in both regions). When glucose infusion was terminated, fluorescence increased almost to values observed before glucose infusion in about 10 min. Fluor-

Fig. 2. Effect of glucose on 7-hydroxycoumarin fluorescence in periportal and pericentral regions of the liver perfused in the anterograde direction

Periportal (light colour) and pericentral (dark colour) regions of the liver lobule were identified by differences in pigmentation (Ji *et al.*, 1980). Two micro-light guides were placed on periportal and pericentral regions 1 mm apart on the left lateral lobe of the liver. Basal fluorescence of the pericentral area was approx. 30% less than that of the periportal area, confirming previous results (Ji *et al.*, 1980). 7-Hydroxycoumarin (HC; $80\mu\text{M}$), glucose (20 mM) and N_2 -saturated perfusate containing 20 mM-ethanol ($\text{N}_2 + \text{E}$) were infused as indicated by horizontal bars and arrows. Numbers in parentheses represent concentrations (μM) of free 7-hydroxycoumarin in the tissue, calculated as described in the Results section. Results are shown of a typical experiment, and are expressed as the photomultiplier current (nA).

escence in both regions returned quickly to basal values when 7-hydroxycoumarin infusion was terminated (Fig. 2). 7-Hydroxycoumarin was then infused in the presence of N_2 -saturated perfusate containing 20 mM-ethanol ($\text{N}_2 + \text{E}$). Fluorescence increased to new, higher, steady-state values in both regions in about 8 min. N_2 -saturated perfusate containing ethanol alone produced only a small increase in fluorescence, owing to reduction of endogenous NAD^+ (Fig. 2).

In all experiments, fluorescence detected in periportal and pericentral regions during perfusion of 7-hydroxycoumarin in the presence of ethanol and N_2 was used to calculate concentrations of 7-hydroxycoumarin in the tissue during normoxic perfusion (Figs. 2 and 3). For example, $80\mu\text{M}$ -7-hydroxycoumarin produced a fluorescence increase of 315% over basal values in the periportal region after correcting for the small increase in NADH fluorescence caused by anoxia plus ethanol (Fig. 2). Under normoxic conditions, the increase was 300% in the same region. Since 7-hydroxycoumarin ($80\mu\text{M}$) increased fluorescence by 315%, the 300% increase detected under normoxic conditions was calculated to be $77\mu\text{M}$ (see parentheses in Fig. 2). Since over 90% of all non-fluorescent products were glucuronides, the decrease in free 7-hydroxycoumarin concentration in any region of the liver lobule was due to production of glucuronides. In this example, the decrease in concentration of free 7-hydroxycoumarin in the periportal region was $80 - 77 = 3\mu\text{M}$. In 13 livers, the fluorescence increase in periportal areas during normoxia was significantly less than the increase during anoxic perfusion of 7-hydroxycoumarin ($P < 0.01$, by matched-pairs *t* test). Therefore we conclude that the production of non-fluorescent glucuronides in periportal areas can be detected with micro-light guides. Pericentral regions are 'downstream' from the periportal regions (e.g. flow is from periportal to pericentral), and therefore pericentral areas are only exposed to the free 7-hydroxycoumarin not conjugated by periportal areas. Since the micro-light guide detected $77\mu\text{M}$ -hydroxycoumarin in the periportal area, but only $49\mu\text{M}$ free 7-hydroxycoumarin in the pericentral area, we calculate that $28\mu\text{M}$ ($77 - 49$) 7-hydroxycoumarin was converted into glucuronides in pericentral regions (Fig. 2). When glucose was infused, the concentration of glucuronides formed in periportal and pericentral areas increased to 18 ($80 - 62$) and 47 ($62 - 15$) μM respectively (Fig. 2). Similar data were obtained from perfusions in the retrograde direction (Fig. 3).

Densitometry of dark spots and light areas in photographs of thin slices of liver indicates that periportal and pericentral regions comprise about equal portions of the liver lobule (J. J. Lemasters & R. G. Thurman, unpublished work). By using the

flow rate, the mass of each region and the decrease in the concentration of free 7-hydroxycoumarin in that region (Table 2), local rates of glucuronidation were calculated (Table 3). To determine the substrate concentration in any specific region of

the liver lobule, the concentration of 7-hydroxycoumarin entering and leaving each sublobular region was averaged (Table 3). For example, in Fig. 2, the periportal area was exposed to 80 μM -7-hydroxycoumarin and 77 μM left the region unmetabolized; therefore the average substrate concentration in that region was 78.5 μM .

When periportal and pericentral areas were exposed to high concentrations of substrate via perfusion in the anterograde and retrograde directions respectively, pericentral regions formed glucuronides 2–3-fold faster than periportal areas (Table 3). Glucose infusion increased rates of glucuronidation in periportal areas from 2.7 to 5.5 $\mu\text{mol/h}$ per g in anterograde perfusions, and from 6.9 to 18.9 $\mu\text{mol/per g}$ in pericentral areas during retrograde perfusions (Table 3). When periportal and pericentral areas are located 'downstream' with respect to direction of flow (i.e. pericentral areas in anterograde perfusions, periportal areas in retrograde perfusions), glucose addition increased the rate of glucuronidation by 75% in the pericentral regions, but had no effect on rates in periportal areas (Table 3).

Discussion

Regulation of glucuronidation by UDP-glucuronic acid supply during starvation

At 7-hydroxycoumarin concentrations below about 20 μM , rates of glucuronidation in livers from starved and fed phenobarbital-treated rats were equivalent, because low concentrations of 7-hydroxycoumarin limit glucuronidation. With higher concentrations of substrate, however, maximal rates were 5-fold greater in livers from fed than in those from starved rats (Fig. 1), confirming earlier work in perfused livers and isolated hepatocytes (Schwartz, 1980; Reinke *et al.*, 1981; Thurman *et al.*, 1981; Aw & Jones, 1984). It is well documented that UDP-glucuronic acid content

Fig. 3. Effect of glucose on 7-hydroxycoumarin fluorescence in periportal and pericentral regions of the liver perfused in the retrograde direction

Conditions as in Fig. 2, except that flow into the liver was via the vena cava. Results are shown of a typical experiment.

Table 2. Effect of glucose on 7-hydroxycoumarin glucuronide formed in periportal and pericentral regions of the liver lobule. Concentrations of 7-hydroxycoumarin glucuronide formed in periportal and pericentral regions were calculated from experiments typified by Figs. 2 and 3 (see the Results section). Results are means $\pm$ S.E.M. for the numbers of experiments in parentheses: ** $P < 0.01$, *** $P < 0.001$, comparing sublobular concentration of glucuronides before and after glucose infusion, with a matched-pairs t test.

Addition	Glucuronide conjugates formed by each region (μM)	
	Periportal	Pericentral
Anterograde perfusion (13)		
None	7.2 $\pm$ 1.8	25.6 $\pm$ 4.3
20 mM-glucose	14.8 $\pm$ 2.4**	45.6 $\pm$ 3.2***
Retrograde perfusions (8)		
None	8.7 $\pm$ 3.6	20.9 $\pm$ 4.0
20 mM-glucose	9.0 $\pm$ 3.8	60.4 $\pm$ 4.8***

Table 3. *Effect of glucose on rates of production of 7-hydroxycoumarin glucuronide in periportal and pericentral regions of the liver lobule*

Concentrations of free 7-hydroxycoumarin in periportal regions during anterograde perfusions and in pericentral regions during retrograde perfusions were calculated from infused 7-hydroxycoumarin and free 7-hydroxycoumarin present in the region during normoxic perfusion from fluorescence changes (Figs. 2 and 3; see the Results section for more detail). Concentrations of free 7-hydroxycoumarin in periportal regions during retrograde perfusions and in pericentral areas during anterograde perfusions were calculated from the 7-hydroxycoumarin concentration in the 'upstream' regions (i.e. periportal area during anterograde perfusion) and free 7-hydroxycoumarin detected in 'downstream' regions. The rate of glucuronidation was calculated from the mass of the lobular region (one-half liver weight), the flow rate of the liver and the concentration of glucuronide formed locally (Table 2). Results are means $\pm$ S.E.M. for the numbers of experiments in parentheses: ** $P < 0.01$, *** $P < 0.001$, comparing sublobular rates of glucuronidation before and after glucose infusion, with a matched-pairs t test.

Addition	Average free 7-hydroxycoumarin concn. during normoxic perfusion (μM)		Rate of glucuronide production ($\mu\text{mol/h per g}$)	
	Periportal	Pericentral	Periportal	Pericentral
Anterograde (13)				
None	76.8 $\pm$ 0.8	59.2 $\pm$ 3.2	2.7 $\pm$ 0.7	9.5 $\pm$ 1.5
20 mM-glucose	72.6 $\pm$ 1.2	42.9 $\pm$ 2.7	5.5 $\pm$ 0.9**	16.6 $\pm$ 1.2***
Difference			2.8 $\pm$ 0.6	7.2 $\pm$ 1.3
Retrograde (8)				
None	55.7 $\pm$ 4.0	69.5 $\pm$ 2.0	2.2 $\pm$ 0.8	6.9 $\pm$ 1.3
20 mM-glucose	19.6 $\pm$ 2.7	51.1 $\pm$ 2.1	2.5 $\pm$ 1.1	18.9 $\pm$ 2.2***
Difference			0.3 $\pm$ 0.2	12.0 $\pm$ 1.2

decreases with starvation (Felsher *et al.*, 1979; Reinke *et al.*, 1981; Aw & Jones, 1984). Lower rates of glucuronidation at high substrate concentrations in livers from starved rats are probably due to limited carbohydrate for the synthesis of UDP-glucuronic acid, since addition of glucose reversed the starvation-induced decrease in glucuronidation completely (Table 1).

The concentration of 7-hydroxycoumarin required for half-maximal rates of glucuronidation in livers from fed phenobarbital-treated rats is 50–60 μM (Fig. 1). This is the same concentration of 7-hydroxycoumarin required for half-saturation of UDP-glucuronosyltransferase assayed with excess UDP-glucuronic acid *in vitro* (Conway *et al.*, 1984). However, glucuronidation in livers from starved rats occurred half-maximally with only 10 μM -7-hydroxycoumarin (Fig. 1). Since glucose infusion into livers from starved rats increased rates of glucuronidation to values observed in the fed state (Table 1, Fig. 1), the apparent lower half-maximal substrate concentration in starved rats does not represent a true kinetic constant. The lower value in livers from starved rats results most likely from rate-limitation of glucuronidation by decreased UDP-glucuronic acid supply, not from intrinsic differences in UDP-glucuronosyltransferase enzymes produced by starvation.

The influence of starvation on glucuronidation *in vivo* is not clear. Mulder *et al.* (1982) observed

that steady-state rates of excretion of harmol glucuronide in the bile and urine were about 2 $\mu\text{mol/h per g}$ of liver in both starved and fed rats. In contrast, Jollow & Price (1984) injected acetaminophen intraperitoneally and observed that starvation decreased the rate of appearance of glucuronides in plasma by 30%. The effect of starvation on glucuronidation depends on the concentration of substrate used. For example, with 20 μM substrate, perfused livers from fed and starved rats produce glucuronides from 7-hydroxycoumarin (Fig. 1) and *p*-nitrophenol (Reinke *et al.*, 1981) at 2–3 $\mu\text{mol/h per g}$. However, with higher concentrations of substrate, starvation decreases glucuronidation. Further studies are needed to define the effects of starvation on glucuronidation *in vivo*.

Effect of starvation and glucose on rates of glucuronide production in periportal and pericentral regions of the liver lobule

We observed previously that pericentral areas have 3-fold higher rates of glucuronidation than do periportal areas in livers from fed, phenobarbital-treated rats, values which correlated with the local activity of UDP-glucuronosyltransferase (Conway *et al.*, 1984). If UDP-glucuronic acid supply was in excess in both regions in the fed state, then differences in enzyme activity could explain the higher rates of glucuronidation observed in peri-

central areas. Since UDP-glucuronic acid supply is an important rate-determinant of glucuronidation during starvation (Reinke *et al.*, 1981; Thurman *et al.*, 1981), we hypothesized that differences in rates between periportal and pericentral regions might be diminished markedly by starvation. Indeed, starvation decreased rates of glucuronidation in both periportal and pericentral regions by 50–70%. Residual rates, however, were still 3-fold higher in pericentral than in periportal regions in livers from starved rats (Table 3). Substrate supply, UDP-glucuronosyltransferase and β -glucuronidase activities and UDP-glucuronic acid supply could influence rates of glucuronide production in both regions of the liver lobule.

In perfusions in the retrograde direction, an increase in rates of glucuronidation due to glucose addition was observed in pericentral areas, but not in periportal regions (Table 3). The failure of glucose to increase glucuronidation in periportal areas under these conditions is most probably due to the limited supply of 7-hydroxycoumarin. The concentration of 7-hydroxycoumarin in the periportal regions in the presence of glucose was only $19.6\mu\text{M}$ (Table 3), a value well below the concentration of substrate required for half-maximal rates ($50\text{--}60\mu\text{M}$) for either the isolated enzyme incubated with excess UDP-glucuronic acid (Conway *et al.*, 1984) or perfused livers from fed rat (Conway *et al.*, 1984; see the Results section).

Rates of glucuronidation measured in intact cells reflect both the formation of glucuronides by UDP-glucuronosyltransferase and the cleavage of glucuronides by β -glucuronidase. β -Glucuronidase has been studied in broken-cell preparations (Levvy & Conchie, 1966); however, the activity of the enzyme in the intact cell is not known. Belinsky *et al.* (1984) showed that adrenaline decreased the production of *p*-nitrophenyl glucuronide by 40% in the perfused liver. It was suggested that the increase in cytosolic Ca^{2+} which follows the binding of adrenaline to the α -receptor caused an activation of microsomal β -glucuronidase, which cleaved newly-formed glucuronides. This hypothesis was supported by the observation that β -glucuronidase was activated by Ca^{2+} in the physiological range *in vitro* (Belinsky *et al.*, 1984; Sokolove *et al.*, 1984). Thus β -glucuronidase activity should be considered when analysing rates of glucuronide export from intact cells. Further characterization of the activity in the two zones of the liver as well as an estimation of the flux through β -glucuronidase in sublobular areas are needed before the contribution of β -glucuronidase to rates of glucuronide production measured with micro-light guides can be quantified.

Starvation decreased rates of glucuronidation by 50–70% in both regions, and glucose infusion

reversed the starvation-induced decrease completely (Table 3). This indicates that the supply of UDP-glucuronic acid is an important rate-determinant in both regions of livers from starved rats; 3-fold higher rates are observed in pericentral areas in the absence of glucose during starvation. Greater rates of UDP-glucuronic acid turnover in the pericentral areas may contribute to the higher rates observed. Higher rates in pericentral areas are probably not due to a greater affinity of UDP-glucuronosyltransferases for UDP-glucuronic acid, since the concentration required for half-saturation of UDP-glucuronosyltransferase was $230\mu\text{M}$ in both periportal and pericentral areas (Conway *et al.*, 1984). Rates 3-fold higher were also observed in pericentral areas in the fed state (Conway *et al.*, 1984) and during starvation in the presence of glucose (Table 3). Therefore greater UDP-glucuronosyltransferase activity in pericentral areas (Conway *et al.*, 1984) is most likely an important rate-determinant in that region. Thus both UDP-glucuronic acid supply and the activity of UDP-glucuronosyltransferase are important determinants of glucuronide production in both regions of the liver lobule.

Formation of UDP-glucuronic acid from glucose requires glucokinase to generate glucose 6-phosphate (Dutton, 1980). Results presented here show that glucose stimulated glucuronidation rapidly ($t_{0.5}$ 1–2 min) in both regions of the liver lobule, indicating that glucose is rapidly phosphorylated in both regions. Hexokinase is probably less important in liver, since it is localized primarily in non-parenchymal cells (Dileepan *et al.*, 1979; Crisp & Pogson, 1972). Microchemical analysis of freeze-dried tissue sections from normal rats indicates that glucokinase activity is higher in pericentral than in periportal regions (Fischer *et al.*, 1982). These data are consistent with our observation that glucose stimulated glucuronide production by twice as much in pericentral as in periportal areas (Table 3). Matsumura & Thurman (1984) observed that glucose infusion increased rates of O_2 uptake in periportal and pericentral regions by 23 and $15\mu\text{mol/h per g}$ respectively. If we assume that this increase in respiration is due to increased ADP supply, owing to the phosphorylation of glucose, we can calculate that both sublobular areas can phosphorylate glucose at $75\mu\text{mol/h per g}$ (5.7mol of ADP/mol of O_2 utilized). Thus rates of glucose phosphorylation greatly exceed rates of stimulation of glucuronidation by glucose. The larger stimulation of glucuronidation by glucose in pericentral areas is most probably due to a higher UDP-glucuronosyltransferase activity and/or increased synthesis of UDP-glucuronic acid from glucose 6-phosphate in that area.

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