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SHORT COMMUNICATION

Heterogeneous Expression of Sulphotransferases in Periportal and Perivenous Hepatocytes Prepared from Male and Female Rat Liver

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ABSTRACT. Sulphotransferase (ST) is a family of enzymes responsible for metabolism and detoxication of endobiotics and xenobiotics. We investigated the hepatic acinar distribution of three sulphotransferases: phenol sulphotransferase (PST), oestrogen sulphotransferase (EST), and hydroxysteroid sulphotransferase (HST) in male and female rat livers by measurement of enzyme activities in isolated periportal and perivenous hepatocytes. The distribution was confirmed by immunohistochemistry. EST activity was located predominantly in the perivenous hepatocytes in male rats but not in female rats, where residual activity is catalysed by another ST. HST activity was not significantly different in periportal and perivenous hepatocytes in either male or female rats. For PST, a more widespread distribution was observed, with slight predominance in the periportal regions. The results indicate heterogeneous distribution of ST isoenzymes in the periportal and perivenous hepatocytes isolated from male and female rat livers. BIOCHEM PHARMACOL 51;3:369–374, 1996.

KEY WORDS. sulphotransferase, hepatocytes, rat, immunohistochemistry, xenobiotics, steroids

Hepatocytes in the periportal (afferent) and perivenous (efferent) zones of the hepatic acinus have different morphological and biochemical characteristics [1, 2]. Periportal hepatocytes have a higher capacity for gluconeogenesis, urea synthesis, and glycogen degradation, whereas perivenous cells display higher rates of lipogenesis, bile acid formation, and glutamine synthesis [e.g. refs. 1–5]. Sulphation is an important pathway of metabolism for bile acids, steroid hormones, monoamine neurotransmitters and xenobiotics, which in general reduces biological activity through the transfer of a polar sulphonate group to various hydroxyl and amine groups [6, 7]. These reactions are catalysed by a family of sulphotransferase isoenzymes (ST) present in the cytosolic fraction of the liver and other tissues. In rats, a number of subfamilies of STs have been discovered that exhibit enzyme activities towards different classes of substrate, including phenol STs (PST), hydroxysteroid STs (HST), and oestrone STs (EST) [7, 8]. We have purified a PST, an EST, and an HST from the livers of male (PST, EST) and female (HST) rats, and raised antibodies against the enzymes [9–11].

To date, there has been little analysis of the acinar zonation of ST families by direct enzyme activity measurements in isolated hepatocytes; however, immunohistochemistry has suggested differential zonation of STs in rat liver [12–14]. Although immunohistochemistry can differentiate periportal and perivenous zones, the technique cannot easily distinguish parenchymal and non-parenchymal cells, nor does it permit the quantification of enzyme activity. To clarify the acinar zonation of STs in male and female rat liver, we determined (a) the activity of the three rat ST subfamilies (PST, HST, EST) in hepatocytes isolated from periportal and perivenous zones of male and female rats, and (b) their distribution by immunohistochemistry.

MATERIALS AND METHODS

Chemicals and Reagents

Digitonin was obtained from BDH Chemicals (Poole, Dorset, U.K.). Collagenase, substrates for enzymes reactions, and cofactors were from Sigma/Aldrich (Poole, U.K.). Other enzymes were from Boehringer Corporation (Lewes, U.K.). Paraformaldehyde was purchased from Agar Scientific Ltd. (Essex, U.K.). Avidin-biotin blocking solutions were from Vector laboratories (Peterborough, U.K.), and biotinylated goat anti-rabbit IgG and streptavidin-peroxidase complex were from Biogenex (Croydon, U.K.).

Preparation of Hepatocytes

Fed male and female Wistar rats (body weight 200–300 g) from the colony maintained in this institute were used. Hepatocytes

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TABLE 1. Marker enzyme activities in periportal and perivenous hepatocytes prepared from male and female rat livers

Enzyme	Sex	Periportal	Perivenous	PP/PV
Alanine aminotransferase	Male	182 ± 6	108 ± 7*	1.69
	Female	169 ± 3	108 ± 5†	1.57
Lactate dehydrogenase	Male	1639 ± 71	1136 ± 35*	1.44
	Female	1628 ± 65	1147 ± 38*	1.42
Glutamate dehydrogenase	Male	2671 ± 127	3673 ± 44*	0.73
	Female	2663 ± 83	3738 ± 24†	0.71

Hepatocytes were isolated from the periportal or perivenous zone of livers of fed male and female rats. Enzyme activities were determined as described in Materials and Methods, and are expressed as nmol per minute per mg of protein (at 30°C) or as the ratio (PP/PV) of activity. Values are means ± SEM for 3 separate preparations.

* $P < 0.005$, † $P < 0.001$ (Student's *t*-test).

were isolated from either the periportal or perivenous zones of the liver by the digitonin-collagenase perfusion technique [e.g. ref. 15], with minor modifications [16]. Rats were anaesthetised by intraperitoneal injection of phenobarbital (60 mg/kg body weight). The portal vein and superior vena cava were cannulated and the liver perfused with Buffer A (150 mM NaCl, 6.7 mM KCl, 5 mM glucose, 10 mM Hepes, and 0.2 mM EDTA). Digitonin (4 mg/mL) was dissolved in 150 mM NaCl by warming in a water bath for 15 minutes. KCl and Hepes were added to final concentrations of 6.7 mM and 50 mM, respectively, the pH was adjusted to 7.5 with 2 mM NaOH, and the solution was filtered (0.2 mM pore size filter). The perfusion was interrupted and digitonin was infused in either the retrograde or antegrade direction to selectively destroy perivenous or periportal hepatocytes. The duration of the digitonin perfusion was determined by the appearance of the destruction pattern observed on the surface of the liver [15]. The destruction pattern took the appearance of either pale spots (for periportal isolation/perivenous destruction) or light rings (perivenous isolation/periportal destruction) [15]. Only livers with uniform destruction patterns were used. After digitonin perfusion, the direction of flow with Buffer A was rapidly reversed to prevent digitonin reaching the intact parenchymal cells. Hepatocytes were then isolated by perfusion in a recirculating manner for 5–7 minutes with Buffer B (150 mM NaCl, 6.7 mM KCl, 5 mM $CaCl_2$, 5 mM glucose, and 30 mM Hepes) containing collagenase [17]. The liver was dissected free of the abdomen and put in a petri dish containing Buffer C (150 mM NaCl, 6.7 mM KCl, 1 mM $CaCl_2$, 5 mM glucose, 10 mM Hepes). The capsule was removed and the liver gently agitated to liberate the cells. Hepatocytes were then filtered through a 40 μm nylon mesh and washed twice with Buffer C by centrifugation for 90 sec at 50 *g*. The hepatocytes were resuspended in Buffer C and viability, assessed by cell refractiveness, was greater than 90%. Hepatocytes were homogenised in 250 mM sucrose, 5 mM Hepes, pH 7.4, and centrifuged at 8,000 *g* for 20 sec. The supernatants were subjected to further centrifugation at 105,000 *g* for 30 minutes, divided into 0.5-mL aliquots, and stored at −70°C.

Enzymatic Analysis and Immunohistochemistry

Standard methods [18] were used for the assay in periportal and perivenous cell pellets of lactate dehydrogenase (EC 1.1.1.27), glutamate dehydrogenase (EC 1.4.1.2), and alanine aminotransferase (EC 2.6.1.2). All spectrophotometric assays were carried out at 30°C with a centrifugal analyser. ST activity towards DHEA (7.5 μM), 1-naphthol (10 μM), and oestrone (7.5 μM) were determined as described previously [9–11], in the presence of 50 μM PAPS. Protein content was estimated by the method of Lowry *et al.* [19]. Adult male and female Wistar rats were killed by cervical dislocation. Blocks of liver tissue (1 cm × 1 cm × 3 mm) were formalin-fixed and paraffin-embedded as described previously [20], and cut in 10-μm sections. Paraffin was removed from the sections with Histoclear, rehydrated in ethanol, and rinsed in water. Endogenous peroxidase was inactivated with 7.5% hydrogen peroxide, and avidin-biotin binding sites were blocked using a kit from Vector Laboratories. After a 5-minute incubation with 20% normal goat serum, sections were incubated for 1 hr at room temperature in primary antibody (rabbit anti-rat liver HST, EST, or PST IgG [9–11]) at a concentration of 0.04 mg/mL, and control sections were exposed to pre-immune rabbit IgG (0.04 mg/mL). Sections were then incubated at room temperature in biotinylated goat anti-(rabbit IgG) diluted 1:25, followed by incubation in streptavidin-peroxidase complex, and the peroxidase reaction was visualised with 0.1% diaminobenzidine and 0.03% H_2O_2. Sections were lightly counterstained with Mayer's Haematoxalin, dehydrated through graded alcohols, and cleared with Histoclear before coverslipping in synthetic resin.

TABLE 2. Sulphotransferase activities in periportal and perivenous hepatocytes prepared from male and female rat livers

Enzyme (Substrate)	Sex	Periportal	Perivenous	PP/PV
Estrogen sulphotransferase (Estrone)	Male	47.1 ± 5.5	126 ± 21*	0.37
	Female	10.3 ± 1.0	7.6 ± 0.7	1.36
Hydroxysteroid sulphotransferase (DHEA)	Male	12.6 ± 3.1	15.0 ± 7.0	0.84
	Female	683 ± 87	437 ± 71	1.56
Phenol sulphotransferase (1-Naphthol)	Male	1591 ± 165	1574 ± 209	1.01
	Female	922 ± 143	766 ± 130	1.20

Hepatocytes were isolated as described in Materials and Methods. Enzyme activities were determined in cytosolic fractions as described in Materials and Methods. Activities are expressed as pmol per min per mg protein and PP/PV as ratio. Results are expressed as means ± SEM for each of 3–5 periportal and perivenous preparations.

* $P < 0.01$ (Student's *t*-test).

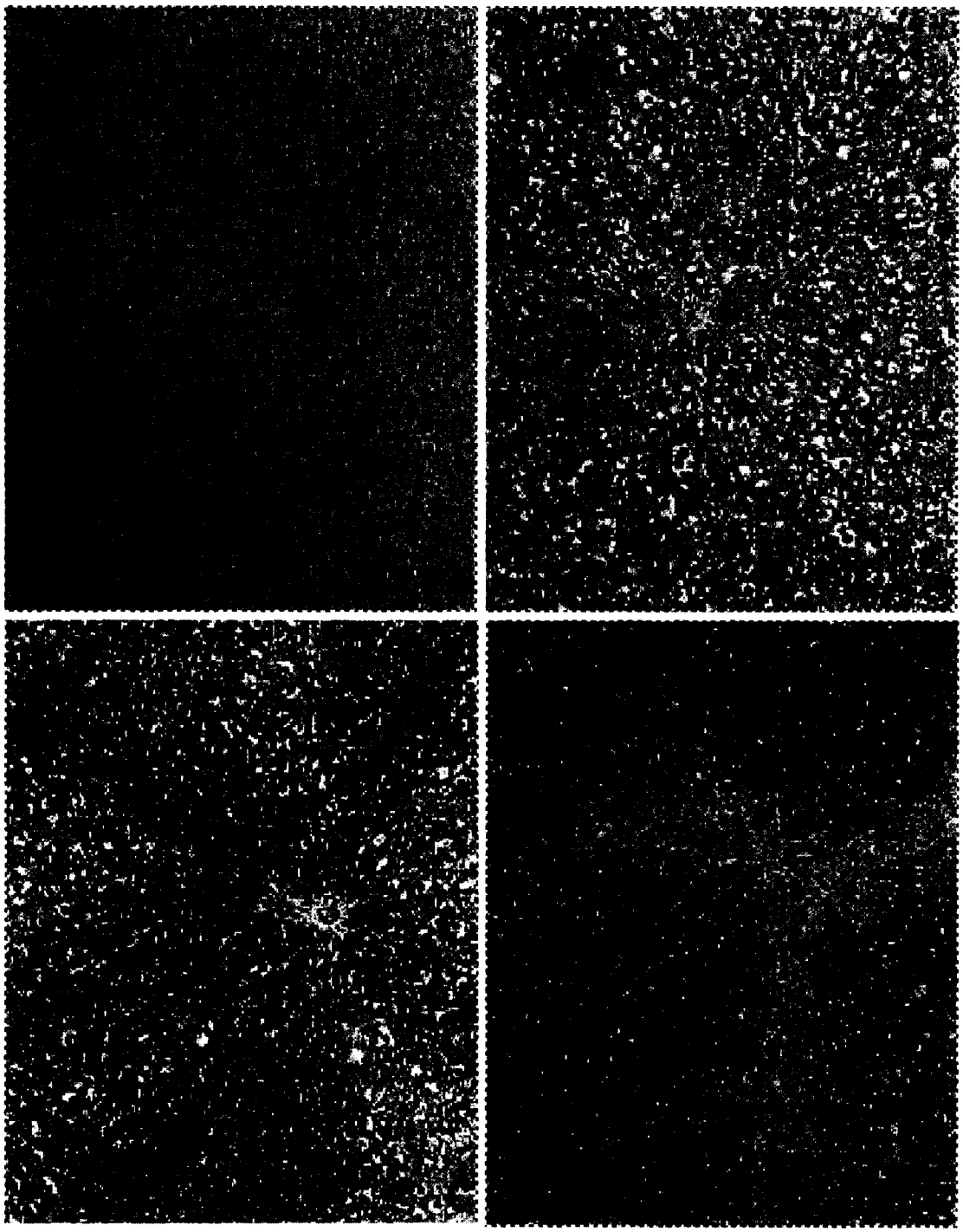

FIG. 1. Immunohistochemical analysis of sulphotransferase expression in male rat liver. Immunohistochemistry was performed as described in Materials and Methods on formalin fixed, paraffin embedded sections of male and female rat liver, with either (A) non-immune rabbit IgG, (B) anti-(rat liver PST), (C) anti-(rat liver HST), or (D) anti-(rat liver EST) as primary antibody.

RESULTS AND DISCUSSION

The concept of hepatic metabolic zonation arose from the discovery that rate-limiting enzymes for a variety of biochemical pathways were not evenly distributed across the liver acinus [2, 21]. In this study, we used enzyme activity measurements on cytosol prepared from isolated periportal and perivenous hepatocytes in combination with immunohistochemistry to examine the acinar zonation of ST isoenzymes. This approach has allowed the functional correlation of enzyme activity measurements with enzyme protein expression.

The degree of enrichment of periportal or perivenous hepa-

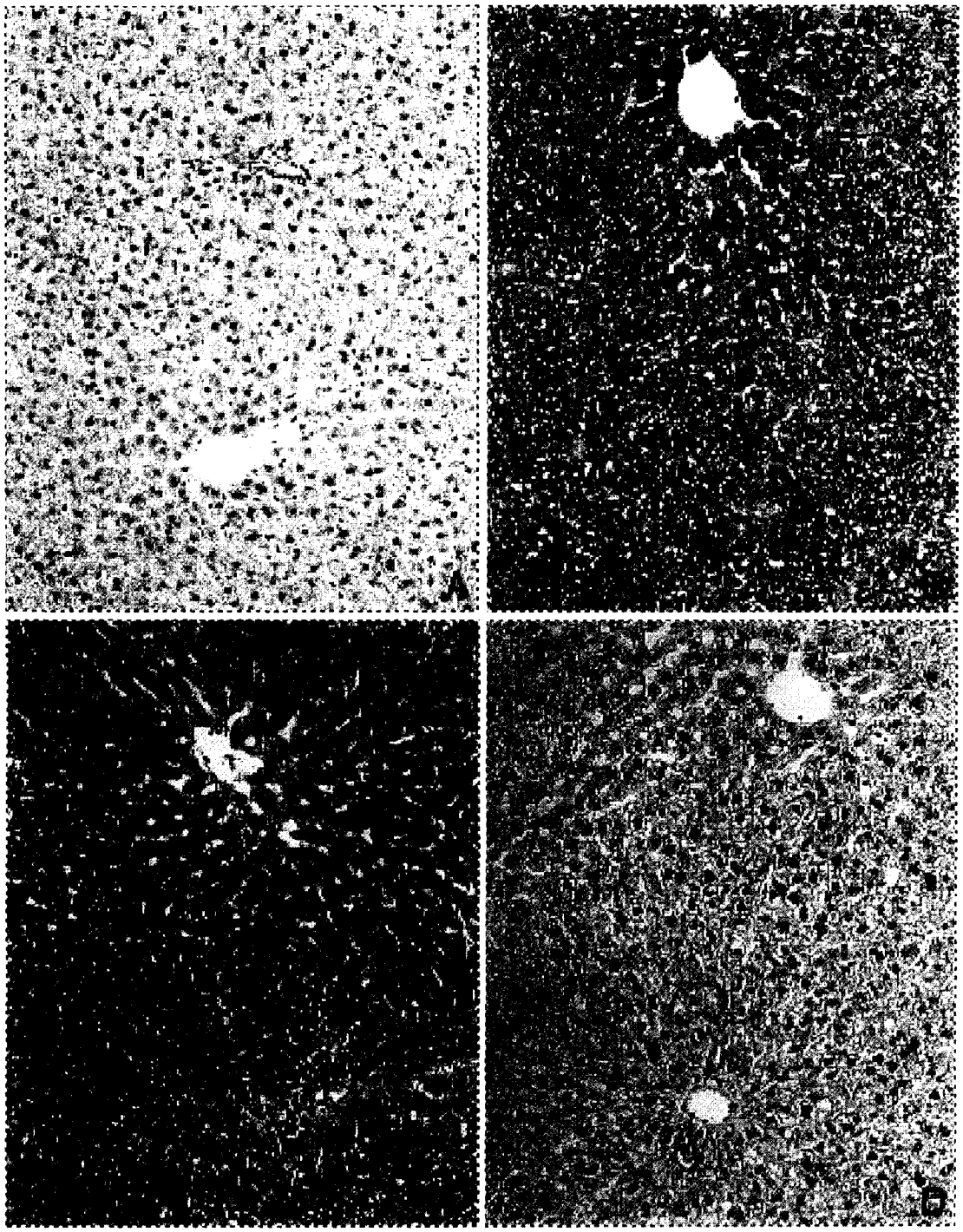

FIG. 2. Immunohistochemical analysis of sulphotransferase expression in female rat liver. Immunohistochemistry was performed as described in Materials and Methods on formalin-fixed, paraffin-embedded sections of male and female rat liver, with either (A) non-immune rabbit IgG, (B) anti-(rat liver PST), (C) anti-(rat liver HST), or (D) anti-(rat liver EST) as primary antibody.

tocyte populations can be demonstrated by differences in activities of marker enzymes (Table 1). Alanine aminotransferase is one of the most commonly used enzyme markers in studies on isolated periportal and perivenous hepatocytes, as there is a relatively sharp gradient of activity between the periportal and perivenous zones of the liver acinus [15]. Other acinar enzyme markers include lactate dehydrogenase [22] and glutamate dehydrogenase [23]. The periportal zone had a higher activity of alanine aminotransferase and lactate dehydrogenase, and a lower activity of glutamate dehydrogenase than did perivenous hepatocytes (Table 1). The ratios obtained are in good agreement with previous results obtained

using this technique [15], and demonstrate that all preparations were significantly enriched in hepatocytes from either the periportal or perivenous zone.

EST activity (oestrone as substrate) was present at significantly higher (2.7-fold) levels in perivenous compared to periportal hepatocytes isolated from male rats (Table 2). In female rat hepatocytes, this perivenous enrichment was not evident (Table 2), with a slightly higher level (1.4-fold) in periportally-derived cells. HST is expressed at higher levels in female rat liver than male rat liver [e.g. ref. 10], and HST activity (DHEA as substrate) was higher (1.6-fold, but not quite statistically significant: $P = 0.06$, Student's t-test) in female periportal hepatocytes than in perivenous cells (Table 2), a similar distribution to the residual EST activity observed in female hepatocytes (Table 2). In hepatocytes prepared from male rats, HST activity was similar in cells derived from either periportal or perivenous regions (Table 2). PST activity, measured with 1-naphthol as substrate, was similar in perivenous and periportal hepatocytes isolated from male rats, but showed a slight (1.2-fold) predominance in periportal cells from female rats (Table 2).

When sections of livers from male and female rats were exposed to anti-rat liver ST antibodies, positive staining for each isoenzyme was evident within parenchymal cells throughout the liver acinus, except for EST in female rat, where no immunostaining was observed due to the lack of expression of this isoenzyme (Figs. 1 and 2). Immunohistochemical staining was not evident within these cells when sections were exposed to normal rabbit IgG (Figures 1 and 2A). Although parenchymal liver cells throughout the acinus were stained for STs, the staining was not always of uniform intensity across the liver acinus. In male rats, EST isoenzyme expression was localised primarily in the perivenous hepatocytes (Figure 1D), whereas HST immunoreactivity showed the opposite pattern of zonation in male rat liver, with predominantly periportal distribution (Fig. 1C). PST enzyme protein expression was more uniformly distributed in both male and female rat livers (Figs. 1 and 2B). EST was not expressed in female rat liver (Fig. 2D).

Previous studies have suggested that umbelliferone is sulphated mainly in periportal hepatocytes [8], and that sulphation of harmol [24, 25], acetaminophen [26], and 7-hydroxycoumarin [27] also takes place predominantly in the periportal zone, as determined by conjugate formation by perfused liver preparations. However, the substrate specificity boundaries of the different ST sub-families are to date somewhat ill defined, and it is distinctly possible that these substrates do not differentiate between different ST isoforms. We observed a slight periportal zonation for PST by immunohistochemistry in both male and female rats, data which are in agreement with the enzyme activity measurements made with 1-naphthol as substrate.

The most dramatic zonation of ST activity was observed with EST in male rats, where an approximately 3-fold higher enzyme activity was found in perivenous hepatocytes (Table 2). Young adult female rats do not express hepatic EST protein or mRNA [10, 12, 28], and we have previously shown by antibody inhibition that the residual sulphation of oestrone by female rat liver cytosol is principally the result of the action of HST [10, 11]. By immunohistochemistry, we observed a predominant perivenous localisation of EST expression in male rat livers. EST in isolated periportal and perivenous hepatocytes of female rat livers has not previously been measured. Although the EST activity is much lower in female compared with male rat livers, we found a slightly higher activity of EST in periportal hepatocytes isolated from female rat livers, which paralleled the expression of HST, the enzyme responsible for the limited oestrogen sulphation observed in female rat liver.

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