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Glucuronidation of 7-Hydroxycoumarin in Periportal and Pericentral Regions of the Lobule in Livers from Untreated and 3-Methylcholanthrene-Treated Rats

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SUMMARY

Rates of production of 7-hydroxycoumarin glucuronide were measured in specific zones of the liver lobule using micro-light guides placed on periportal and pericentral regions on the surface of livers from untreated and 3-methylcholanthrene-treated rats. Livers were perfused with sulfate-free buffer under normoxic conditions and fluorescence of free 7-hydroxycoumarin was monitored. The formation of nonfluorescent 7-hydroxycoumarin glucuronide was then inhibited completely by perfusion with N_2-saturated perfusate containing 20 mM ethanol. The difference between fluorescence readings under normoxic and hypoxic conditions was used to calculate rates of glucuronidation. Maximal rates of glucuronidation (11.9–13.5 μmol/g/hr) did not differ significantly in periportal and pericentral regions in livers from either 3-methylcholanthrene-treated or untreated rats. In all regions of the liver lobule, glucuronidation was half-maximal with about 20 μM 7-hydroxycoumarin. Glucuronosyltransferase assayed in lyophilized tissue sections with saturating concentrations of UDPGA (9 mM) was 2.3-fold greater in pericentral than in periportal areas in livers from untreated rats. In livers from 3-methylcholanthrene-treated rats, activities were similar in periportal and pericentral regions but were 4- to 7-fold higher than values from untreated rats. In addition, glucuronosyltransferase activity assayed in native microsomes with physiological concentrations of UDP-glucuronic acid (UDPGA) (0.4 mM) with UDP-*N*-acetylglucosamine (0.3 mM) was 2-fold higher in preparations from 3-methylcholanthrene-treated than untreated rats. Thus, 3-methylcholanthrene treatment increased glucuronosyltransferase activity *in vitro* but did not alter rates of glucuronide formation in periportal and pericentral regions of the liver lobule of intact liver. Infusion of epinephrine (50 nM) into perfused livers from untreated and 3-methylcholanthrene-treated rats increased rates of glucuronidation by about 35%. Since epinephrine probably acts by increasing the supply of the cofactor UDPGA due to increased breakdown of glycogen, it follows that UDPGA supply limits rates of glucuronidation in perfused livers from both untreated and 3-methylcholanthrene-treated rats.

Glucuronidation is an important pathway in the detoxification of several hepatotoxins that damage specific zones of the liver lobule (1–3). For example, increases (4, 5) and decreases (6) in glucuronidation were associated with protection and potentiation, respectively, of pericentral necrosis caused by acetaminophen. Therefore, information on regulation of glucuronidation in periportal and pericentral regions of the liver lobule is important in understanding mechanisms of zone-specific hepatotoxicity.

Rates of glucuronide production in periportal and pericentral regions of the liver lobule can now be measured noninvasively with a new fluorescence technique employing fiberoptic micro-light guides (7). The advantage of this method is that rates of glucuronide production can be measured under nearly physiological conditions in the perfused liver. In livers from fed, phenobarbital-treated rats, maximal rates of glucuronidation as well as glucuronosyltransferase activities were 3-fold higher in pericentral than in periportal areas (7). Fasting decreased rates of glucuronidation by 60% in both regions of the liver lobule (8). Since glucose reversed the fasting-induced decrease in glucuronidation, Conway *et al.* (8) suggested that UDPGA supply is an important rate determinant of glucuronidation in both periportal and pericentral regions of the liver lobule in the fasted state. However, regulation of glucuronidation in sublobular regions of livers from fed, untreated rats is less clear. Measurements of residual microsomal glucuronosyltransferase activity (9) and glucuronidation in perfused liver (10) after treatment with hepatotoxins *in vivo* provided evidence consistent with the hypothesis that glucuronidation was localized predominantly in periportal areas. In contrast, glucuronosyltransferase activity in microdissected samples of sublobular

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ABBREVIATIONS: UDPGA, uridine 5′-diphosphate-glucuronic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

areas (11) and immunohistochemical localization of glucuronosyltransferase (12) suggested a more or less even distribution of the enzyme across the liver lobule. Pharmacokinetic analysis of harmol conjugation in perfused livers from untreated rats and glucuronidation of *p*-nitrophenol in fractions of hepatocytes enriched in periportal and pericentral hepatocytes also suggested that glucuronidation was distributed equally across the lobule (13, 14). Some of the discrepancies in the above results may be due to differential localization of glucuronosyltransferases with different substrate specificities across the liver lobule. In addition, use of broken cell preparations does not allow one to evaluate regulatory factors such as cofactor supply. Accordingly, the goal of the present study was to study factors regulating glucuronidation in periportal and pericentral regions of the liver lobule in perfused livers from untreated and 3-methylcholanthrene-treated rats. 3-Methylcholanthrene treatment was employed since it is known to increase hepatic glucuronosyltransferase activity (15, 16) and the concentration of UDPGA (16, 17) as well as rates of glucuronidation in perfused livers (15, 16). Rates of formation of 7-hydroxycoumarin glucuronide were measured using micro-light guides placed on periportal and pericentral regions of the liver lobule. This information was compared to direct measurements of enzyme activities measured *in vitro* to identify factors important in regulation of glucuronidation in intact cells.

Materials and Methods

Animals. Female Sprague-Dawley rats weighing 250–400 g were injected intraperitoneally with 3-methylcholanthrene (80 mg/kg; Eastman Kodak) in corn oil 72 hr before perfusion experiments. Corn oil had no effect on rates of glucuronidation of 7-hydroxycoumarin (data not shown); therefore, untreated rats were used as controls. All rats employed in this study received rat chow *ad libitum*.

Liver perfusion and rates of glucuronidation of 7-hydroxycoumarin. Details of the non-recirculating perfusion technique have been described elsewhere (18). Livers from untreated rats were perfused with Krebs-Henseleit bicarbonate buffer, pH 7.4, saturated with a gas mixture of oxygen (95%) and carbon dioxide (5%). Sulfate-free perfusate used in these experiments was prepared by replacing magnesium sulfate with magnesium chloride. Under these conditions, rates of sulfation were less than 0.25 μmol/g/hr (19). Livers from 3-methylcholanthrene-treated rats were perfused with Krebs-Henseleit bicarbonate buffer containing α-naphthoflavone (35 μM) and albumin (final concentration, 0.37%; No. 8022, Sigma Chemical Co., St. Louis, MO). α-Naphthoflavone was employed to inhibit the metabolism of 7-hydroxycoumarin by mixed function oxidases in livers from 3-methylcholanthrene-treated rats (see Results). 7-Hydroxycoumarin (Sigma) was dissolved in dimethylformamide and added to the perfusate at final concentrations indicated in the figure and table legends. The final dimethylformamide concentration infused into the liver was less than 0.025% and had no effect on hepatic oxygen uptake or on rates of glucuronidation. In some experiments, sodium bitartrate epinephrine (final concentration of 50 nM) was dissolved in an ascorbate solution before being pumped into the inflow perfusate. The final concentration of ascorbate (15 μM) had no effect on the pH of the perfusate or on rates of conjugation. For perfusions in the anterograde direction, the perfusate was pumped into the portal vein at rates ranging from 2.5 to 3.5 ml/g/min and effluent perfusate was collected via a cannula placed in the inferior vena cava. In some experiments, the direction of flow was reversed (retrograde perfusions) to maximize substrate delivery to pericentral regions. Oxygen tension in the effluent perfusate was monitored continuously with a Teflon-shielded, Clark-type platinum O_2 electrode, and tissue viability was assessed from values of oxygen uptake which were calculated from the influent minus effluent oxygen concentration difference, the flow rate, and the liver wet weight (18).

Free 7-hydroxycoumarin in the effluent perfusate was measured fluorometrically (366 → 450 nm) with a quartz light guide (tip diameter, 2 mm) attached to a Johnson Foundation fluorometer. Glucuronides of 7-hydroxycoumarin were hydrolyzed enzymatically with β-glucuronidase as described elsewhere (7) and liberated free 7-hydroxycoumarin was measured fluorometrically. Fluorescence of unknown samples was compared with authentic 7-hydroxycoumarin standards incubated under identical conditions. Rates of glucuronidation by the whole organ were calculated from the concentration of glucuronide in the effluent perfusate, the flow rate, and the liver wet weight.

Micro-light guides. Light and dark spots visible on the surface of the hemoglobin-free perfused rat liver correspond to periportal and pericentral regions of the liver lobule, respectively (20). Micro-light guides consisting of two strands of glass fiber (tip diameter, 170 μm) were constructed as described previously (20, 21). Fluorescence of 7-hydroxycoumarin in the tissue was monitored simultaneously in periportal and pericentral areas of the left lateral lobe of the perfused liver with micro-light guides placed on the liver surface with micromanipulators. One strand of the micro-light guide was connected to a 100-W mercury arc lamp and the other strand to a photomultiplier. The liver was illuminated with a 366-nm light, and fluorescence of 7-hydroxycoumarin was measured at 450 nm as described in detail elsewhere (20, 22).

Glucuronosyltransferase activity in microsomes. Rats were anesthetized with pentobarbital (60 mg/kg) and livers were perfused briefly with Krebs-Henseleit buffer to remove blood. Livers were then homogenized in 0.25 M sucrose and microsomes were prepared by differential centrifugation as described by Bock and White (16). Microsomal glucuronosyltransferase was assayed by adding 0.25 ml of microsomal suspension (0.2 mg of protein) to 2.0 ml of 0.1 M Tris-HCl buffer (pH 7.4, 37°), containing 5 mM $MgCl_2$ and 100 μM 7-hydroxycoumarin. The reaction was initiated by the addition of 0.25 ml of various concentrations of UDPGA (0.05–6.4 mM) in 0.1 M Tris-HCl buffer containing 5 mM $MgCl_2$. After 10 min, 0.5 ml of the assay mixture was added to 1 ml of toluene/butanol (3:1) and extracted three times to remove free 7-hydroxycoumarin. Assays were linear for at least 10 min. An aliquot of the aqueous layer (100 μl) was then added to 0.9 ml of 0.5 M Tris-HCl, pH 7.4, containing 10 units of β-glucuronidase (Sigma, No. 8888). After a 1-hr incubation to cleave glucuronides completely, liberated 7-hydroxycoumarin was measured fluorometrically at 366 → 450 nm. Protein was determined by the biuret reaction (23). Glucuronosyltransferase activity was expressed per g of liver using a value of 40 mg of microsomal protein/g of wet liver (16, 24).

Glucuronosyltransferase activity in periportal and pericentral regions of the liver lobule. The assay of glucuronosyltransferase activity in lyophilized sections of periportal and pericentral regions of the liver lobule using microchemical techniques is described in detail elsewhere (7). Briefly, individual samples of periportal and pericentral areas weighing between 0.1 and 0.5 μg were dissected from lyophilized sections of liver and incubated at 37° in 10 μl of reagent containing: 25 mM HEPES buffer (pH 7.3), 0.1 mM dithiothreitol, 1 mM MgCl, 0.02% bovine serum albumin, 9 mM UDPGA, and 100 μM 7-hydroxycoumarin. After 2 hr, 100 μl of 0.05 M sodium acetate buffer (pH 5) were added and the unreacted 7-hydroxycoumarin was removed by three extractions with toluene/butanol (3:1). Finally, 100 μl of 0.01 M sodium acetate buffer, pH 5, containing 0.02% bovine serum albumin and 1000 units/ml β-glucuronidase, were added to the aqueous phase to hydrolyze glucuronides. After a 30-min incubation at 37°, 1 ml of 0.02 M sodium carbonate (pH 10) was added and the liberated 7-hydroxycoumarin was measured fluorometrically. Glucuronosyltransferase activity in periportal and pericentral areas was expressed as μmol/g wet wt/hr using a dry weight/wet weight ratio of 0.25 for data from both sublobular regions (25).

Results

Rates of Glucuronidation of 7-Hydroxycoumarin in Perfused Livers from Untreated and 3-Methylcholanthrene-Treated Rats

Greater than 95% of the 7-hydroxycoumarin infused into perfused livers from untreated rats was recovered in the effluent perfusate as the free substrate or the glucuronide. Double-reciprocal analysis of data indicated that glucuronidation was half-maximal with about 20 μM 7-hydroxycoumarin when substrate was infused in either the anterograde (via the portal vein) or retrograde (via the vena cava) directions (Fig. 1). Maximal rates of glucuronidation were 9.0 and 11.5 μmol/g/hr in perfusions in the anterograde and retrograde directions, respectively. These values were not statistically different in spite of slight differences in the concentrations of free 7-hydroxycoumarin leaving the liver (Table 1).

In contrast to untreated rats, recovery of 7-hydroxycoumarin (80 μM) infused into livers from 3-methylcholanthrene-treated rats was very low. Only 34 ± 4% ($n = 4$) of the infused substrate was recovered in the effluent perfusate as free 7-hydroxycoumarin and glucuronides. Previous work has shown that 7-hydroxycoumarin can be metabolized by mixed function oxidases to nonfluorescent products (26). To investigate this possibility, microsomes were incubated with 7-hydroxycoumarin and an NADPH-generating system (27). Microsomes from 3-methylcholanthrene-treated rats converted 7-hydroxycoumarin (80 μM) into nonfluorescent product(s) at rates of 16.6 nmol/min/mg of protein (data not shown). α-Naphthoflavone (5 μM), an inhibitor of the 3-methylcholanthrene-induced form of cytochrome P-450 (28), inhibited formation of nonfluorescent products completely. The above reaction was undetectable in microsomes from untreated rats.

When livers from 3-methylcholanthrene-treated rats were perfused with 80 μM 7-hydroxycoumarin, apparent rates of glucuronidation increased from 5.1 ± 2.9 ($n = 4$) to 8.7 ± 2.2 ($n = 10$) μmol/g/hr upon addition of 35 μM α-naphthoflavone. In the presence of α-naphthoflavone, 7-hydroxycoumarin could be accounted for totally as either the free substrate or the glucuronide. Since measurement of sublobular rates of glucuronidation in the perfused liver requires that all products be recovered (see below), livers from 3-methylcholanthrene-treated rats were perfused routinely in the presence of α-naphthoflavone. To evaluate whether α-naphthoflavone had nonspecific effects, six livers from untreated rats were perfused in the anterograde direction with 80 μM 7-hydroxycoumarin in the presence of 0.37% albumin and 35 μM α-naphthoflavone. Rates of glucuronidation in the absence (6.1 ± 0.5, $N = 15$) and presence (6.9 ± 0.8 μmol/hr, $N = 6$) of α-naphthoflavone were similar, allowing us to reject this possibility.

Maximal rates of glucuronidation in livers from 3-methylcholanthrene-treated rats perfused in the presence of α-naphthoflavone were 10.7 μmol/g/hr in perfusions in the anterograde direction and 12.1 μmol/g/hr when perfusions were in the retrograde direction (Fig. 2). Thus, rates of glucuronidation in perfused livers from 3-methylcholanthrene-treated rats were not statistically different from rates observed in perfused livers from untreated rats (Figs. 1 and 2). Glucuronidation was also half-maximal with about 20 μM 7-hydroxycoumarin with perfusions in either direction.

Epinephrine was infused into livers to activate glycogen phosphorylase a, increase the breakdown of glycogen, and thus increase the supply of glucose 1-phosphate for UDPGA synthesis. Livers from untreated and 3-methylcholanthrene-treated rats were perfused in the anterograde direction in the presence of 35 μM α-naphthoflavone and 100 μM 7-hydroxycoumarin. After establishment of steady state rates of glucuronidation, 50 nM epinephrine was infused and new steady state rates of glucuronidation were measured. Basal rates of glucuronidation were similar in livers from untreated and 3-methylcholanthrene-treated rats (Table 1). Epinephrine increased the rates

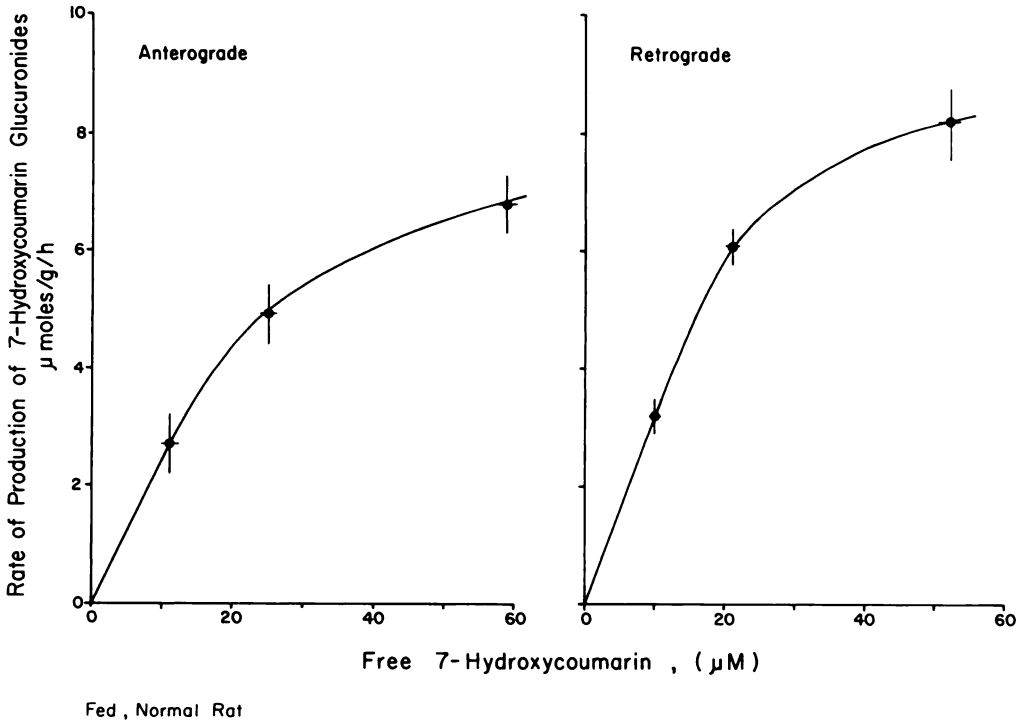

Fig. 1. Rates of production of 7-hydroxycoumarin glucuronide by livers from untreated rats perfused in the anterograde and retrograde directions. Livers from untreated rats were perfused with Krebs-Henseleit buffer in either the anterograde ($n = 37$) or retrograde ($n = 24$) direction. Effluent perfusate was collected every 2 min and assayed for glucuronides as described under Materials and Methods. The average free 7-hydroxycoumarin concentration in the liver was calculated from inflow + outflow concentrations/2. Data from 5–21 livers are averaged at each concentration of 7-hydroxycoumarin studied (mean ± standard error).

TABLE 1

Effect of epinephrine on glucuronidation in perfused livers from untreated and 3-methylcholanthrene-treated rats

Livers from untreated and 3-methylcholanthrene-treated rats were perfused with Krebs-Henseleit buffer containing 0.37 g% albumin and 35 μM α-naphthoflavone. Basal steady state rates of glucuronidation were measured 15–25 min after infusion at 100 μM 7-hydroxycoumarin. Epinephrine (50 nM) was then infused and rates were determined 10–20 min later. Each value is the average ± standard errors.

Treatment (*N*)	Rates of glucuronidation		Percentage increase
	Basal	+ Epinephrine	
	μmol/g/hr		
Untreated (3)	6.8 ± 0.6	8.9 ± 0.8[a]	30 ± 5
3-Methylcholanthrene (4)	7.1 ± 0.6	9.9 ± 0.8[a]	39 ± 6

[a] $p < 0.01$ compared to basal rates using a matched pair *t* test.

of glucuronidation in perfused livers from control and 3-methylcholanthrene-treated rats 30 ± 5 ($N = 3$) and 39 ± 6% ($N = 4$), respectively. This increase was statistically significant in livers from both untreated and 3-methylcholanthrene-treated rats at $p < 0.01$ using a matched pairs *t* test. Under these conditions, the recovery of 7-hydroxycoumarin was greater than 95%.

Rates of Glucuronidation in Periportal and Pericentral Regions in Livers from Untreated and 3-Methylcholanthrene-Treated Rats

Calibration of 7-hydroxycoumarin fluorescence from the liver surface. Measurement of sublobular rates of glucuronide production with micro-light guides is based on the conversion of fluorescent 7-hydroxycoumarin to its nonfluorescent glucuronide (7). Briefly, micro-light guides are placed on periportal and pericentral regions of the liver lobule. 7-Hydroxycoumarin is then infused and fluorescence is monitored during steady state rates of glucuronidation. The fluorescence signals are then converted into concentrations of free 7-hydroxycoumarin in tissue, and local rates of production of nonfluorescent glucuronides are calculated from the concentration differences of free substrate entering and leaving each sublobular region.

To convert fluorescence signals into concentrations of free 7-hydroxycoumarin in the tissue, the fluorescence of 7-hydroxycoumarin was calibrated in each liver by infusing 7-hydroxycoumarin in the presence of 20 mM ethanol and N_2-saturated perfusate ($N_2 + ET$, Figs. 3 and 4). Under these conditions, all infused 7-hydroxycoumarin was recovered in the effluent perfusate unmetabolized (i.e., glucuronide production was inhibited completely). Fluorescence detected from the liver surface increased in a linear fashion when 7-hydroxycoumarin concentration in the tissue was increased in the presence of nitrogen and ethanol (7). Because 7-hydroxycoumarin was not metabolized in anoxic livers in the presence of ethanol, fluorescence arising from the liver surface was due to free 7-hydroxycoumarin in the tissue after correction for small (<5%) increases in NADH fluorescence due to ethanol and nitrogen alone.

Determination of 7-hydroxycoumarin concentration in tissue during normoxia. Micro-light guides were placed on periportal and pericentral regions on the surface of a liver from an untreated rat, and a stable fluorescence baseline was established (Fig. 3). When 7-hydroxycoumarin (80 μM) was infused, fluorescence reached higher, steady state values in both regions in about 8 min and returned quickly to basal levels when 7-hydroxycoumarin infusion was terminated (Fig. 3). 7-Hydroxycoumarin was then infused in the presence of nitrogen-saturated perfusate containing 20 mM ethanol (Fig. 3, $N_2 + ET$). 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 due to reduction of NAD^+ (Fig. 3).

In all experiments, fluorescence detected in periportal and pericentral regions during perfusion of 7-hydroxycoumarin in the presence of ethanol and nitrogen was used to calculate concentrations of 7-hydroxycoumarin in the tissue during perfusion under normoxic conditions (Figs. 3 and 4). For example, 80 μM 7-hydroxycoumarin produced a fluorescence increase of 163% over the basal value in the periportal region during the

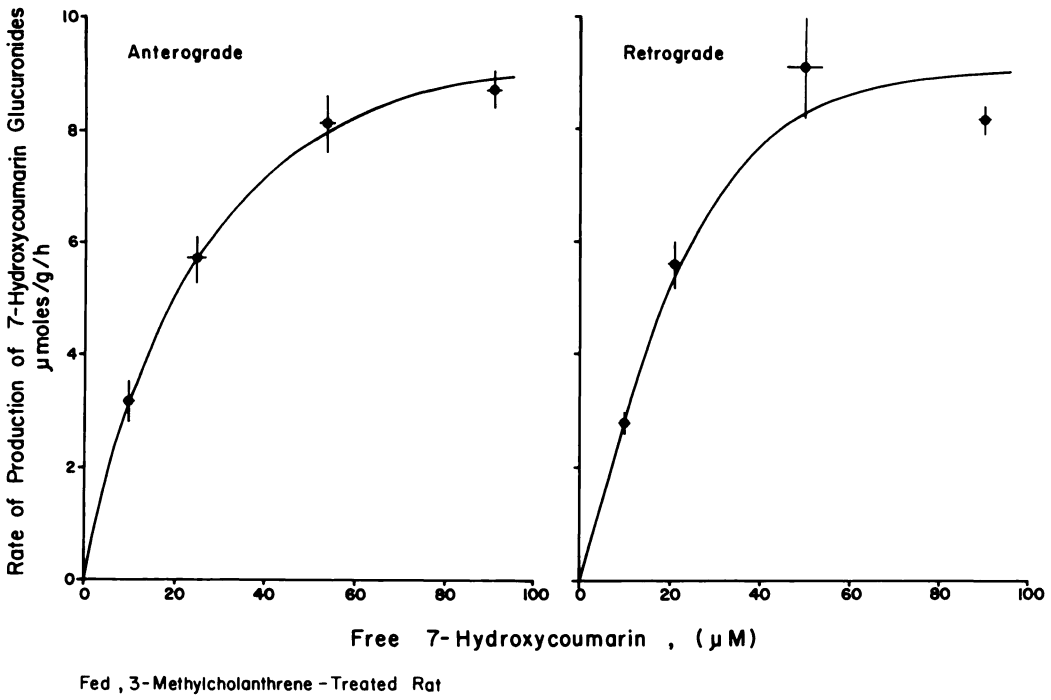

Fig. 2. Rates of production of 7-hydroxycoumarin glucuronide by livers from 3-methylcholanthrene-treated rats perfused in the anterograde and retrograde directions. 3-Methylcholanthrene (80 mg/kg in corn oil) was injected intraperitoneally 72 hr before perfusion experiments. Livers were perfused with Krebs-Henseleit buffer containing 0.37% albumin and 35 μM α-naphthoflavone in the anterograde direction ($n = 31$) or the retrograde direction ($n = 25$). Data from 5–11 livers are averaged at each concentration of 7-hydroxycoumarin studied. Other conditions are as in Fig. 1.

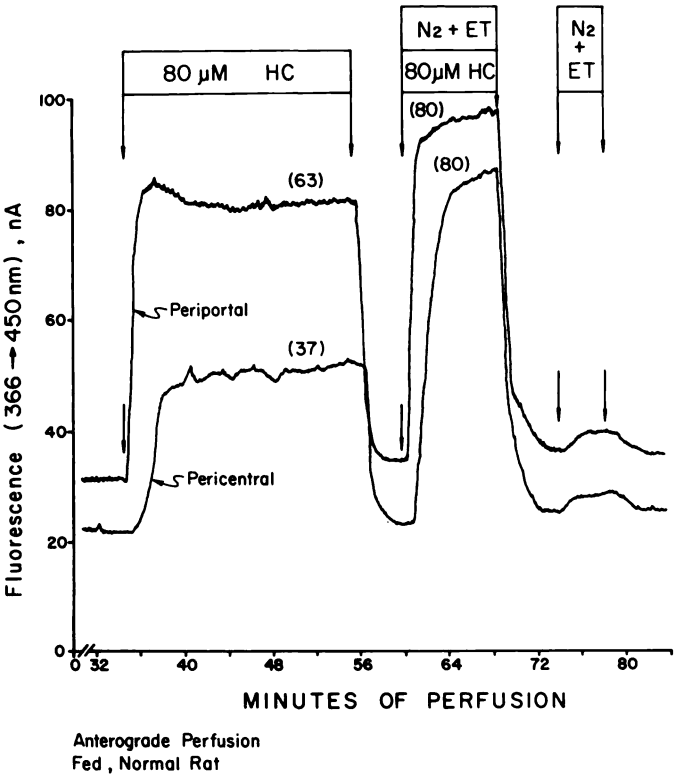

Fig. 3. Fluorescence increase due to 7-hydroxycoumarin (*HC*) and N_2-saturated perfusate containing 20 mM ethanol (*N_2 + ET*) in periportal and pericentral regions of a liver from an untreated rat perfused in the anterograde direction. Two micro-light guides were placed on periportal and pericentral regions 1 mm apart on the left lateral lobe of the liver (20). 7-Hydroxycoumarin and N_2-saturated perfusate containing 20 mM ethanol were infused as indicated by *horizontal bars* and *arrows*. *Numbers in parentheses* represent μM concentrations of free 7-hydroxycoumarin in the tissue calculated as described in Results. A typical experiment is shown.

anoxia plus ethanol period (Fig. 3). Under normoxic conditions, the increase was 128% in the same region. From these fluorescence values, it was calculated that 63 μM 7-hydroxycoumarin was free in the tissue under normoxic conditions (see values in parentheses, Fig. 3). Greater than 95% of all nonfluorescent products were glucuronides in livers from untreated and 3-methylcholanthrene-treated rats; therefore, it was concluded that the decrease in free 7-hydroxycoumarin concentration in any region of the liver lobule was due to the production of glucuronides. In the above example, the decrease in the concentration of free 7-hydroxycoumarin in the periportal region was 80 − 63 = 17 μM. Pericentral regions are "downstream" from periportal regions (e.g., flow is from periportal to pericentral); therefore, pericentral areas are only exposed to free 7-hydroxycoumarin not conjugated by periportal areas. Since the micro-light guide detected 63 μM hydroxycoumarin in periportal areas, but only 37 μM free 7-hydroxycoumarin appeared in the pericentral regions, it was calculated that 26 μM (63 − 37) 7-hydroxycoumarin was converted to glucuronides in pericentral regions (Fig. 3). Similar data were obtained from perfusions in the retrograde direction (not shown) and in livers from 3-methylcholanthrene-treated rats (Fig. 4).

Densitometry of dark spots and light areas in photographs of thin slices of liver indicate that periportal and pericentral regions comprise about equal portions of the liver lobule (25); therefore, we assume that each sublobular region is one-half the liver wet weight. Local rates of glucuronidation (Figs. 5 and 6) were calculated using the flow rate of perfusate, the mass of each region, and the concentration of 7-hydroxycoumarin glucuronide formed in that region (Tables 2 and 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.

Maximal rates of glucuronidation were 12–13 μmol/g/hr in periportal and pericentral regions, respectively, in livers from untreated rats (Fig. 5). In livers from 3-methylcholanthrene-treated rats, maximal rates were around 13 μmol/g/hr in both regions (Fig. 6). Thus, rates of glucuronidation were not statistically significantly different in periportal and pericentral regions in livers from untreated and 3-methylcholanthrene-treated rats regardless of the direction of perfusion. Double-reciprocal analysis of the data indicated that glucuronidation was half-maximal with 10–20 μM 7-hydroxycoumarin in livers from both untreated and 3-methylcholanthrene-treated rats.

Microchemical Analysis of Glucuronosyltransferase Activity in Periportal and Pericentral Areas

Glucuronosyltransferase activity was measured in lyophilized samples microdissected from periportal and pericentral areas. In the presence of 100 μM 7-hydroxycoumarin and 9 mM UDPGA, glucuronosyltransferase activity was 10 and 23 μmol/g/hr in periportal and pericentral areas, respectively, in liver samples from untreated rats (Table 4). In contrast, maximal glucuronosyltransferase activity was about 80 and 95 μmol/g/hr in periportal and pericentral regions, respectively, in samples from 3-methylcholanthrene-treated rats (Table 4).

In homogenates of freeze-dried liver (Table 4), glucuronosyltransferase activity was about 8-fold higher than rates of glucuronidation in perfused livers from 3-methylcholanthrene-treated rats (Figs. 2 and 6). Because of this disparity between rates measured in freeze-dried tissue and the whole organ, the effects of UDPGA concentration and UDP-*N*-acetylglucosamine on glucuronosyltransferase activity in native hepatic microsomes prepared from untreated and 3-methylcholanthrene-treated rats were also investigated. Glucuronosyltransferase activity was 2- to 3-fold higher in native microsomes prepared from 3-methylcholanthrene-treated than untreated rats at all UDPGA concentrations studied (Fig. 7). Concentrations of UDP-*N*-acetylglucosamine normally found in rat liver (0.3 mM) (29) increased glucuronosyltransferase activity by about 50% in native microsomes from both 3-methylcholanthrene-treated and untreated rats (Fig. 7).

Discussion

Glucuronidation in periportal and pericentral regions of the lobule in livers from untreated and 3-methylcholanthrene-treated rats. Rates of production of glucuronides from 7-hydroxycoumarin were similar in periportal and pericentral regions of the lobule in livers from untreated rats (Fig. 5). This finding agrees with conclusions from studies on the kinetics of harmol glucuronidation in livers perfused in anterograde and retrograde directions (13) and glucuronidation in preparations enriched with hepatocytes from periportal and pericentral areas (14). 3-Methylcholanthrene treatment had no effect on rates of glucuronidation in periportal and pericentral areas of the perfused liver (cf. Figs. 5 and 6). Tonda and Hirata (14) reported equal rates of glucuronidation in preparations

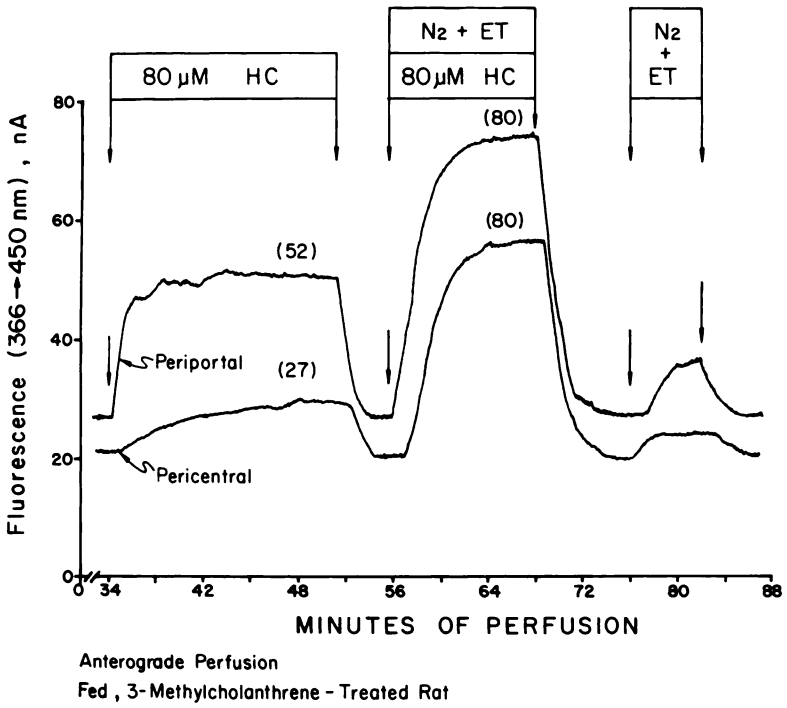

Fig. 4. Fluorescence increase due to 7-hydroxycoumarin (*HC*) and N_2-saturated perfusate containing 20 mM ethanol (N_2 + *ET*) in periportal and pericentral regions of a liver from a 3-methylcholanthrene-treated rat perfused in the anterograde direction. Conditions are as in Fig. 3. A typical experiment is shown.

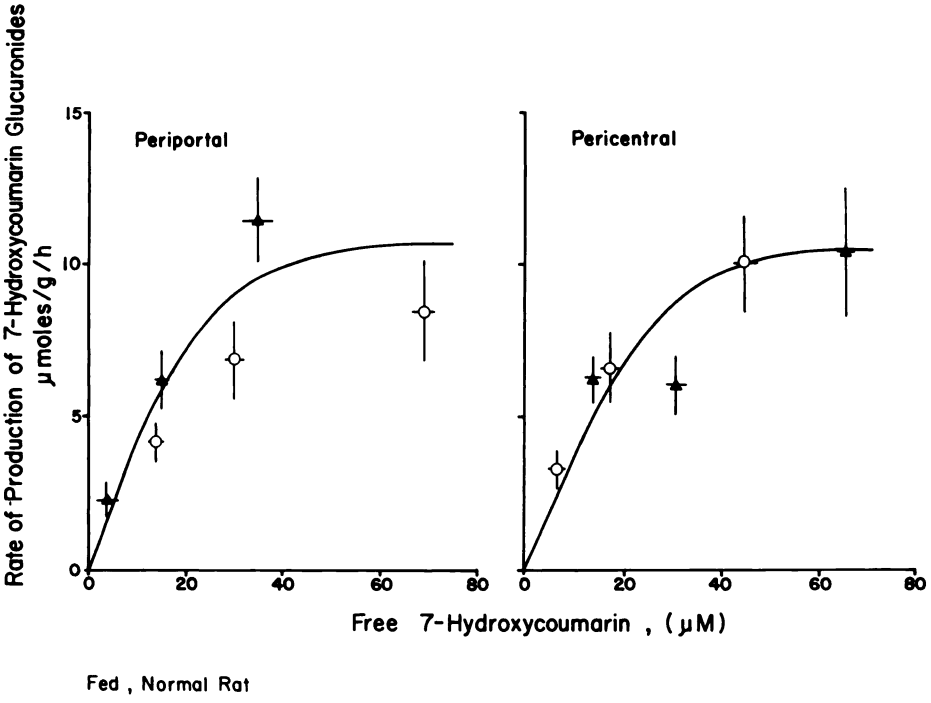

Fig. 5. Rates of production of glucuronides from 7-hydroxycoumarin in periportal and pericentral regions of the liver lobule in perfused livers from untreated rats. The concentration of glucuronide conjugates formed in each region during anterograde (○) and retrograde (▲) perfusions was derived from fluorescence measurements of free 7-hydroxycoumarin in the tissue (see Fig. 3 and Table 2). Rates were calculated using the flow rate and the wet weight of each sublobular region (liver wet weight/2). Concentrations of substrate are the average of the free 7-hydroxycoumarin (μM) entering and leaving each sublobular region (see Results). Data from 5–21 livers were averaged at each concentration of 7-hydroxycoumarin studied (mean ± standard error).

enriched with hepatocytes from periportal and pericentral areas of livers from 3-methylcholanthrene-treated rats. 3-Methylcholanthrene treatment also increased glucuronidation about 3-fold in both preparations (14). These data along with our findings (Figs. 5 and 6) indicate that glucuronidation does not differ across the lobule in preparations from untreated and 3-methylcholanthrene-treated rats.

The activity of glucuronosyltransferase and β-glucuronidase as well as the supply of UDPGA may regulate rates of glucuronide formation and export from intact hepatocytes. In addition, glucuronosyltransferase isoenzymes may have different sensitivities to regulation by cofactor supplies and endogenous activators such as UDP-*N*-acetylglucosamine. Studies with phenobarbital and 3-methylcholanthrene treatments have demonstrated a correlation between increases in glucuronosyltransferase activity *in vitro* and rates of glucuronidation in perfused livers (15, 30) and isolated hepatocytes (31). Because the above work implicated enzyme activity as an important determinant of rates of glucuronidation in intact cells, we compared maximal enzyme activities in periportal and pericentral areas dissected from lyophilized sections of liver with rates of glucuronidation in intact periportal and pericentral regions. In livers from untreated rats, glucuronosyltransferase activity was 2.3-fold higher in pericentral than periportal areas (Table 4), yet rates

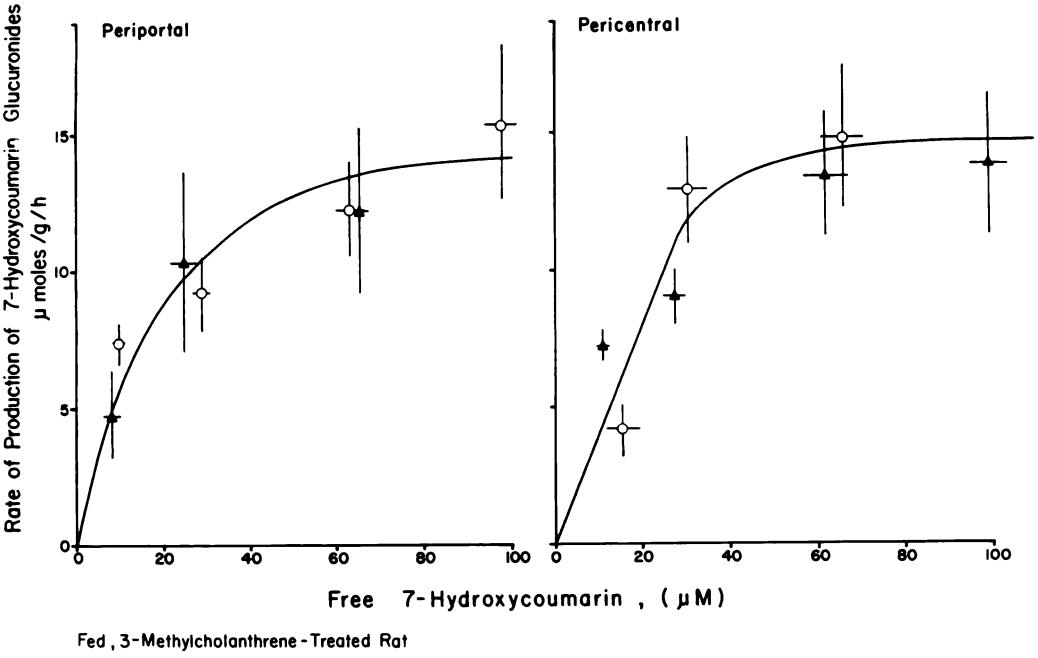

Fig. 6. Rates of production of glucuronides of 7-hydroxycoumarin in periportal and pericentral regions of the liver lobule in perfused livers from 3-methylcholanthrene-treated rats. Rats were pretreated and livers were perfused as in Fig. 2. All other conditions are as in Fig. 5. Data from 5–11 livers were averaged at each concentration of 7-hydroxycoumarin studied (mean ± standard error).

TABLE 2

Effect of substrate concentration on free 7-hydroxycoumarin and its glucuronide in periportal and pericentral regions of perfused livers from normal rats

Concentrations of glucuronide conjugates formed in periportal and pericentral regions were calculated from experiments typified by Fig. 3 (see Results). Concentrations of free 7-hydroxycoumarin in the effluent perfusate were measured as described under Materials and Methods. Values are means ± standard errors for the number of experiments shown in parentheses in column 1.

I 7-Hydroxycoumarin infused (Influent perfusate)	II Glucuronide conjugates formed in each region		III Free 7-hydroxycoumarin present (Effluent perfusate)	II + III (Sum)
	Periportal	Pericentral		
			μM	
Anterograde				
20* (6)	8.9 ± 1.3	8.8 ± 1.2	3.2 ± 1.3	20.8 ± 1.3
40 (10)	11.5 ± 3.2	20.8 ± 3.8	9.9 ± 1.2	42.1 ± 2.6
80 (21)	16.5 ± 2.8	26.3 ± 3.5	41.2 ± 2.2	86.0 ± 3.3
Retrograde				
20 (5)	6.3 ± 1.5	12.9 ± 1.4	0.8 ± 0.5	20.0 ± 0.4
40 (7)	18.6 ± 1.3	18.2 ± 2.9	3.3 ± 0.6	40.1 ± 2.4
80 (12)	35.6 ± 5.0	27.6 ± 5.7	25.2 ± 1.5	88.4 ± 3.3

* μM concentration.

TABLE 3

Effect of substrate concentration on free 7-hydroxycoumarin and its glucuronide in periportal and pericentral regions of the lobule in perfused livers from 3-methylcholanthrene-treated rats

Concentrations of glucuronide conjugates formed in periportal and pericentral regions were calculated from experiments typified by Fig. 4 (see Results). Concentrations of free 7-hydroxycoumarin in the effluent perfusate were measured as described under Materials and Methods. Values are means ± standard errors of the number of experiments shown in parentheses in column 1.

I 7-Hydroxycoumarin infused Influent perfusate	II Glucuronide conjugates formed in each region		III Free 7-hydroxycoumarin present (Effluent Perfusate)	II + III (Sum)
	Periportal	Pericentral		
		μM		
Anterograde				
20* (5)	18.8 ± 0.1	1.2 ± 0.8	0.3 ± 0.3	20.2 ± 0.2
40 (5)	22.0 ± 3.6	10.4 ± 2.5	9.7 ± 3.0	42.1 ± 4.5
80 (10)	33.7 ± 6.1	31.8 ± 5.4	29.2 ± 3.0	89.4 ± 4.1
120 (11)	45.0 ± 8.0	35.1 ± 4.7	63.6 ± 4.4	134.6 ± 13.8
Retrograde				
20 (5)	1.5 ± 1.2	18.5 ± 1.2	2.8 ± 2.1	20.0 ± 0.1
40 (6)	12.9 ± 4.1	25.9 ± 3.6	1.3 ± 0.8	40.2 ± 1.1
80 (6)	28.6 ± 10.3	40.3 ± 8.0	20.8 ± 5.7	89.7 ± 3.2
120 (6)	37.2 ± 9.4	42.6 ± 8.0	50.2 ± 1.0	129.8 ± 6.5

* μM concentration.

TABLE 4

Glucuronosyltransferase activity in microdissected periportal and pericentral regions of perfused livers from untreated, corn oil-treated, and 3-methylcholanthrene-treated rats

Six to eight samples of periportal and pericentral regions were dissected from individual livers and were assayed with 100 μM 7-hydroxycoumarin and 9 mM UDPGA (Materials and Methods). Data are expressed per g wet weight using a dry weight/wet weight ratio of 0.25 for data from both periportal and pericentral regions (25). Each value is the average ± standard error obtained from a single liver. Two untreated, corn oil-treated and 3-methylcholanthrene-treated rats were analyzed. 3-Methylcholanthrene (80 mg/kg) in corn oil was injected intraperitoneally 72 hr before liver perfusion.

Treatment	Periportal	Pericentral	Pericentral/periportal
	μmol/g wet wt/hr		
None	5.7 ± 0.6	12.4 ± 1.5*	2.2
	11.4 ± 1.3	27.7 ± 1.4*	2.4
Corn Oil	9.2 ± 1.4	22.1 ± 1.1*	2.4
	15.2 ± 1.2	32.2 ± 1.9*	2.1
3-Methylcholanthrene	83.2 ± 0.9	103.3 ± 0.2*	1.2
	76.4 ± 5.2	93.5 ± 8.6	1.2

*$p < 0.01$ for comparison between periportal and pericentral areas.

of glucuronide production in the two regions in perfused livers were nearly identical (Fig. 5). Furthermore, treatment with 3-methylcholanthrene increased glucuronosyltransferase activity 4- to 7-fold in lyophilized samples of periportal and pericentral areas (Table 4) but did not alter rates of glucuronidation in sublobular areas in perfused livers significantly (Figs. 5 and 6). In addition, glucuronosyltransferase activity measured in native microsomes from 3-methylcholanthrene-treated rats was 2- to 3-fold higher than that in controls at all UDPGA concentrations used (Fig. 7). Taken together, it appears that factors other than absolute activities of glucuronosyltransferase are major determinants of rates of glucuronidation in intact cells.

β-Glucuronidase may influence glucuronidation in intact hepatocytes (32, 33) and therefore could possibly contribute to the above discrepancies between glucuronosyltransferase activities and rates of glucuronidation observed in intact cells. However, β-glucuronidase activity was distributed evenly in periportal and pericentral regions of the liver lobule (34) and therefore cannot explain the lower than expected rates of glucuronidation observed in pericentral areas. In addition, Bock and White (16) failed to detect a difference in β-glucuronidase activity in liver homogenates from normal and 3-methylcholanthrene-treated rats. Taken together, the above data suggest that β-glucuronidase does not preferentially limit glucuronide production in sublobular zones or in livers from 3-methylcholanthrene-treated rats.

Lyophilization causes an activation of glucuronosyltransferase (7), making comparisons between data obtained from isolated sublobular areas and perfused liver dangerous. Preferential activation of glucuronosyltransferases by lyophilization could contribute to the high activity observed in pericentral regions of normal livers and the large increases in enzyme activity observed after 3-methylcholanthrene treatment (Table 4). Bock and White (16) also suggested that 3-methylcholanthrene-induced glucuronosyltransferases may be sensitive to activation since glucuronosyltransferase activity toward naphthol was much greater in lypholized liver (11), detergent-activated microsomes (16), and native microsomes (16) than in perfused livers from 3-methylcholanthrene-treated rats (16).

UDPGA is an obligatory cofactor for glucuronidation. Infusion of the α_1 receptor agonist epinephrine increased rates of glucuronidation in perfused livers from untreated and 3-methylcholanthrene-treated rats by about 35% (Table 1). This increase is likely due to increased formation of glucose 1-phosphate from glycogen via α_1 receptors and resultant increases in UDPGA levels (33). Rate limitation of glucuronidation by UDPGA supply is in accord with previous observations that fasting-refeeding (30) and orotic acid treatment (35) increased UDPGA concentrations and rates of glucuronidation. In addition, glucuronosyltransferase activity in native microsomes prepared from untreated and 3-methylcholanthrene-treated rats was 2- to 3-fold below maximal values when assayed with physiological concentrations of UDPGA (about 0.4 mM; Fig. 7). This finding suggests that cofactor supply is an important rate-limiting factor for glucuronidation in intact cells.

Relative rates of mixed function oxidation and glucuronidation in periportal and pericentral regions of the liver lobule. Substrates for glucuronidation are in many cases generated intracellulary via mixed function oxidase reactions, and efficient conjugation may be important in maintaining low levels of toxic intermediates in the cell. Studies with 7-ethoxycoumarin and 7-hydroxycoumarin allow us to compare relative

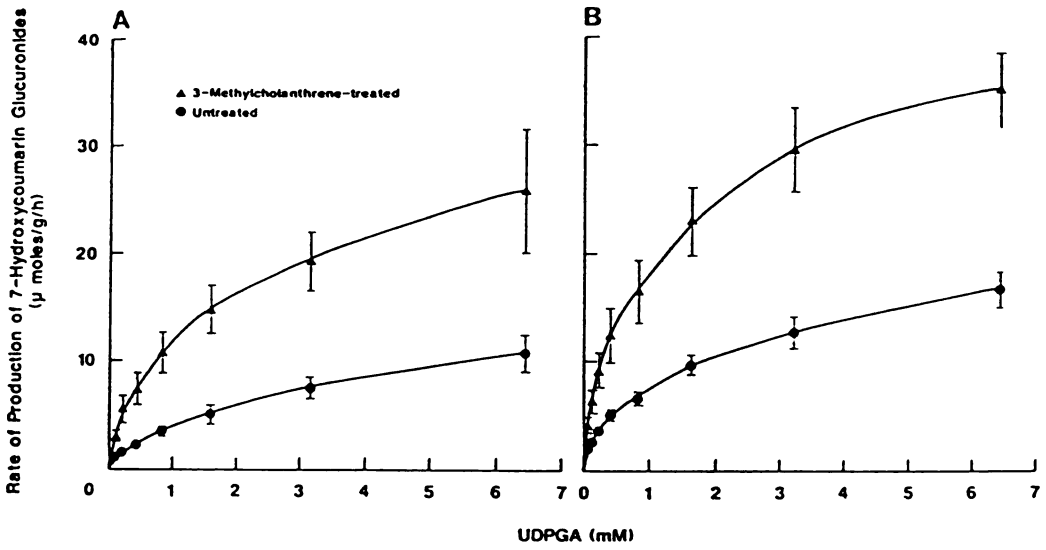

Fig. 7. Effect of UDPGA on rates of glucuronidation of 7-hydroxycoumarin in hepatic microsomes from untreated and 3-methylcholanthrene-treated rats. Rats were treated, microsomes were prepared, and glucuronidation was assayed as described under Materials and Methods. A. Average data ± standard errors from five experiments with native microsomes. B. Three experiments with native microsomes incubated in the presence of 0.3 mM UDP-*N*-acetylglucosamine. In each experiment microsomes from three rats were pooled.

rates of mixed function oxidation and glucuronidation in sublobular areas for the first time.

Micro-light guides have been used to measure maximal rates of generation of 7-hydroxycoumarin via *O*-deethylation of 7-ethoxycoumarin noninvasively in sublobular areas of perfused livers from untreated (36), phenobarbital-treated (22), and β-naphthoflavone-treated rats (36). Maximal rates of 7-ethoxycoumarin *O*-deethylation were about 1.2 μmol/g/hr in periportal and pericentral regions of livers from untreated rats (22), whereas maximal rates of glucuronidation were about 11 μmol/g/hr in both sublobular areas of livers from untreated rats (Fig. 5). When rats were pretreated with phenobarbital, rates of 7-ethoxycoumarin *O*-deethylation increased to 3.6 and 7.0 μmol/g/hr in periportal and pericentral areas (22), and maximal rates of glucuronidation were 10 and 35 μmol/g/hr in periportal and pericentral regions, respectively (7). Thus, in livers from untreated and phenobarbital-treated rats, maximal rates of glucuronidation in each sublobular area were at least 4-fold greater than rates of mixed function oxidation. Treatment with β-naphthoflavone, a 3-methylcholanthrene-like inducer, increased rates of 7-ethoxycoumarin *O*-deethylation to 21 μmol/g/hr in both sublobular areas (36); however, maximal rates of glucuronidation were only about 12 μmol/g/hr in both regions (Fig. 6). Thus, changes induced by 3-methylcholanthrene, and possibly other carcinogens, are unique in that rates of generation of toxic products from monooxygenases may exceed rates of glucuronidation in both regions of the liver lobule. This change in mixed function oxidation relative to the capacity to conjugate following administration of polycyclic aromatic hydrocarbons may result in higher concentrations of reactive intermediates and thus increased hepatotoxicity.

Acknowledgments

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