0026-895X/86/060599-07$02.00/0

MOLECULAR PHARMACOLOGY, 29:599-605

Hydrolysis of Organic Sulfates in Periportal and Pericentral Regions of the Liver Lobule: Studies with 4-Methylumbelliferyl Sulfate in the Perfused Rat Liver

IRENE M. ANUNDI, FREDERICK C. KAUFFMAN, MOHAMED EL-MOUELHI, and RONALD G. THURMAN

Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514 (I.M.A., R.G.T.) and Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, Maryland 21201 (F.C.K., M.E.-M.)

Received September 30, 1985; Accepted February 26, 1986

SUMMARY

The hydrolysis of 4-methylumbelliferyl sulfate by liver sulfatases to free fluorescent 4-methylumbelliferone and the subsequent formation of the glucuronide conjugate were studied in the isolated perfused rat liver. In livers from fed, phenobarbital-treated rats, 4-methylumbelliferyl sulfate (0.25–1.5 mM) was hydrolyzed rapidly to free 4-methylumbelliferone at maximal rates of about 5 μmol/g/hr. A major fraction of the free 4-methylumbelliferone formed was converted to the glucuronide at maximal rates around 20 μmol/g/hr. Similar rates of hydrolysis were observed in livers from fasted, phenobarbital-treated or normal rats, although the ratio of glucuronide to free product was decreased markedly by fasting. In liver homogenates, however, rates of organic sulfate hydrolysis exceeded those observed in the perfused liver by at least 2-fold, suggesting that 4-methylumbelliferyl sulfate content is an important determinant of rates of hydrolysis in the perfused liver. There was a good correlation ($r = 0.91$) between rates of product formation and fluorescence of 4-methylumbelliferone detected from the liver surface with fiber optic light guides. Fluorescence of 4-methylumbelliferone produced from hydrolysis of 4-methylumbelliferyl sulfate was also monitored with micro-light guides placed on periportal and pericentral areas of the liver lobule for the estimation of local rates of product formation. When perfusions were in the anterograde direction, desulfation of 4-methylumbelliferyl sulfate was about 50% higher in pericentral (28.8 ± 9.3 μmol/g/hr) than in periportal (18.2 ± 2.7 μmol/g/hr) areas. Furthermore, 4-methyl umbelliferyl sulfate content determined in microdissected samples was 1.5- to 2-fold higher in pericentral than in periportal regions of the liver lobule but the activity of 4-methylumbelliferyl sulfate sulfatase was identical in both zones of the liver lobule. We conclude, therefore, that the local substrate content is an important determinant of rates of 4-methylumbelliferyl sulfate hydrolysis in sublobular zones of the liver.

Previous studies have established that hepatocytes in periportal and pericentral regions of the liver lobule are metabolically different (1–3). Histochemical, microchemical, and noninvasive techniques employing micro-light guides and miniature oxygen electrodes have been used to study this metabolic heterogeneity (4–7). For example, miniature oxygen electrodes have been used to demonstrate that rates of gluconeogenesis are higher in periportal than in pericentral areas of the liver lobule, whereas glycolysis predominates in pericentral regions (8, 9). Since rates can be shifted to the opposite region of the liver lobule by changing the direction of the flow, both glycolysis and gluconeogenesis seem to be regulated by events other than the distribution of enzyme activities (9, 10). Mechanisms responsible for the rapid reversal of metabolic compartmentation are not clear but may be linked to the oxygen gradient across the liver lobule (11).

This work was supported in part by Grant ES 02759 from the National Institute of Environmental Health Sciences and Grants CA 23080 and CA 20807 from the National Cancer Institute.

Several xenobiotic metabolizing enzymes have also been shown to be distributed unevenly across the liver lobule. Glucuronosyltransferases are localized preferentially in pericentral regions where rates of glucuronide formation are higher (12–14). Sulfation, however, is more active in periportal regions (14, 15), and sulfation and glucuronidation are generally thought to be competing reactions. At high substrate concentrations glucuronidation predominates, whereas at low substrate concentrations the sulfate is the major conjugate formed (16, 17). The supply of UDP-glucuronic acid for glucuronidation and adenosine 3′-phosphate 5′-sulphatophosphate for sulfation are also important determinants of rates of glucuronidation and sulfation in intact cells (18, 19). Unlike carbohydrate metabolism, however, maximal rates of glucuronidation are observed in pericentral areas also during perfusions in the retrograde direction (i.e., enzyme activity probably is a major rate determinant of this process).

It is known that multiple forms of sulfatases exist which are localized in different fractions of the cells (20). However, rela-

ABBREVIATION: HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

tively little information is available regarding the sublobular distribution of sulfatases, their regulation, and physiological significance. Numerous exogenous and endogenous sulfate conjugates such as steroid sulfates and glycosaminoglycans (21) are hydrolyzed by these enzymes. Furthermore, lack of sulfatase activity has also been implicated in metabolically inherited diseases such as metachromatic leucodystrophy (20). It is possible that desulfation of sulfate conjugates may also regulate the net production of sulfate conjugates by futile cycling of sulfate ester formation followed by sulfate ester hydrolysis. Since information on the distribution of sulfatases in sublobular regions of the liver lobule is lacking and little is known about the metabolism of sulfate conjugates, we have investigated hepatic desulfation using 4-methylumbelliferyl sulfate as substrate. The data indicate that the liver takes up this conjugate and metabolizes it at very high rates predominantly in pericentral regions of the liver lobule when perfusion is in the anterograde direction. A preliminary account of this work has appeared elsewhere (22).

Materials and Methods

Animals. Female Sprague-Dawley rats weighing 200–250 g were given phenobarbital (1 g/liter) in their drinking water 10–20 days prior to perfusion experiments (23). Phenobarbital treatment enhanced the color contrast between periportal and pericentral regions of the liver lobule. All rats had free access to laboratory chow unless otherwise stated.

Liver perfusion. Details of the non-recirculating perfusion technique have been described elsewhere (24). 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 was prepared by replacing magnesium sulfate with magnesium chloride on an equimolar basis. Perfusions were performed with sulfate-free perfusate except in experiments depicted in Figs. 3 and 4A. 4-Methylumbelliferyl sulfate (Sigma, St. Louis, MO) was dissolved directly in the perfusate at concentrations indicated in the figure and table legends. For perfusions in the anterograde direction, perfusate was pumped into the portal vein at rates ranging from 3 to 4.5 ml/min/g of liver, and the effluent perfusate was collected via a cannula placed in the inferior vena cava. During perfusions in the retrograde direction the flow was reversed. In some experiments the bile duct was cannulated with PE-10 tubing and bile was collected. The oxygen tension in the effluent perfusate was monitored continuously with a Teflon-shielded, Clark-type platinum electrode and the rate of oxygen uptake was used to assess tissue viability. Free 4-methylumbelliferone 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 or with an Eppendorf fluorometer. Sample fluorescence was compared with authentic 4-methylumbelliferone standards incubated under identical conditions. Glucuronide conjugates were hydrolyzed enzymatically as described elsewhere (16). The difference between total products formed (free 4-methylumbelliferone plus the glucuronide) and free 4-methylumbelliferone was used to calculate the concentration of glucuronide conjugates in perfusate and bile. Rates of production were calculated from the influent-effluent concentration difference of metabolite, the flow rate, and the liver wet weight.

Micro-light guides. 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 were as described previously (5, 25). Light and dark spots visible on the surface of the hemoglobin-free perfused liver correspond to periportal and pericentral regions, respectively (26). Fluorescence changes were 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 was 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 4-methylumbelliferone was measured at 450 nm (25).

Sulfatase activity in periportal and pericentral regions. The perfused liver was frozen by immersion in liquid nitrogen and tissue adjacent to the surface was sectioned at 20-μm intervals in a cryostat at −20°. The sections were then freeze-dried at −40° to preserve histological structure (27). Periportal and pericentral areas of lyophilized tissue sections were dissected under a microscope and weighed on a quartz-fiber balance (27). Sulfatase activity in the lyophilized samples was assayed by incubating samples weighing 0.2–1 μg with 200 μM 4-methylumbelliferyl sulfate in a reagent consisting of: 75 mM HEPES buffer (pH 7.3), 5 mM $MgCl_2$, and 0.02% bovine serum albumin in a final volume of 10 μl. After incubation for 30 min at 37°, the reaction was stopped by chilling the samples to 4° and transferring them to 1 ml of 0.02 M carbonate buffer, pH 10. The liberated 4-methylumbelliferone was measured fluorometrically (366 → 450 nm). Under these conditions, the reaction was linear for at least 2 hr and was proportional to tissue added from 0.1 to 4 μg of dry weight. Activity of sulfatase was diminished by about 50% by lyophilization of liver sections.

Assay of 4-methylumbelliferyl sulfate in liver homogenates and in periportal and pericentral areas of the liver lobule. Livers from fasted, phenobarbital-treated rats were perfused with 0.5 mM 4-methylumbelliferyl sulfate for 14 min followed by a 3-min washout period with perfusate alone. The liver was then immediately minced and homogenized in Krebs-Henseleit buffer. Aliquots (0.5 ml) of a 20% homogenate were diluted with 0.75 ml of Tris-HCl buffer (0.5 M, pH 7.4) and deproteinized by adding 0.25 ml of perchloric acid (0.6 M). The mixture was then centrifuged for 5 min in an Eppendorf microcentrifuge. For the assay of total 4-methylumbelliferone products, 0.2 ml of the resulting supernatant was added to 0.8 ml of Tris-HCl buffer (0.5 M, pH 7.4) and, when necessary, the pH was adjusted to 7.4. The reaction was initiated by adding 0.1 ml of an enzyme solution containing 250 units of β-glucuronidase (type H-2, *Helix pomatia*) which also contained sulfatase activity (400–1000 units/ml). Samples were incubated in triplicate for 3 hr at room temperature. Subsequently, the fluorescence of the liberated 4-methylumbelliferone was measured at 366 → 450 nm in an Eppendorf fluorometer. For the assay of 4-methylumbelliferyl glucuronide, 0.2 ml of the supernatant was added to 0.8 ml of Tris-HCl buffer (0.5 M, pH 7.4) and 0.1 ml of a solution of pure β-glucuronidase (type VIII from *Escherichia coli*) was added to initiate the reaction. For the assay of free 4-methylumbelliferone in the supernatant, samples were incubated without added enzymes. The amount of 4-methylumbelliferyl sulfate was calculated based on appropriate standards incubated under identical conditions.

In a second series of experiments using the same experimental protocol as above, livers were frozen after the 3-min washout period by pressing on an aluminium mallet chilled in liquid nitrogen on the left lateral lobe. The frozen liver was sectioned at 20 μm and samples were microdissected from periportal and pericentral regions of lyophilized tissue as described previously (27). Assays were performed with pooled samples (3–5 μg) of 5 periportal or pericentral regions. Samples were incubated in 100 μl of a reagent containing 0.02 M Tris-HCl in the presence or absence of β-glucuronidase/aryl sulfatase and 10 mM saccharolactone for 1 hr at 37°. The reaction was stopped by the addition of 1 ml of 0.02 M Na_2CO_3 buffer, pH 10.3, and the fluorescence of liberated 4-methylumbelliferone was determined in a Farrand fluorometer (366 → 450 nm).

Results

Metabolism of 4-methylumbelliferyl sulfate in the perfused liver. Livers were perfused with different concentrations of 4-methylumbelliferyl sulfate in sulfate-free perfusate and products (free and glucuronides) were measured in the effluent perfusate and bile. Inorganic sulfate was omitted to minimize resulfation of 4-methylumbelliferone formed from 4-methylumbelliferyl sulfate. Under these conditions, it is likely that

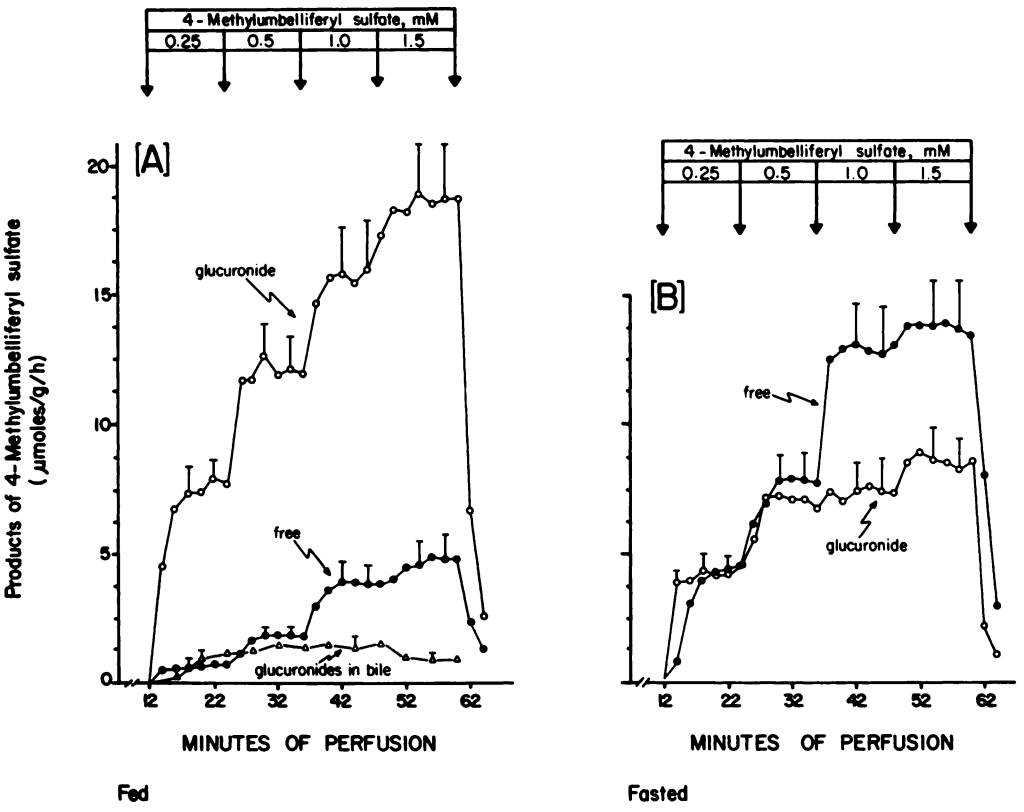

Fig. 1. A. Rates of product formation from 4-methylumbelliferyl sulfate in perfused livers from fed, phenobarbital-treated rats. 4-Methylumbelliferyl sulfate was added directly to the perfusate at concentrations and time points indicated by the *horizontal bars* and *arrows*. Effluent perfusate and bile were collected and assayed for metabolites as described in Materials and Methods. ●, free 4-methylumbelliferone; ○, the glucuronide conjugate; △, glucuronides excreted into the bile. Values are means ± SE of data from five to eight livers. B. Same conditions as in A except that livers were from fasted, phenobarbital-treated rats.

little if any resulfation occurred since sulfate conjugation with this product was minimal *in vivo* (28). Indeed, rates of desulfation in this study were not altered by omission of sulfate from the perfusion medium (data not shown). Infusion of 4-methylumbelliferyl sulfate from 0.25 to 1.5 mM produced step-wise increases in the release of 4-methylumbelliferone and 4-methylumbelliferyl glucuronide into the effluent perfusate (Fig. 1A). In livers from fed, phenobarbital-treated rats, glucuronides were released into the effluent perfusate at rates around 7 μmol/g/hr during infusion of 0.25 mM 4-methylumbelliferyl sulfate. Values increased in steps to about 18 μmol/g/hr when the substrate concentration was increased to 1.5 mM (Fig. 1A). When the concentration of 4-methylumbelliferyl sulfate was increased above 1.5 mM, rates of product formation were not altered significantly. Glucuronides were also excreted into the bile at maximal rates around 2 μmol/g/hr; however, free 4-methylumbelliferone or 4-methylumbelliferyl sulfate could not be detected in the bile. In livers from fed rats, free 4-methylumbelliferone represented about 20% of the total products in the effluent perfusate. The amount of 4-methylumbelliferone varied from 0.8 to 5 μmol/g/hr when substrate was infused in concentrations ranging from 0.5 to 1.5 mM (Fig. 1A). When 4-methylumbelliferyl sulfate infusion was terminated, all values returned rapidly to zero.

Rates of production of total metabolites (i.e., glucuronides in effluent perfusate and bile and free 4-methylumbelliferone in effluent perfusate) from 4-methylumbelliferyl sulfate were comparable in livers from fed and fasted, phenobarbital-treated rats (Table 1). However, more free metabolite and less glucuronide was detected in the fasted state as expected due to diminished UDP-glucuronic acid levels in glycogen-depleted livers (16). During the step-wise infusion of 4-methylumbelliferyl sulfate, rate of glucuronide production increased from 4 to 8 μmol/g/hr (Fig. 1B), whereas free 4-methylumbelliferone increased from 4 to 14 μmol/g/hr (Fig. 1B). Rates of total product formation from 4-methylumbelliferyl sulfate were similar in livers from normal and phenobarbital-treated rats (Table 1), and maximal rates of product formation were comparable whether perfusions were in the anterograde or retrograde direc-

TABLE 1

Oxygen uptake and formation of products by the isolated perfused rat liver following the addition of 4-methylumbelliferyl sulfate

Values are means ± SE. Number of experiments are for: fasted normal, 3–6; fed, phenobarbital treated, 4–11; fasted, phenobarbital treated, 9–14; and for retrograde perfusion, 9. Product = 4-methylumbelliferone and 4-methylumbelliferyl glucuronide.

Treatment	4-Methylumbelliferyl sulfate	Oxygen uptake	Formation of products
	mM	*μmol/g/hr*	
Fasted, normal	0	132 ± 10	
	0.25	138 ± 8	7.8 ± 1.3
	0.5	152 ± 11	13.7 ± 2.4
	1.0	148 ± 12	16.7 ± 2.9
	1.5	137 ± 13	21.2 ± 6.8
Fed, phenobarbital treated	0	118 ± 11	
	0.25	144 ± 13	8.8 ± 0.6
	0.5	154 ± 17	14.9 ± 1.3
	1.0	154 ± 15	20.7 ± 2.5
	1.5	146 ± 10	24.4 ± 2.9
Fasted, phenobarbital treated	0	98 ± 8	
	0.25	104 ± 8	8.7 ± 0.9
	0.5	114 ± 8	14.7 ± 2.1
	1.0	117 ± 7	18.9 ± 1.6
	1.5	115 ± 7	21.7 ± 2.7
Fasted, phenobarbital treated, retrograde perfusion	1.5	ND*	20.8 ± 2.0

* ND, not determined.

tion (Table 1). The direction of flow did not influence the free product/glucuronide ratio.

4-Methylumbelliferyl sulfate increased oxygen uptake in livers from all three groups of rats studied (Table 1). In livers from fed, phenobarbital-treated rats, the infusion of 0.25 and 0.5 mM substrate increased rates of oxygen uptake by more than 36 μmol/g/hr (Fig. 2). Further increases in substrate concentration up to 1.5 mM had little additional effect on oxygen uptake. Maximal increases in oxygen uptake were around 20 μmol/g/hr in livers from fasted rats and about 36 μmol/g/hr in livers from fed rats (Table 1). When infusion of 4-methylumbelliferyl sulfate was terminated, rates of oxygen uptake returned to levels slightly below initial basal values.

Lobular distribution of organic sulfate hydrolysis and sulfatase activity. 4-Methylumbelliferyl sulfate is nonfluorescent but is cleaved by liver sulfatases to the fluorescent free 4-methylumbelliferone which can be detected fluorometrically from the liver surface with fiber optic light guides. Fig. 3 shows a typical experiment in which a large-tipped fiber optic light guide was used to monitor fluorescence of 4-methylumbelliferone. When 4-methylumbelliferyl sulfate was infused in a step-wise manner, fluorescence of 4-methylumbelliferone detected from the liver surface increased correspondingly. Steady state fluorescence values were reached within 8 min after each infusion step (Fig. 3). Furthermore, a good correlation was observed between changes in fluorescence and rates of product formation both in the fed and fasted state. Similar results were obtained using a large-tipped fiber optic light guide ($r = 0.91$) or micro-light guides ($r = 0.89$; Fig. 4, A and B). In the fed state, no fluorescence was detected from the liver surface (see intersect on *abscissa* Fig. 4A) when product formation was below 4 μmol/g/hr, probably due to more efficient glucuronidation in the fed state. In contrast, when experiments were performed with livers from fasted rats, the line representing

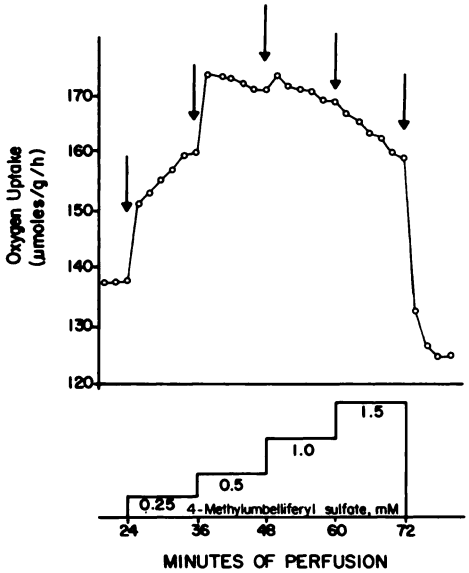

Fig. 2. Typical experiment showing the effect of 4-methylumbelliferyl sulfate on oxygen uptake in the perfused liver from a fed, phenobarbital-treated rat. 4-Methylumbelliferyl sulfate was infused in concentration steps depicted by the *horizontal bars*. Oxygen tension was monitored in the effluent perfusate polarographically and oxygen uptake was calculated from the arterious-venous concentration difference, the liver wet weight, and the flow rate.

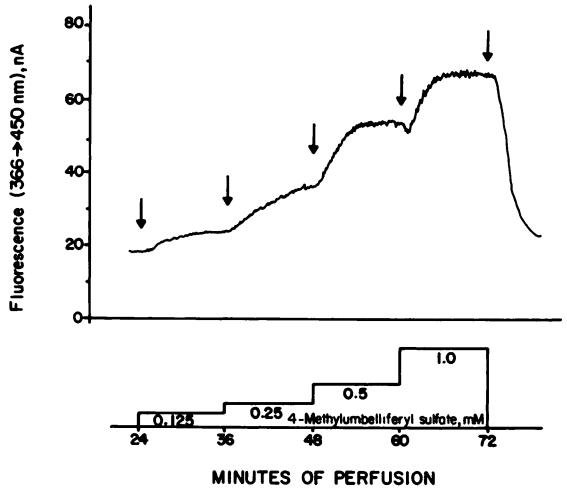

Fig. 3. Typical experiment of fluorescence of 4-methylumbelliferone from the liver surface following the addition of 4-methylumbelliferyl sulfate. One large-tipped fiber optic light guide (tip diameter 2 mm) was placed on the left lateral lobe of a liver from a fed, normal rat. 4-Methylumbelliferyl sulfate was infused at concentrations and times indicated by the *vertical arrows* and *horizontal bars*.

the relationship between fluorescence and product formation intersected the origin (Fig. 4B).

Micro-light guides placed on adjacent periportal and pericentral regions of the liver lobule were used to record fluorescence of free 4-methylumbelliferone in the two sublobular zones. In livers from fasted, phenobarbital-treated rats, fluorescence increased in both regions of the liver lobule in a step-wise manner when 4-methylumbelliferyl sulfate was infused in increasing concentration steps (Fig. 5). Fluorescence, expressed as percentage of basal fluorescence, was about 50% higher in pericentral than in periportal regions.

Rates of product formation in sublobular zones were calculated from changes in fluorescence and the proportionality between rates of product formation and fluorescence. This calculation is based on the assumption that periportal and pericentral regions each constitute 50% of the liver mass (13). Rates of product formation in pericentral areas were about 50% higher than in periportal regions in livers from both fed and fasted rats (Fig. 6). With 1.5 mM substrate, rates of organic sulfate hydrolysis in the fed state were 20.3 and 31.7 μmol/g/hr ($p < 0.05$) in periportal and pericentral regions of the liver lobule, respectively (Fig. 6). Half-maximal rates were detected with 0.7 mM and 1.2 mM substrate in pericentral and periportal regions, respectively, in livers from fasted rats. Maximal rates of 41 and 29 μmol/g/hr were calculated from reciprocal transformations of the data for pericentral and periportal regions, respectively, in livers from fasted rats. When livers were perfused with buffer saturated with 95% N_2 and 5% CO_2, rates of product formation with 1.0 mM 4-methylumbelliferyl sulfate were 17.4 ± 2.4 μmol/g/hr in periportal areas and 17.1 ± 3.4 μmol/g/hr in pericentral regions ($n = 6$).

4-Methylumbelliferyl sulfate content and sulfatase activity in microdissected samples of periportal and pericentral regions. Average sulfatase activity measured in six livers was 19.9 ± 2.1 μmol/g of dry weight/hr in periportal regions and 20.4 ± 2.3 μmol/g of dry weight/hr in pericentral zones (Table 2). Thus, sulfatase activity was essentially the same in both regions of the liver lobule *in vitro*. These values were lower than those calculated for the perfused liver due, in

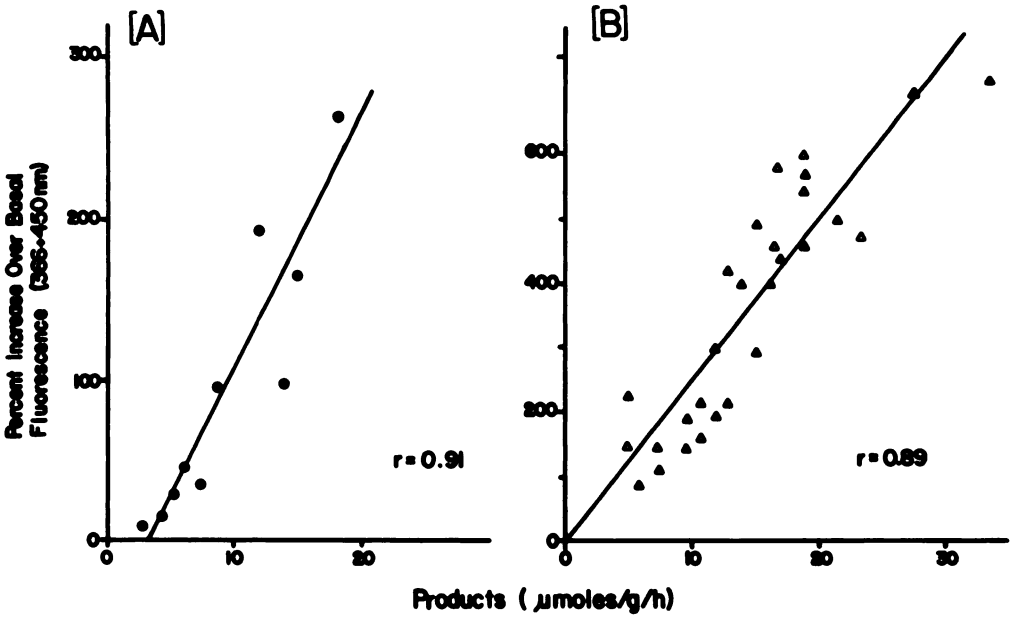

Fig. 4. A. Relationship between fluorescence increases detected from tissue and product formation from 4-methylumbelliferyl sulfate by the liver. The *ordinate* represents the increase in fluorescence expressed as percentage of basal values. Data are from fed, normal rats, $n = 10$. A large-tipped fiber optic light guide was used. B. Similar to A except that livers were from fasted, phenobarbital-treated rats and fluorescence was collected with micro-light guides. △, periportal areas; ▲, pericentral regions. $n = 14$.

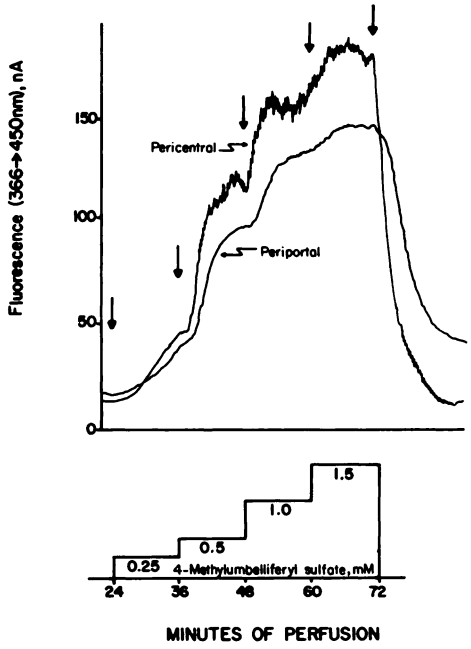

Fig. 5. Typical experiment showing fluorescence of 4-methylumbelliferone in periportal and pericentral regions of the liver lobule from a fasted, phenobarbital-treated rat following the addition of 4-methylumbelliferyl sulfate. Two micro-light guides were placed on two adjacent periportal and pericentral areas and 4-methylumbelliferone fluorescence was detected as described in Materials and Methods.

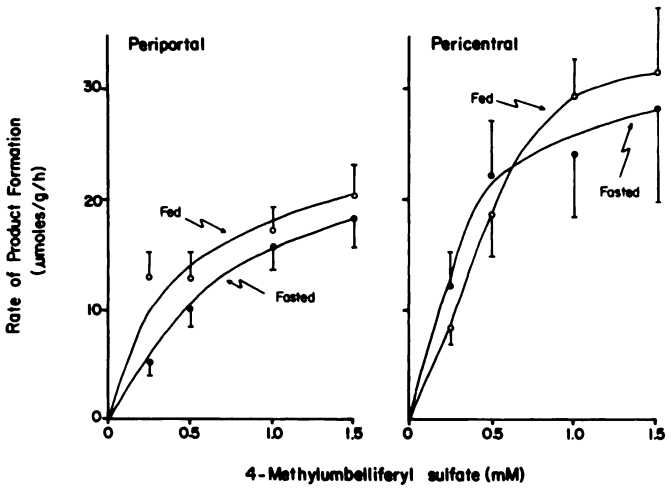

Fig. 6. Rates of organic sulfate hydrolysis in periportal and pericentral areas of the liver lobule. Data represent rates of product formation (glucuronide and free 4-methylumbelliferone) from 4-methylumbelliferyl sulfate detected with micro-light guides. ○, fed, phenobarbital-treated rats; ●, fasted, phenobarbital-treated rats. Data are means ± SE of data from five to eight livers per group.

part, to a loss of activity which occurred during lyophilization of frozen sections and also due to the low substrate concentration (200 μM) required for the assay to minimize blank values. Measurement of 4-methylumbelliferyl sulfate sulfatase in fresh liver homogenates with saturating amounts of sulfate substrate at pH 7.4 yielded values above 50 μmol/g of wet weight/hr. 4-Methylumbelliferyl sulfate content was also assayed in liver homogenates and lyophilized samples of periportal and pericentral regions. Livers from fasted, phenobarbital-treated rats were perfused with 0.5 mM 4-methylumbelliferyl sulfate for 14 min followed for 3 min with perfusate alone to minimize substrate

TABLE 2

4-Methylumbelliferyl sulfate sulfatase activity in periportal and pericentral regions of livers from phenobarbital-treated rats

Values are averages ± SE of five to eight samples microdissected from periportal and pericentral regions from 20-μm lyophilized sections prepared from livers of fed, phenobarbital-treated rats. Assays were performed as described in Materials and Methods.

Rat	Sulfatase	
	Periportal	Pericentral
	μmol/g/hr	
1	24.6 ± 1.4	25.3 ± 1.8
2	23.8 ± 2.5	24.8 ± 1.7
3	18.3 ± 1.3	20.4 ± 1.6
4	18.9 ± 1.8	14.7 ± 0.9
5	22.8 ± 1.9	25.1 ± 2.8
6	11.0 ± 0.9	12.2 ± 0.3
Average	19.9 ± 2.1	20.4 ± 2.3

content in the vascular space. Homogenates from these livers contained 87 ± 18 nmol of 4-methylumbelliferyl sulfate/g of wet weight (n = 4). In microdissected samples the content of 4-methylumbelliferyl sulfate was 0.152 pmol/g of dry weight in periportal regions and 0.297 in pericentral areas. In another liver, comparable values were 0.295 and 0.454 pmol/g of dry weight for periportal and pericentral regions, respectively.

Discussion

Hydrolysis of 4-methylumbelliferyl sulfate in the perfused liver. The isolated perfused liver (Fig. 1, Table 1) and isolated hepatocytes (29) can take up and metabolize sulfate conjugates at very high rates. Compared to maximal rates of other xenobiotic metabolizing systems (30), this activity is very high. In perfused liver, 4-methylumbelliferyl sulfate hydrolysis was half-maximal with 0.7 mM substrate, and calculated maximal rates were around 36 μmol/g/hr. These values are lower than kinetic constants calculated for sulfatase assayed at pH 8.0 in liver homogenates (K_m = 1.4 mM and V_{max} = 151 μmol/g/hr) with p-nitrophenyl sulfate as substrate (31) and are also lower than values reported for 4-methylumbelliferyl sulfate sulfatase measured in ox liver (20). Maximal sulfatase activity with 4-methylumbelliferyl sulfate as substrate in rat liver homogenate was about 50 μmol/g/hr when assayed at pH 7.4. Under these conditions, the K_m was about 0.6 mM. Since maximal rates were greater in broken cells than in the perfused liver it is probable that substrate delivery is an important determinant of the rate of desulfation in intact cells. Indeed, 4-methylumbelliferyl sulfate content in liver homogenates was estimated to be far below the K_m of the sulfatase (Results).

Sulfation has been reported to be a high affinity, low capacity system and data presented above indicate that desulfation is a low affinity process with a high capacity. Marked differences in substrate affinity for these systems [K_m for phenolsulfotransferase: 8–11 μM (14, 21)] and for sulfatase [0.7 mM (this study) to 1.4 mM (31)] as well as compartmentation of enzymes explain why water-soluble sulfate esters are formed and released from cells. These considerations suggest that further studies are needed to evaluate the significance of possible futile cycling via sulfation and desulfation.

The existence of highly active sulfatases may be important in the regulation of metabolism of endogenous substrates such as steroids as well as xenobiotics. Steroid sulfates are generally metabolically inactive and desulfation yields active metabolites. During conditions of decreased sulfate availability due to fasting or a low protein diet (32, 33), sulfate may be liberated from intracellular molecules such as sulpholipids by the action of sulfatases (34).

Fasting has been shown to increase sulfatase activity in liver homogenates (31). In contrast, fasting for 24 hr had no effect on overall rates of desulfation in the perfused liver (Fig. 1, Table 1). Therefore, sulfate hydrolysis in intact cells may be limited by uptake of organic sulfates, and manipulations such as fasting would not be expected to influence maximal rates of hydrolysis.

Desulfation in periportal and pericentral regions of the liver lobule. Desulfation of 4-methylumbelliferyl sulfate was about 50% higher in pericentral than in periportal areas of the liver lobule (Fig. 6). Desulfation was half-maximal at 0.7 and 1.2 mM substrate in periportal and pericentral regions, respectively. Maximal calculated rates were 41 and 29 μmol/g/hr for pericentral and periportal areas, respectively, in livers from fasted rats. Several explanations for the lobular distribution of sulfate hydrolysis were considered. The first possibility, that enzyme activities might be distributed unevenly across the liver lobule, was not supported with data from microdissected samples, which demonstrated nearly identical activities in periportal and pericentral regions (Table 2). Second, it is possible that the fluorescence signal in downstream, pericentral regions is increased due to release of 4-methylumbelliferone from upstream, periportal regions; however, the sublobular differences in hydrolysis of 4-methylumbelliferyl sulfate were abolished by perfusion with nitrogen-saturated buffer. One could not expect a gradient of free 4-methylumbelliferone to be altered by N_2. Furthermore, free 4-methylumbelliferone concentrations were about 10 times higher in the cell than in the vascular space. Since the vascular space occupies only about 30% of the total volume of the perfused liver (35), less than 5% of the fluorescence detected by micro-light guides in pericentral areas could be free 4-methylumbelliferone released from periportal regions. The third and most likely explanation for the different rates of hydrolysis of 4-methylumbelliferyl sulfate in periportal and pericentral regions of the liver lobule is differences in substrate content. Since 4-methylumbelliferyl sulfate levels were 1.5- to 2-fold higher in pericentral than periportal regions, it is concluded that local organic sulfate uptake is responsible for the local rates of hydrolysis observed. Reasons for the higher 4-methylumbelliferyl sufate content in pericentral regions are not clear; however, they may be due to the organic anion uptake system. About 50% of the uptake of inorganic sulfate is known to be carrier mediated. Part is sodium-dependent and involves the Na^+/K^+-ATPase (36). It has also been suggested that sulfate conjugates may be exported from hepatocytes by a carrier-mediated process (37). It is possible that sulfate conjugates such as 4-methylumbelliferyl sulfate utilize the same transport systems. Since desulfation was diminished about 30% when livers were perfused with nitrogen-saturated buffer, which presumably decreases energy-dependent substrate uptake (data above), we hypothesize that desulfation of specific sulfate conjugates by intact hepatocytes is limited by energy-dependent uptake of substrate.

References

1. Jungermann, K., and N. Katz. Metabolic heterogeneity of liver parenchyma, in *Metabolic Compartmentation* (H. Sies, ed.). Academic Press, London, 411–435 (1982).
2. Rappaport, A. M. The microcirculatory acinar concept of normal and pathological hepatic structure. *Beitr. Pathol.* **157**:215–243 (1976).
3. Jungermann, K., and N. Katz. Functional hepatocellular heterogeneity. *Hepatology* **2**:385–395 (1982).
4. Lowry, O. H., and J. V. Passonneau. *A Flexible System of Enzymatic Analysis.* Academic Press, New York (1972).
5. Ji, S., J. J. Lemasters, and R. G. Thurman. A fluorometric method to measure sublobular rates of mixed-function oxidation in the hemoglobin-free perfused rat liver. *Mol. Pharmacol.* **19**:513–516 (1981).
6. Thurman, R. G., F. C. Kauffman, S. Ji, J. J. Lemasters, J. G. Conway, S. A. Belinsky, T. Kashiwagi, and T. Matsumura. Metabolic heterogeneity in the perfused rat liver. *Pharmacol. Biochem. Behav.* **18**:415–419 (1983).
7. Pette, D., and M. Wimmer. Kinetic microphotometric activity determination in enzyme containing gels and model studies with tissue sections. *Histochemistry* **64**:11–22 (1979).
8. Guder, W. G., and U. Schmidt. Liver cell heterogeneity. The distribution of pyruvate kinase and phosphoenolpyruvate carboxykinase (GTP) in the liver lobule of fed and starved rats. *Z. Physiol. Chem.* **357**:1793–1800 (1976).
9. Matsumura, T., and R. G. Thurman. Predominance of glycolysis in pericentral regions of the liver lobule. *Eur. J. Biochem.* **140**:229–234 (1984).
10. Matsumura, T., T. Kashiwagi, H. Meren, and R. G. Thurman. Gluconeogenesis predominates in periportal regions of the liver lobule. *Eur. J. Biochem.* **144**:409–415 (1984).
11. Ji, S., J. J. Lemasters, and R. G. Thurman. Ethanol-induced changes in the

intralobular oxygen gradient of perfused rat liver. *Pharmacol. Biochem. Behav.* **13**:41–45 (1980).
12. Pang, K. S., H. Koster, I. C. M. Halsema, E. Scholtens, G. J Mulder, and R. N. Stillwell. Normal and retrograde perfusion to probe the zonal distribution of sulfation and glucuronidation activities of harmol in the perfused rat liver preparation. *J. Pharmacol. Exp. Ther.* **224**:647–653 (1983).
13. Conway, J. G., F. C. Kauffman, T. Tsukada, and R. G. Thurman. Glucuronidation of 7-Hydroxycoumarin in periportal and pericentral regions of the liver lobule. *Mol. Pharmacol.* **25**:487–493 (1984).
14. Conway, J. G., F. C. Kauffman, S. Ji, and R. G. Thurman. Rates of sulfation and glucuronidation in periportal and pericentral regions of the liver lobule. *Mol. Pharmacol.* **22**:509–516 (1982).
15. Dawson, J. R., J. G. Weitering, G. J. Mulder, R. N. Stillwell, and K. S. Pang. Alteration of transit time and direction of flow to probe the heterogeneous distribution of conjugating activities for harmol in the perfused rat liver preparation. *J. Pharmacol. Exp. Ther.* **234**:691–697 (1985).
16. Reinke, L. A., S. A. Belinsky, R. K. Evans, F. C. Kauffman, and R. G. Thurman. Conjugation of *p*-nitrophenol in the perfused rat liver: the effect of substrate concentration and carbohydrate reserves. *J. Pharmacol. Exp. Ther.* **217**: 863–870 (1981).
17. Thurman, R. G., L. A. Reinke, S. A. Belinsky, R. K. Evans, and F. C. Kauffman. Co-regulation of mixed-function oxidation and glucuronidation of *p*-nitrophenol in the perfused rat liver by carbohydrate reserves. *Arch Biochem. Biophys.* **209**:137–142 (1981).
18. Singh, J., and L. R. Schwartz. Dependence of glucuronidation rate on UDP-glucuronic acid levels in isolated hepatocytes. *Biochem. Pharmacol.* **30**:3252–3254 (1981).
19. Roy, A. B. Sulphate conjugation enzymes, in *Handbook of Experimental Pharmacology* (B. B. Brodie and J. R. Gillette, eds.), Vol. 28, Part 2. Springer-Verlag, Berlin, 536–563 (1971).
20. Roy, A. B. Sulphate esters and the sulphatases, in *Sulfate Metabolism and Sulfate Conjugation* (G. J. Mulder, J. Caldwell, G. M. J. van Kempen, and R. J. Vonk, eds.). Taylor and Francis, London, 299–306 (1982).
21. Mulder, G. J. *Sulfation of Drugs and Related Compounds.* CRC Press, Boca Raton, FL (1981).
22. Anundi, I., R. G. Thurman, M. El-Mouelhi and F. Kauffman. Differential distribution of sulfatase activity in pericentral and periportal regions of the liver lobule. *Pharmacologist* **27**:205 (1985).
23. Marshall, W. J., and A. E. M. McLean. The effect of oral phenobarbital on hepatic microsomal cytochrome P-450 and demethylation activity in rats fed normal and low protein diets. *Biochem. Pharmacol.* **18**:153–157 (1969).
24. Scholz, R., W. Hansen, and R. G. Thurman. Interaction of mixed-function oxidation with biosynthetic processes: inhibition of gluconeogenesis in perfused rat liver by aminopyrine. *Eur. J. Biochem.* **38**:64–72 (1973).
25. Ji, S., B. Chance, K. Nishiki, T. Smith, and T. Rich. Micro-light guides: a new method for measuring tissue fluorescence and reflectance. *Am. J. Physiol.* **216**:C144–C156 (1979).
26. Ji, S., J. J. Lemasters, and R. G. Thurman. A non-invasive method to study metabolic events within sublobular regions of the hemoglobin-free perfused liver. *FEBS Lett.* **113**:37–41 (1980).
27. Lowry, O. H. The quantitative histochemistry of the brain: histological sampling. *J. Histochem. Cytochem.* **1**:420–428 (1953).
28. Mulder, G. J., S. Brouwer, J. G. Weitering, E. Scholtens, and K. S. Pang. Glucuronidation and sulfation in the rat *in vivo*. The role of the liver and the intestine in the *in vivo* clearance of 4-methylumbelliferone. *Biochem. Pharmacol.* **34**:1325–1329 (1985).
29. Norling, A., P. Moldeus, B. Anderson, and O. Hanninen. Passage of glucuronides and sulphates into isolated hepatocytes and action on conjugation reactions, in *Conjugation Reactions in Drug Biotransformation* (A. Aito, ed.). Elsevier/North-Holland Biomedical Press, Amsterdam, 303–310 (1978).
30. Sato, R., and R. Kato. *Microsomes, Drug Oxidations, and Drug Toxicity.* Japan Scientific Societies Press, Tokyo/Wiley-Interscience, New York (1982).
31. Belanger, P. M., M. Lalande, G. Labrecque, and F. M. Dore. Diurnal variation in the transferases and hydrolases involved in glucuronide and sulfate conjugation of rat liver. *Drug Metab. Dispos.* **13**:386–389 (1985).
32. Krijgsheld, K. R., E. Scholtens, and G. J. Mulder. An evaluation of methods to decrease the availability of inorganic sulphate for sulphate conjugation in the rat *in vivo*. *Biochem. Pharmacol.* **30**:1973–1979 (1981).
33. Krijgsheld, K. R., E. J. Glazenburg, E. Scholtens, and G. J. Mulder. The oxidation of L- and D-cysteine to inorganic sulfate and taurine in the rat. *Biochim. Biophys. Acta* **677**:7–12 (1981).
34. Curtis, C. G. The origins of intra-cellular sulphate for conjugation reactions, in *Sulfate Metabolism and Sulfate Conjugation* (G. J. Mulder, J. Caldell, G. M. J. van Kempen, and R. J. Vonk, eds.). Taylor and Francis, London, 67–71 (1982).
35. Conway, J. G., J. A. Popp, and R. G. Thurman. Microcirculation in periportal and pericentral regions of lobule in perfused rat liver. *Am. J. Physiol.* **249**:G449–G456 (1985).
36. von Dippe, P. and D. Levy. Analysis of the transport system for inorganic anions in normal and transformed hepatocytes. *J. Biol. Chem.* **257**:4381–4385 (1982).
37. De Vries, M. H., G. M. M. Groothuis, G. J. Mulder, H. Nguyen and D. K. F. Meijer. Secretion of the organic anion harmol sulfate from liver into blood. *Biochem. Pharmacol.* **34**:2129–2135 (1985).