MOLECULAR PHARMACOLOGY, 25:487–493

Glucuronidation of 7-Hydroxycoumarin in Periportal and Pericentral Regions of the Liver Lobule

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Received August 23, 1983; Accepted December 7, 1983

SUMMARY

Rates of glucuronidation were measured at high substrate concentrations in specific zones of the liver lobule using micro-light guides placed on periportal and pericentral regions on the surface of livers from phenobarbital-treated rats. Livers were perfused with sulfate-free buffer under normoxic conditions, and fluorescence of free 7-hydroxycoumarin was monitored in the tissue. The formation of nonfluorescent 7-hydroxycoumarin glucuronide was then inhibited completely by perfusion with N_2-saturated perfusate containing 20 mM ethanol. Under these conditions, fluorescence recorded from the surface of the liver was directly proportional to the concentration of substrate infused. The difference in 7-hydroxycoumarin fluorescence between N_2 plus ethanol and normoxic perfusion was due to glucuronidation. Maximal rates of glucuronidation in periportal and pericentral regions of the liver lobule calculated with this new method were 9.6 and 35 μmoles/g/hr, respectively. Glucuronidation was half-maximal with 25–50 μM 7-hydroxycoumarin in both regions. Glucuronosyltransferase activity assayed in microdissected, freeze-dried tissue samples *in vitro* was 3-fold greater in pericentral areas than in periportal areas. This activity was half-maximal with 0.2 mM UDP-glucuronic acid and 54 μM 7-hydroxycoumarin in both regions of the liver lobule. Thus, the maximal capacity of the glucuronidation system determined *in vitro* is about 3-fold greater in pericentral than in periportal regions of the liver lobule, a difference which correlates well with measured rates of glucuronidation of 7-hydroxycoumarin in the two zones of the lobule in the intact, perfused liver.

INTRODUCTION

Glucuronidation is an important pathway in the hepatic metabolism of a large array of xenobiotics as well as endogenous substances (1). A number of factors, including substrate concentration (2–9), activities of a family of transferase enzymes (2, 3, 10), and supply of the cofactor UDP-glucuronic acid (2, 3, 5, 7, 11, 12) may influence rates of glucuronidation in the intact liver. Since there is considerable metabolic microheterogeneity across the liver lobule (9, 13, 14), events regulating glucuronidation in periportal and pericentral regions of the liver lobule may differ. Information regarding regulation in specific lobular zones is needed, since a variety of compounds which damage specific regions of the liver lobule are detoxified by glucuronidation (1, 15).

This research was supported, in part, by United States Public Health Service Grants CA-23080, CA-20807, and ES-02759.

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Previous work from this laboratory (9) demonstrated that rates of glucuronidation of 7-hydroxycoumarin could be measured in specific zones of the liver lobule with low substrate (<20 μM) concentrations. Glucuronosyltransferase has a relatively high K_m for substrate; thus the earlier studies were performed under conditions where substrate was most likely rate-limiting. The purpose of the present work was to analyze rates of glucuronidation of the highly fluorescent substrate, 7-hydroxycoumarin, in specific zones of the liver lobule as a function of substrate concentration. Data obtained in the perfused liver are compared with activities of 7-hydroxycoumarin glucuronosyltransferase determined in microdissected samples of the two zones of the liver studied under optimal conditions *in vitro*. The results indicate that rates of glucuronidation in perfused liver are about 3-fold higher in pericentral than in periportal regions of the lobule in livers from fed, phenobarbital-treated rats. Preliminary accounts of this work have appeared elsewhere (16).

METHODS

Animals. Female Sprague-Dawley rats weighing 250–400 g were pretreated with phenobarbital (1 g/liter) in drinking water for 15–30

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days prior to perfusion experiments (17) to enhance the color contrast between periportal and pericentral regions of the liver lobule. All rats had free access to laboratory chow.

Liver perfusion. Details of the nonrecirculating perfusion technique have been described elsewhere (18). The perfusate was 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. The maximal rate of sulfation under these conditions was 0.25 μmoles/g/hr. 7-hydroxycoumarin (Sigma Chemical Company, St. Louis, Mo.) was dissolved in dimethylformamide before being added to the perfusate. The final dimethylformamide concentration was less than 0.025% and had no effect on hepatic oxygen uptake or rates of glucuronidation. For perfusions in the anterograde direction, the perfusate was pumped into the portal vein (3–4 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). Effluent perfusate was collected for measurement of 7-hydroxycoumarin and its glucuronide. The oxygen tension in the effluent perfusate was monitored continuously with a Teflon-shielded, Clark-type platinum electrode, and oxygen uptake was used to assess tissue viability.

Free 7-hydroxycoumarin was measured fluorometrically (366 → 450 nm) with a quartz light guide (tip diameter 2 mm) attached to a Johnson Foundation fluorometer. Sample fluorescence was compared with authentic 7-hydroxycoumarin standards incubated under identical conditions. Glucuronide conjugates were hydrolyzed enzymatically as described elsewhere (14, 19). The difference between total and free 7-hydroxycoumarin represented glucuronide conjugates. Rates of conjugation were calculated from the concentration of glucuronides in the effluent perfusate, the flow rate, and liver wet weight.

Micro-light guide. The construction and use of micro-light guides, consisting of two strands of glass fiber (diameter 70 μm) held together at the tip with epoxy glue, have been described previously (19). 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). Fluorescence changes in periportal and pericentral regions are monitored simultaneously after placing micro-light guides on light and dark areas of the left lateral lobe of the perfused liver 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 is lluminated with 366-nm light, and fluorescence of 7-hydroxycoumarin is measured at 450 nm (14, 20).

Glucuronosyltransferase activity in periportal and pericentral regions. The perfused liver was frozen by immersion in liquid nitrogen. Tissue adjacent to the surface was sectioned at 20 μm in a cryostat at −20°. The sections were then freeze-dried at −40° to preserve histological structure (21). Periportal and pericentral areas in lyophilized tissue sections were dissected under a microscope and weighed on a quartz-fiber balance (21). Glucuronosyltransferase activity in the lyophilized samples (0.2–0.5 μg dry weight) was assayed by incubating samples in 10 μl of reagent containing 25 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid buffer (pH 7.3), 0.1 mM dithiothritol, 1 mM $MgCl_2$, 0.02% bovine serum albumin, 9 mM UDP-glucuronic acid, and 100 μM 7-hydroxycoumarin. After 2 hr at 37°, the reaction was stopped by immersing the tubes in ice and adding 100 μl of 0.05 M sodium acetate (pH 5). Unreacted 7-hydroxycoumarin was removed by three successive extractions with toluene:butanol (3:1). Finally, 100 μl of 0.01 M sodium acetate (pH 5), 0.02% bovine serum albumin, and β-glucuronidase (1000 units/ml) were added to the aqueous phase. 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 fluorimetrically. Under these conditions, the assay was linear for at least 3 hr and was proportional to tissue added from 0.1 to 0.5 μg dry weight.

RESULTS

Rates of glucuronidation of 7-hydroxycoumarin in perfused livers. Rates of formation of nonfluorescent glucuronide conjugates by the whole liver were not statistically significantly different (Fig. 1) when a given concentration of 7-hydroxycoumarin was infused in either the anterograde (via the portal vein) or retrograde (via the vena cava) direction. Double-reciprocal analysis of data from anterograde perfusions indicated that glucuronidation was half-maximal with around 55 μM substrate. Maximal rates were 21 μmoles/g/hr, and the slope of the Hill plot was 1. Since the double-reciprocal plot of data from retrograde perfusions was not linear, simple kinetic analysis was not possible.

Calibration of 7-hydroxycoumarin fluorescence from the liver surface. To achieve our goal of measuring sublobular rates of glucuronidation at high substrate concentrations, micro-light guides were first placed on periportal and pericentral regions of the liver lobule. 7-hydroxycoumarin was then infused, and its fluorescence in periportal and pericentral regions was monitored during normoxic perfusions. Finally, the fluorescence signals were converted into concentrations of free 7-hydroxycoumarin in tissue (described below), and local rates of formation of nonfluorescent glucuronide conjugates were calculated from the concentration differences of free substrate entering and leaving each sublobular region.

Following the addition of 7-hydroxycoumarin during normoxic perfusion, the fluorescence of 7-hydroxycoumarin was calibrated in each liver by infusing 20 mM ethanol in N_2-saturated perfusate (N_2 + E, Figs. 2 and 3). Under these conditions, all infused 7-hydroxycoumarin was recovered in the effluent unmetabolized (i.e., glucuronidation was inhibited completely). Because 7-hydroxycoumarin was not metabolized in the presence of nitrogen and ethanol, the 7-hydroxycoumarin fluorescence arising from the liver surface was assumed to represent free 7-hydroxycoumarin in the tissue. This assumption is supported by the observation that the fluorescent signal from the liver surface increased in a linear fashion when 7-hydroxycoumarin concentration in the tissue was increased (Fig. 4). A correlation coefficient of 0.70 ($p < 0.001$) was obtained between the fluorescence increase and the free 7-hydroxycoumarin concentration in the tissue when data from 54 separate perfusions were analyzed (Fig. 4). Data were routinely corrected for the small fluorescence increase due to N_2 and ethanol alone (Figs. 2 and 3).

Determination of 7-hydroxycoumarin concentration in the tissue during normoxia. When 7-hydroxycoumarin was infused under normoxic conditions, the fluorescent signals detected by micro-light guides placed on periportal and pericentral regions of the liver lobule were always less than the values detected in the presence of N_2-saturated perfusate and ethanol (Figs. 2 and 3). After 7-hydroxycoumarin infusion was terminated under either normoxic or anoxic conditions, fluorescence signals returned rapidly to their respective baselines (Figs. 2 and 3). Since a linear relationship between fluorescence and 7-hydroxycoumarin concentration was observed (Fig. 4), maximal fluorescence changes due to 7-hydroxycoumarin during anoxia plus ethanol can be used to calculate the concentrations of free 7-hydroxycoumarin in discrete areas of the liver lobule during normoxia. For example,

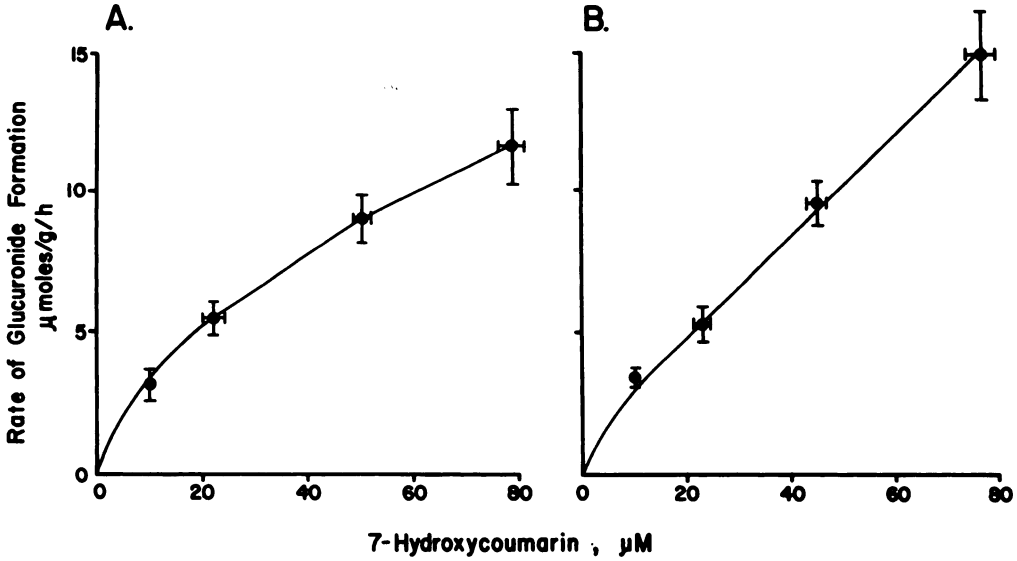

FIG. 1. *Rate of glucuronide formation by intact liver during anterograde (A) and retrograde (B) perfusions*
Thirty-three livers from fed, phenobarbital-treated rats were perfused with Krebs-Henseleit buffer in the anterograde directions (A) and 21 in the retrograde direction (B). Perfusate was collected and assayed for glucuronides (see Methods). The average free 7-hydroxycoumarin concentration in the liver was calculated from inflow + outlfow concentrations/2. Data from 4–13 livers are averaged for each concentration of 7-hydroxycoumarin (mean ± standard error of the mean).

80 μM 7-hydroxycoumarin produced a fluorescence increase of 160% over basal (i.e., anoxia plus ethanol infusion) in the periportal region (Fig. 2). Under normoxic conditions, the increase was only 142% in the same region. Therefore, the 7-hydroxycoumarin concentration in the tissue under normoxic conditions was calculated to be 71 μM (see parentheses, Fig. 2). The decrease in free 7-hydroxycoumarin concentration in any region was assumed to be due to glucuronidation. This assumption is based on the observation that free and nonfluorescent products leaving the liver equaled 7-hydroxycoumarin infused (Table 1). Furthermore, more than 85% of the nonfluorescent products formed in all perfusions were glucuronides. In this example, the concentration of glucuronide conjugates formed by the periportal region was 80 − 71 = 9 μM. Since the pericentral region is "downstream" from the periportal region (e.g., flow is from periportal to pericentral), it is only exposed to the free 7-hydroxycoumarin not conjugated by periportal areas (i.e., 71 μM). Since the micro-light guide detected only 12 μM free 7-hydroxycoumarin in the pericentral area, we conclude that pericentral hepatocytes converted 59 μM (71 − 12) of the available 71 μM 7-hydroxycoumarin to glucuronides (Fig. 2).

Almost identical results were found when pericentral hepatocytes were exposed to known concentrations of 7-hydroxycoumarin during retrograde perfusions (see Fig. 3). In both anterograde and retrograde perfusions, pericentral regions formed 0.5- to 4-fold more glucuronides than periportal areas depending on the concentration of substrate infused (20–120 μM) (Table 1).

In all perfusions, the concentration of 7-hydroxycoumarin detected in the region last exposed to flow during anterograde (i.e., pericentral areas) and retrograde perfusion (flow via vena cava; periportal areas) was nearly identical with the concentration of 7-hydroxycoumarin in the effluent perfusate (Fig. 5). Furthermore, total glucuronides formed by periportal and pericentral areas plus free 7-hydroxycoumarin leaving the liver equaled the 7-hydroxycoumarin infused irrespective of the direction of flow (Table 1). Thus, the method described above can be used to measure glucuronidation in periportal and pericentral regions of the liver lobule.

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.[5] On the basis of these data, we assume that periportal and pericentral regions constitute about one-half of the liver's mass. Using the flow rate, the mass of each region, and the concentration of glucuronide formed by that region (Table 1), local rates of glucuronidation were calculated (Table 2). 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 2). For example, in Fig. 2, the periportal area was exposed to 80 μM 7-hydroxycoumarin, and 71 μM left the region unmetabolized; therefore, the average substrate concentration in that region was 75.5 μM.

Sublobular rates of glucuronidation in the intact liver are plotted against local 7-hydroxycoumarin concentrations in Fig. 6. Double-reciprocal analysis of data in Fig. 6 showed that glucuronidation by periportal regions was maximal at 9.6 μmoles/g/hr and was half-maximal with 26 μM substrate. In contrast, rates of glucuronidation by pericentral hepatocytes were maximal at 35 μmoles/g/hr and half-maximal with 47 μM substrate. There were no significant differences in half-maximal concentrations in the two regions. The relationship between local rates of glucuronidation in periportal or pericentral regions and

[5] J. J. Lemasters and R. G. Thurman, unpublished data.

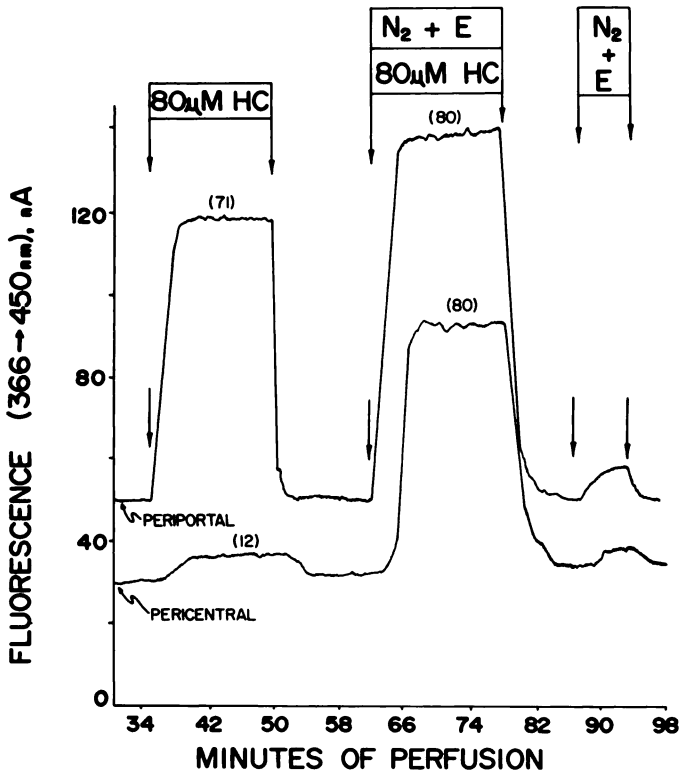

FIG. 2. *Fluorescence increase upon infusion of 7-hydroxycoumarin (HC) and N_2-saturated perfusate containing 20 mM ethanol (N_2 + E) in periportal and pericentral regions of the liver perfused in the anterograde direction.*

Two micro-light guides were placed on periportal and pericentral regions (1 mm apart) on the left lateral lobe of the liver. Periportal and pericentral regions were identified by differences in native pigmentation as well as by differential responses to anoxia (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 micromolar concentrations of free 7-hydroxycoumarin in the tissue calculated as described under Results. Typical experiment.

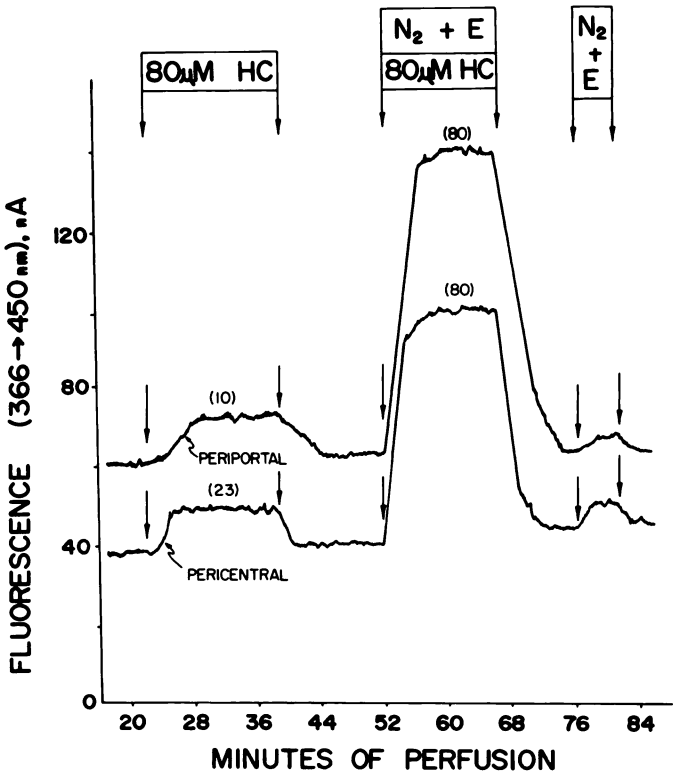

FIG. 3. *Fluorescence increase upon infusion of 7-hydroxycoumarin (HC) and N_2-saturated perfusate containing 20 mM ethanol (N_2 + E) in periportal and pericentral regions of the liver perfused in the retrograde direction.*

Conditions as in Figs. 1 and 2 except that flow was via the vena cava. Typical experiment.

free substrate concentrations was identical in anterograde and retrograde perfusions (Fig. 6).

Glucuronosyltransferase activity in microdissected periportal and pericentral areas. Periportal and pericentral areas were microdissected and assayed for glucuronosyltransferase activity (Table 3). The activity in pericentral regions was about 3.2-fold greater than that in periportal areas. In both regions the glucuronosyltransferase activity was half-maximal with about 230 μM UDP-glucuronic acid and 54 μM 7-hydroxycoumarin (Table 3). The activity of glucuronosyltransferase was about 10-fold higher in homogenates from freeze-dried liver than in homogenates from fresh liver (data not shown). Furthermore, glucuronosyltransferase activity in fresh homogenates was activated by detergent (Triton- X-100, 0.01%) but not in freeze-dried homogenates (data not shown). Thus, we conclude that the freeze-drying procedure, like detergent and freezing (1, 22), activates glucuronosyltransferase.

DISCUSSION

A new method to measure rates of glucuronidation in periportal and pericentral regions of the liver lobule. Mi-

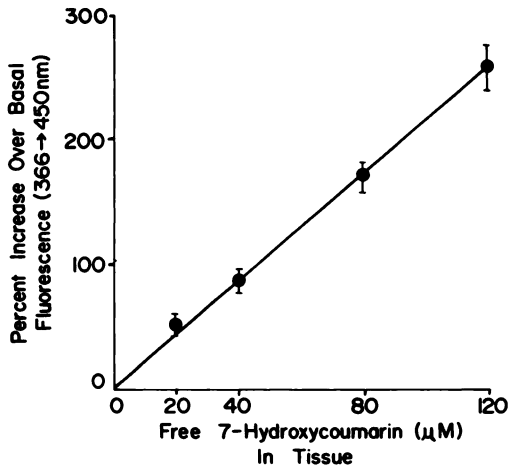

FIG. 4. *Relationship between fluorescence increase and free 7-hydroxycoumarin concentration in the tissue*

The ordinate represents the increase in fluorescence due to free 7-hydroxycoumarin in the presence of N_2-saturated perfusate containing 20 mM ethanol minus the fluorescence due to N_2-saturated perfusate and 20 mM ethanol alone. Data points represent averages of 20–42 measurements ± standard error of the mean at the respective 7-hydroxycoumarin concentrations. A correlation coefficient ($r = 0.80$; $p < 0.001$) was calculated from 108 periportal and pericentral measurements obtained from 54 anterograde and retrograde perfusions typified by Figs. 2 and 3.

TABLE 1

Effect of substrate concentration on free and glucuronidated 7-hydroxycoumarin in periportal and pericentral regions of the liver lobule

Concentrations of glucuronide conjugates formed in periportal and pericentral regions were calculated from experiments typified by Figs. 2 and 3 (see Results). Concentrations of free 7-hydroxycoumarin in effluent perfusate were measured as described under Methods. Values are means ± standard error of the mean. Numbers in parentheses are perfusions.

I. 7-Hydroxycoumarin infused	II. Glucuronide conjugates formed in each region		III. Free 7-hydroxycoumarin present	II + III.
Influent perfusate	Periportal	Pericentral	Effluent perfusate	Sum
μM	*μM*	*μM*	*μM*	*μM*
Anterograde				
20 (6)	8.0 ± 1.5	12.0 ± 1.5	0.8 ± 0.3	20.8 ± 0.3
40 (7)	11.6 ± 4.6	24.1 ± 4.9	5.8 ± 2.0	43.0 ± 0.6
80 (13)	24.8 ± 5.9	36.7 ± 5.9	21.2 ± 2.3	83.6 ± 4.2
120 (7)	16.3 ± 6.0	72.1 ± 9.5	38.0 ± 3.4	130.4 ± 7.7
Retrograde				
20 (4)	3.3 ± 1.5	15.7 ± 1.2	0.4 ± .3	19.4 ± 1.0
40 (5)	9.7 ± 4.6	27.5 ± 3.6	3.5 ± 1.0	36.9 ± 0.8
80 (7)	16.1 ± 6.3	51.7 ± 7.5	12.1 ± 1.5	76.0 ± 2.5
120 (5)	25.5 ± 10.7	67.3 ± 6.1	30.4 ± 6.0	123.2 ± 3.6

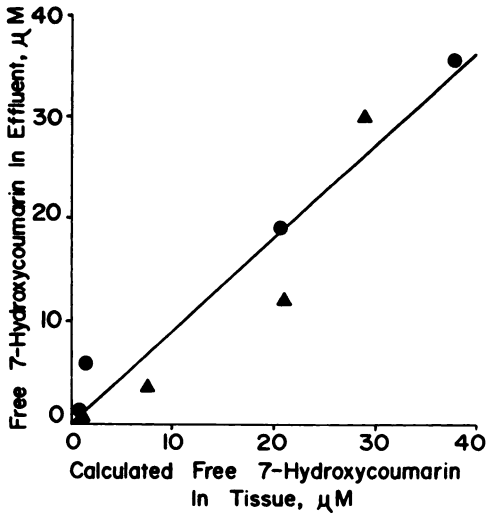

FIG. 5. *Relationship between calculated free 7-hydroxycoumarin in the tissue and free 7-hydroxycoumarin in effluent perfusate.*

Average free 7-hydroxycoumarin concentrations in pericentral areas (●) during normoxic anterograde perfusions (N = 33) and in periportal areas (▲) during retrograde perfusions (n = 21) were calculated from measurements of 7-hydroxycoumarin fluroescence with micro-light guides (Figs. 2 and 3). Free 7-hydroxycoumarin in effluent was assayed as described under Methods. The correlation coefficient (r = 0.71; $p <$ 0.001) was calculated from 54 perfusions.

cro-light guides placed on periportal and pericentral regions of the liver lobule (20) have been used previously to monitor pyridine nucleotide and 7-hydroxycoumarin fluorescence within different zones of the liver lobule. With this methodology we have estimated the lobular oxygen gradient (23), local rates of alcohol dehydrogenase-dependent ethanol metabolism (24), and aldehyde dehydrogenase-dependent acetaldehyde metabolism (25). Furthermore, we were able to demonstrate that rates of monooxygenation of 7-hydroxycoumarin predominated in pericentral regions (14), whereas sulfation of 7-hydroxycoumarin was primarily localized in periportal areas in livers from phenobarbital-treated rats (9).

In the present study, micro-light guides were used to estimate the concentration of nonfluorescent glucuronide conjugates formed in periportal and pericentral regions of the liver lobule. To calculate the sublobular concentration of glucuronide conjugates, fluorescence detected by micro-light guides was calibrated with a known concentration of 7-hydroxycoumarin in the tissue. This was achieved by eliminating all 7-hydroxycoumarin metabolism by anoxia and ethanol infusion. This calibration was then used to calculate the concentration of free 7-hydroxycoumarin present in each region during normoxic perfusions (Figs. 2 and 3). Therefore, the difference in the concentration of 7-hydroxycoumarin entering and leaving each sublobular region was due to the local formation of glucuronide conjugates (Table 1). Thus, with this method we can quantitate both the substrate concentration and the rate of glucuronidation in periportal and pericentral regions of the liver lobule.

Characterization of glucuronidation in periportal and pericentral regions of the liver lobule. There is evidence that conjugating enzymes are distributed unevenly across the liver lobule. For example, sulfation appears to be localized primarily in periportal regions of the liver lobule (9, 26–28). The sublobular localization of glucuronidation is less clear. James *et al.* (29) assayed glucuronosyltransferase activity after damaging periportal and pericentral regions with hepatotoxins and concluded that glucuronosyltransferase activity in normal rats is localized primarily in midzonal and periportal hepatocytes. On the other hand, Pang *et al.* (8) showed that the kinetics of harmol conjugation in perfused livers from normal rats is consistent with more active glucuronidation in pericentral hepatocytes. Our previous approach to this problem involved titrating periportal and pericentral hepatocytes with low (<20 μM) concentrations of 7-hydroxycoumarin in anterograde and retrograde perfusions, respectively (9). Micro-light guides only detected free 7-hydroxycoumarin in the regions first exposed to perfusate (i.e., periportal region in anterograde perfusion), and more than 90% of the infused 7-hydroxycoumarin was recovered in the effluent as the glucuronide (9). Under

TABLE 2

Effect of 7-hydroxycoumarin on rates of glucuronidation 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 micro-light guide measurements (Figs. 2 and 3) (see results). Concentrations of free 7-hydroxycoumarin in periportal regions during retrograde perfusions and in pericentral regions during anterograde perfusions were calculated from the free 7-hydroxycoumarin concentration in the "upstream" regions (i.e., periportal area during anterograde perfusion) and free 7-hydroxycoumarin detected in "downstream" regions. With infusion of 20 μM 7-hydroxycoumarin, free 7-hydroxycoumarin could not be detected in pericentral regions during anterograde perfusions or in periportal regions during retrograde perfusions. The rate of glucuronidation was calculated from the mass of the lobular region (one-half of liver weight), the flow rate of the liver, and the concentration of glucuronide formed locally (Table 1). Values are means ± standard error of the mean. Numbers in parentheses are perfusions.

7-Hydroxycoumarin infused	Average free-7-hydroxycoumarin concentration during normoxic perfusion		Rate of glucuronide formation	
	Periportal	Pericentral	Periportal	Pericentral
μM	*μM*	*μM*	*μmoles/g/hr*	
Anterograde				
20 (6)	16.0 ± 0.8	—	3.6 ± 0.6	5.6 ± 0.9
40 (6)	33.3 ± 2.5	13.3 ± 2.5	5.0 ± 4.3	11.1 ± 2.4
80 (13)	67.6 ± 2.9	36.6 ± 3.8	10.1 ± 2.3	15.2 ± 2.5
120 (7)	112.9 ± 2.8	69.7 ± 5.6	6.4 ± 2.4	28.8 ± 4.6
Retrograde				
20 (4)	—	12.2 ± 0.6	1.1 ± 0.5	6.3 ± 0.5
40 (5)	10.7 ± 2.9	26.2 ± 3.9	3.5 ± 1.6	8.8 ± 1.9
80 (8)	24.0 ± 5.6	55.5 ± 3.7	5.5 ± 2.4	19.8 ± 3.3
120 (5)	46.5 ± 6.7	86.3 ± 3.1	10.5 ± 4.9	26.9 ± 2.9

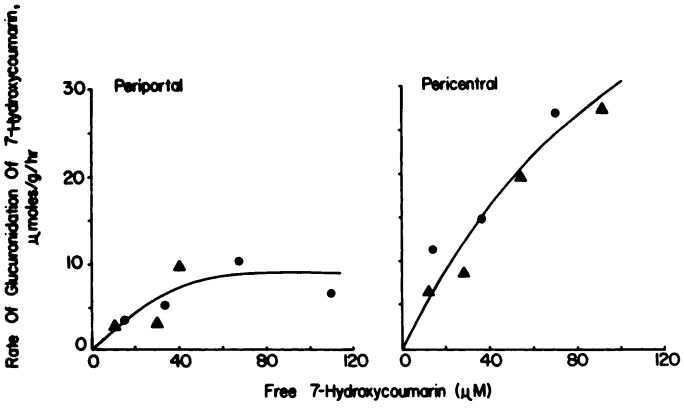

FIG. 6. *Rates of glucuronidation of 7-hydroxycoumarin in periportal and pericentral regions of perfused rat livers*

Average data from Table 2. 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 Figs. 2 and 3, Results). Rates were calculated using the flow rate and the wet weight of each sublobular region (wet weight/2). Concentrations of substrate are the average of the free 7-hydroxycoumarin entering and leaving each sublobular region (Table 2).

TABLE 3

Glucuronosyltransferase activity in microdissected periportal and pericentral regions of perfused livers from phenobarbital-treated, fed rats

For livers 1, 2, and 3, values are averages ± standard error of the mean of six to eight periportal and pericentral regions assayed from each liver with 100 μM 7-hydroxycoumarin and 9 mM UDP-glucuronic acid (see Methods). To determine K_m values, about 50 periportal, and pericentral regions were pooled from one liver. Assays were performed with a range of UDP-glucuronic acid concentrations (50–800 μM) in the presence of 100 μM 7-hydroxycoumarin and a range of 7-hydroxycoumarin (10–110 μM) concentrations in the presence of 9 mM UDP-glucuronic acid.

Liver	Glucuronosyltransferase activity		Pericentral/periportal
	Periportal	Pericentral	
	μmoles/g (wet wt.)/hr		
1	14.9 ± 0.7	50.4 ± 2.4*	3.4
2	29.4 ± 2.4	73.3 ± 2.0*	2.5
3	17.4 ± 0.9	61.8 ± 2.4*	3.6
K_m for 7-hydroxycoumarin	54 μM	55 μM	
K_m for UDP-glucuronic acid	230 μM	200 μM	

*$p < 0.001$, for comparison between periportal and pericentral regions in the same liver.

these conditions, hepatocytes from phenobarbital-treated rats had about equal rates of glucuronidation at low substrate concentration (<20 μM) in periportal and pericentral areas. These rates are probably limited by substrate supply, since 7-hydroxycoumarin concentrations are below the K_m of glucuronosyltransferase for substrate (Table 3). This conclusion is supported by the observation that pericentral hepatocytes have much greater rates of glucuronidation than periportal areas at high substrate concentrations (Fig. 6). Maximal rates of glucuronidation calculated with the new method described here were 9.6 and 35 μmoles/g/hr in periportal and pericentral regions of the liver lobule, respectively. Glucuronidation was half-maximal in both regions with 25–50 μM substrate.

The high rates of glucuronidation by hepatocytes in pericentral regions correlate well with activities of glucuronosyltransferase in the two zones measured *in vitro* (Table 3). On the surface, one might conclude that enzyme activity is the major determinant of rates of glucuronidation in different regions of the liver lobule. How-

ever, this interpretation should be made cautiously, since the enzyme activity measured in homogenates of freeze-dried tissue (see Results) is "activated" and measured in the presence of unphysiologically high concentrations of UDP-glucuronic acid (9 mM). In the intact hepatocyte, glucuronosyltransferase is most likely in the less active membrane-bound form and may be modulated by a number of effectors (1, 10, 30). Moreover, intracellular UDP-glucuronic acid concentrations (300 μM) are near the K_m of glucuronosyltransferase for cofactor (Table 3) (2, 3, 10–12). Furthermore, rates of glucuronidation have been shown to correlate with intracellular UDP-glucuronic acid concentrations in isolated hepatocytes and perfused liver (2, 3, 11). For these reasons, activities of glucuronosyltransferase measured *in vitro* cannot be related directly to rates of glucuronidation in the perfused liver. Thus, although these data demonstrate clearly that rates of glucuronidation occur at higher rates in pericentral than in periportal regions of the liver lobule when adequate substrate is supplied, we still do not know whether this is due predominantly to differences in enzyme activity or cofactor supply.

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