Propranolol Elimination by Right and Left Fetal Liver: Studies in the Intact Isolated Perfused Fetal Sheep Liver

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ABSTRACT

Propranolol extraction in vivo by the left lobe of the fetal sheep liver is greater than that by the right lobe, and this may be due to the fact that oxygenation of the left lobe is greater than that of the right lobe. To explore this hypothesis, we studied the elimination of (R)-(−)-propranolol (PROP) by right and left lobes of the intact isolated perfused fetal sheep liver model, in which there is equal oxygenation of both liver lobes. After isolation of the liver, near-term fetal sheep livers (n = 11) were perfused (2.68 ± 0.10 ml/g liver/min) in situ via the umbilical vein in a 1-liter recirculating system. PROP was infused (1.2 mg/hr) into the reservoir after an initial bolus dose (2.3 mg). Perfusate samples were taken from the common and right and left hepatic veins every 10 min for determination of PROP concentrations and oxygen consumption over the 180-min experimental period. Mean ductus venosus shunt through the liver was 42 ± 21% of perfusate flow. Oxygen consumption was not significantly different between the left and right lobes of the liver (0.79 ± 0.46 and 0.67 ± 0.44 μmol/g liver/min, respectively, P > .05), nor was there any significant difference between lobes in PROP hepatic extraction at steady state (0.25 ± 0.20 and 0.25 ± 0.23, respectively, P > .05). This supports the hypothesis that the difference between lobes in PROP extraction in vivo may be due to the degree in difference of oxygenation of the left and right lobes that is known to be present in vivo.

The fetal liver is capable of many drug biotransformation reactions (Juchau et al., 1980; Krauer and Dayer, 1991; Raucy and Carpenter, 1993; Rurak et al., 1991), but the extent to which fetal hepatic metabolism contributes to overall fetal drug elimination has yet to be defined (Krauer and Dayer, 1991). In utero, in an environment very different from that of its adult counterpart, the fetal liver is unique anatomically and metabolically. For example, it has an extra input (the umbilical vein), a significant proportion of the total blood flow to the liver is supplied with poorly oxygenated blood from the ductus venosus and the liver obtains most of its oxygen supply from the umbilical vein rather than the hepatic artery (Edelstone et al., 1978; Jones and Rolph, 1985; Wilson et al., 1963). Furthermore, there is evidence that the right and left lobes of the fetal liver may act as quite separate functional units in utero (Bristow et al., 1983; Chianale et al., 1988; Germain et al., 1987).

An in vivo study of PROP disposition in the pregnant sheep model showed that PROP extraction by the left lobe of the fetal liver was ~0.3, whereas extraction by the right lobe of the fetal liver was negligible (Mihaly et al., 1982). The reason for this difference was not identified, but it could have resulted from a different level of expression between the lobes of the oxidative metabolizing enzymes responsible for PROP metabolism (Chianale et al., 1988). An alternative explanation arises when differences in oxygen delivery rates between the left and right lobes of the fetal liver in vivo are considered (Edelstone et al., 1978; Germain et al., 1987). The contribution to total oxygen delivery from the hepatic artery is minor, delivering desaturated blood evenly to both lobes. The umbilical vein delivers well oxygenated blood to both lobes, but the right lobe of the liver is additionally supplied with poorly oxygenated blood from the portal vein. Because total blood flow to the right and left lobes is equal in vivo, the net rate of oxygen delivery to the right lobe is lower than that to the left (Edelstone et al., 1978; Reuss and Rudolph, 1981; Rudolph and Heymann, 1970). Thus, the right lobe of the fetal liver can be considered relatively deprived of oxygen compared with the left lobe as a consequence of the characteristics of blood flow in the fetal hepatic circulation. Although the effect of hypoxia on fetal hepatic drug elimination has not been investigated, studies in our laboratory show that the elimination of PROP by the adult liver is extremely sensitive to...
the level of hepatic oxygenation because the oxidative metabolism of PROP requires a ready supply of molecular oxygen (Elliott et al., 1993b; Hickey et al., 1996; Jones et al., 1984).

PROP is a commonly prescribed nonselective beta adrenoceptor antagonist that has been associated with fetal and neonatal bradycardia and neonatal hypoglycemia (Rubin, 1981). PROP is metabolized by multiple oxidation and conjugation pathways that have been characterized in adult humans, rats, dogs and sheep (Bargar et al., 1983; Ring et al., 1995; Walle et al., 1985). Previously, we demonstrated that in the isolated perfused fetal sheep liver preparation, PROP is primarily metabolized via ring and side-chain oxidation reactions, and that significant sulfation and glucuronidation also occur (Ring et al., 1995).

If the difference in hepatic extraction of PROP between the left and right lobes of the fetal sheep liver in vivo (Mihaly et al., 1982) were a direct result of the difference in oxygenation of the two lobes, then in the isolated perfused fetal sheep liver preparation, in which oxygenation of the two lobes is equal, the extraction of PROP by each lobe should be the same. Accordingly, we compared the extractions of PROP by the left and right lobes of the isolated perfused fetal sheep liver preparation.

Materials and Methods

Chemicals and drugs. PROP, sodium taurocholate, \( \beta \)-d-glucuronidase/aryl sulfatase (in the ratio 20:1; from Helix pomatia type H-I), bovine serum albumin (fraction V) and labetalol were purchased from Sigma Chemical (St. Louis, MO). D-Glucose was purchased from BDH Chemicals (Melbourne, Victoria, Australia). \( ^{153} \)Gd-radiolabeled 15-\( \mu \)m latex microspheres were bought from DuPont NEN (Sydney, NSW, Australia). HPLC grade chromatography solvents were purchased from Rhone-Poulenc (Melbourne, Australia). All other reagents were of analytical grade.

Animals. Experiments were conducted on the fetuses of 11 Merino-Dorset Horn sheep. Fetal age (125–145 days; term, 147 days) was determined from joining dates and confirmed through fetal metatarsal length at surgery (Santucci et al., 1993). The experiments were approved by the Austin Hospital Animal Welfare Committee.

Perfusion of isolated fetal livers. The fetal livers were isolated and perfused in situ in a recirculating system using the technique we described previously (Ring et al., 1994). Briefly, fetal sheep were delivered by cesarean section with the mother under general anesthesia with halothane and thiopental (Mihaly et al., 1982). After cannulation of the umbilical vein (the inflow) and the inferior vena cava (draining left and right hepatic veins as the outflow) and ligation of hepatic artery, portal vein, and suprarenal inferior vena cava (fig. 1), the isolated liver was connected to the perfusion circuit. The outflow cannula contained a side port allowing the introduction of sampling cannulae into the right and left hepatic veins that were left in situ during each experiment (fig. 1). Their correct positioning was verified by dissection of the liver at the conclusion of each experiment. The bile duct also was cannulated. The circuit was a recirculating system consisting of a reservoir containing 1 liter of oxygenated Krebs-Henseleit buffer with both 10% washed human red blood cells (experiments 1–6) and 20% washed human red blood cells (experiments 7–11), 1% bovine serum albumin and 0.1% glucose. The temperature was maintained at 37°C and pH 7.4. An infusion of sodium taurocholate solution (7.5 mM) was delivered to the reservoir at a rate of 4 ml/hr to maintain bile flow (Ring et al., 1994). The perfusate flow rate used was 300 ml/min (2.68 \pm 0.5 ml/min/g liver) (Edelstone et al., 1978). The viability and stability of each experiment were verified at 30-min intervals by monitoring organ appearance (uniformly perfused, nondistended capsule, without perfusate leakage), perfusion pressure, bile flow, oxygen consumption and perfusate potassium (Ring et al., 1994).

Experimental design. In 11 experiments, after an initial 15-min equilibration period, 2.3 mg of PROP was administered as a bolus loading dose into the reservoir, followed by a constant infusion of PROP at a rate of 1.2 mg/hr, which was calculated from pilot studies designed to achieve a steady state perfusate concentration of \( \approx 1 \mu \)M. Perfusionate samples (2 ml) were taken at 0, 2, 4, 6, 10, 15, 20, 40, 60, 80, 100, 120, 130, 140, 150, 160, 170 and 180 min from the reservoir and right and left hepatic veins from 120 min on. Sample volumes were replaced with equivalent amounts of drug-free perfusate, and bile was collected in 30-min aliquots. Perfusionate and bile samples were collected into light-protected tubes containing 2% ascorbic acid (w/v) and stored at \(-20^\circ\)C until analyzed. Oxygen delivery and consumption were calculated at 30-min intervals from 500-ml samples of inflow (umbilical vein) and outflow (suprarenal IVC, right and left hepatic vein) perfusate (fig. 1) with the use of a blood gas and pH analyzer (Instrumentation Laboratory System 1302, Lexington, MA) (Jones et al., 1984). At the conclusion of each experiment, \( ^{153} \)Gd-labeled microspheres were used to assess intrahepatic perfusate distribution. A 200-ml bolus containing \( \approx 2 \times 10^5 \)Gd-labeled microspheres was injected into the liver inflow cannula using a 1-ml syringe at a distance of 15 cm from the lIVER to allow adequate mixing of microspheres before entering the liver (Ring et al., 1994). The outflow perfusate was collected in 10-sec aliquots (50 ml each) for 1 min and counted using a Packard Auto-Gamma 5005 Count (Meridian, CT) as described previously (Heymann et al., 1977). All of the microspheres that eluted from the liver were eluted within the first 10-sec aliquot. Each liver was carefully removed from the carcass, and the right and left lobes were weighed after dissection as follows. The middle hepatic vein divides the liver into right and left lobes.
along “Cantlie’s line,” which runs from the middle of the gallbladder fossa to the left of the inferior vena cava posteriorly. With the use of these landmarks, the liver was separated into two units with independent arterial supply and venous and biliary drainage (Bismuth et al., 1991). The total number of \(^{153}\text{Gd}\)-labeled microspheres remaining in the right and left liver lobes was counted after homogenization of the entire liver. Comparison of outflow perfusate and hepatic tissue microsphere contents allowed the proportion of perfusate being shunted via the ductus venosus to be determined.

**Sample assay.** PROP and its metabolites were assayed in 500 \(\mu\)l of perfusate and 100 \(\mu\)l of bile using the methods described previously (Ring et al., 1995) with minor modifications. Labetolol (50 \(\mu\)g/ml of 50 \(\mu\)g/ml) as internal standard, 1 ml of carbonate buffer (1 M, pH 10.3) and 5 ml of acid-washed diethyl ether were added to the sample, and the sample was vortexed and centrifuged. The ether phase was aspirated and back extracted into 125 \(\mu\)l of 0.5% phosphoric acid. Ten microliters of the phosphoric acid was injected into the chromatograph. The chromatography system was a Novapak phenyl 4- \(\mu\)m (8 \(\times\) 100 mm; Waters and Associates, Milford, MA) plastic radial compression column eluted at 3 ml/min with acetonitrile/water/triethylamine (23:77:1, v/v/v) (adjusted to pH 3.6 with concentrated phosphoric acid). In addition, a Shimadzu RF-551 programmable wavelength fluorescence detector (Shimadzu, Japan), with an excitation wavelength of 295 nm and an emission wavelength of 360 nm, was used. Elution times were 4.0 and 5.5 min for labetolol and PROP, respectively, and all peaks were resolved to baseline. The between-day assay coefficient of variation for PROP was 2.5% at 250 ng/ml. The standard curve concentrations ranged from 62.5 to 2000 ng/ml.

**Pharmacokinetic and statistical analysis.** The hepatic Cl was calculated as:

\[
\text{Cl} = \frac{R}{Q} 
\]

(1)

where R is the infusion rate of PROP into the reservoir, and \(Q\) is the liver inflow (i.e., reservoir) PROP concentration. The net hepatic extraction ratio (\(E_n\)) was calculated as:

\[
E_n = \frac{C_{in} - C_{out}}{C_{in}} 
\]

(2)

where \(C_{in}\) is the liver inflow concentration, \(C_{out}\) is the liver outflow concentration at steady state, and \(C_{in}\) is the hepatic vein inflow concentration at steady state. A corrected \(E_n\), defined as \(E_n^*\), also was calculated to take account of shunting of perfusate through the ductus venosus as:

\[
E_n^* = \frac{Q}{(1 - S)}[\text{Cl}] 
\]

(3)

where \(S\) is the fraction of perfusate shunted. This shunt fraction was determined as the ratio of total counts of \(^{153}\text{Gd}\) in outflow perfusate divided by the sum of total counts in liver tissue and outflow perfusate. This was done at the end of all experiments. Oxygen extraction for total (\(E_{ot}\)), right (\(E_{or}\)) and left (\(E_{ol}\)) liver was calculated as:

\[
E_{ot} = \frac{O_{\text{ot}}}{O_{\text{at}}} 
\]

(4)

\[
E_{or} = \frac{O_{\text{or}}}{O_{\text{at}}} 
\]

(5)

\[
E_{ol} = \frac{O_{\text{ol}}}{O_{\text{at}}} 
\]

(6)

where \(O_{\text{ot}}\) is the oxygen consumption by the total (t), right (r) and left (l) lobes of the liver, and \(O_{\text{at}}\) is the oxygen delivery to the total (t), right (r) and left (l) lobes of the liver. Per fusate oxygen content, delivery and consumption were calculated according to standard methods (Jones et al., 1984).

Data are presented as mean and S.D. unless otherwise specified. Linear regression was used to analyze changes with time in mean values of viability data. Correlations between variables were examined by linear regression analysis. A value of \(P < .05\) was considered statistically significant.

**Results**

**Viability.** Mean gestational age was 135 \(\pm\) 6 days; mean perfusion pressure was 12.9 \(\pm\) 4.3 mm Hg; mean bile flow was 0.36 \(\pm\) 0.19 \(\mu\)mol/min/g liver (table 1); and mean oxygen consumption was 0.55 \(\pm\) 0.22 \(\mu\)mol/min/g liver (range, 0.55–1.04, \(P < .05\)). These values indicated the viability of the preparation (Ring et al., 1995) and did not alter significantly over the experimental period. Mean liver weight was 70.7 \(\pm\) 13.1 g, and the proportion of perfusate shunted through the ductus venosus was 42.0 \(\pm\) 21.5% of umbilical vein flow (range, 17.5–67.6%). Liver weight, bile production, ductus venosus shunt, oxygen extraction and perfusion pressure did not correlate with fetal age.

**Perfusate distribution.** The weight of the left lobe of the liver was significantly greater than that of the right lobe by \(\approx 22\%\), and the two lobes received the same perfusate flow per gram of liver (table 2, \(P > .05\)). There was no significant difference in perfusate distribution to left and right lobes between the 10% and 20% hematocrit experiments (table 2).

**Oxygen delivery and consumption.** Perfusate oxygen content, saturation and partial pressure are shown in table 3. As expected, mean oxygen delivery to the liver was significantly greater in the 20% hematocrit experiments than in the 10% hematocrit experiments (table 4). Mean oxygen consumption (right lobe, left lobe and total liver) was not significantly different between the 10% and 20% hematocrit experiments. Mean oxygen consumption was not significantly different between right and left lobes of the liver in both the 10% and 20% hematocrit experiments (table 4). The combined results for all experiments showed no significant differences in either the delivery or consumption of oxygen between the right and left lobes of the fetal liver.

**PROP pharmacokinetics.** Steady-state PROP concentrations were achieved at all sampling sites by 120 min in all experiments (fig. 2). In the 10% hematocrit experiments, the mean steady-state PROP inflow concentration was 2.64 \(\pm\) 1.84 \(\mu\)M, and in the 20% hematocrit experiments, the con-

**TABLE 1**

<table>
<thead>
<tr>
<th>Physiological parameters</th>
<th>Sheep</th>
<th>Sex</th>
<th>Gestational age (days)</th>
<th>Liver weight (g)</th>
<th>Perfusion pressure (mm Hg)</th>
<th>Mean bile flow ((\mu)mol/min/g liver)</th>
<th>Ductus venosus shunt</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sheep</strong></td>
<td><strong>Sex</strong></td>
<td><strong>Gestational age</strong></td>
<td><strong>Liver weight</strong></td>
<td><strong>Perfusion pressure</strong></td>
<td><strong>Mean bile flow</strong></td>
<td><strong>Ductus venosus shunt</strong></td>
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<tr>
<td>1</td>
<td>M</td>
<td>145</td>
<td>46.7</td>
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<tr>
<td>2</td>
<td>F</td>
<td>130</td>
<td>62.7</td>
<td>8.3</td>
<td>0.494</td>
<td>17.5</td>
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<tr>
<td>3</td>
<td>F</td>
<td>130</td>
<td>56.2</td>
<td>10.2</td>
<td>0.264</td>
<td>67.6</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>135</td>
<td>81.0</td>
<td>8.2</td>
<td>0.351</td>
<td>56.3</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>135</td>
<td>80.2</td>
<td>12.9</td>
<td>0.697</td>
<td>6.8</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>135</td>
<td>70.1</td>
<td>11.6</td>
<td>0.433</td>
<td>58.4</td>
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<tr>
<td>7</td>
<td>F</td>
<td>135</td>
<td>94.2</td>
<td>19.0</td>
<td>0.032</td>
<td>39.3</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>130</td>
<td>79.9</td>
<td>14.0</td>
<td>0.263</td>
<td>41.2</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>125</td>
<td>64.3</td>
<td>20.8</td>
<td>0.172</td>
<td>71.3</td>
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</tr>
<tr>
<td>10</td>
<td>M</td>
<td>138</td>
<td>71.8</td>
<td>10.0</td>
<td>0.311</td>
<td>41.1</td>
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</tr>
<tr>
<td>11</td>
<td>F</td>
<td>143</td>
<td>70.9</td>
<td>16.7</td>
<td>0.391</td>
<td>14.2</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td></td>
<td>135</td>
<td>70.7</td>
<td>12.9</td>
<td>362</td>
<td></td>
<td>42.0</td>
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<tr>
<td><strong>S.D.</strong></td>
<td></td>
<td></td>
<td>5.89</td>
<td>13.1</td>
<td>4.31</td>
<td>0.185</td>
<td></td>
<td>21.5</td>
</tr>
</tbody>
</table>
The mean steady-state PROP inflow concentration for all 11 experiments was \(2.34 \pm 1.59\, \text{mM} \) (Table 2). The mean net hepatic PROP extraction ratio at steady state was 0.143 \(\pm\) 0.111 and, when corrected for ductus venosus shunt, 0.309 \(\pm\) 0.208. Mean PROP extraction ratio was similar in right and left lobes of the liver (0.249 \(\pm\) 0.229 and 0.247 \(\pm\) 0.203, respectively; Table 5; \(P > .05\)). There was no significant difference in PROP extraction by right and left lobes of the liver (Table 5; \(P > .05\)).
and left outflow (\(\text{L}\)), hepatic venous perfusate PROP concentration vs.
time in a typical isolate perfused fetal sheep liver experiment.

**Table 5**

<table>
<thead>
<tr>
<th>Hematocrit</th>
<th>Sheep</th>
<th>Hepatic extraction ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Net</td>
</tr>
<tr>
<td>10%</td>
<td>1</td>
<td>0.220</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.0687</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.0920</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.0654</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.0113</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.320</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.133</td>
</tr>
<tr>
<td>S.D.</td>
<td></td>
<td>0.115</td>
</tr>
</tbody>
</table>

| 20%        | 7     | 0.0396 | 0.207 | 0.221 | 0.220 |
|            | 8     | 0.236 | 0.620 | 0.382 | 0.203 |
|            | 9     | 0.131 | 0.207 | 0.0511 | 0.0517 |
|            | 10    | 0.0535 | 0.186 | 0.117 | 0.147 |
|            | 11    | 0.312 | 0.492 | 0.559 | 0.529 |
| Mean       |       | 0.155 | 0.342 | 0.266 | 0.230 |
| S.D.       |       | 0.118 | 0.200 | 0.206 | 0.179 |

| Overall mean |       | 0.143 | 0.309 | 0.247 | 0.249 |
| S.D.         |       | 0.111 | 0.208 | 0.203 | 0.229 |

There is considerable evidence to suggest that the fetal liver operates as two distinct subunits. For example, hemopoiesis is greater in the right lobe, there is a tendency to greater vacuolation of hepatocytes in the right lobe and the cells of the left lobe receive more highly oxygenated blood than those of the right lobe (Edelstone et al., 1978; Emery, 1963; Reuss and Rudolph, 1981; Rudolph and Heymann, 1970). The right lobe is darker in appearance and to the naked eye obviously less well oxygenated. In another study (Bristow et al., 1981; 1983), it was reported that there is little difference between left and right lobes with respect to hepatic oxygen delivery and consumption. This brings into question whether differences in oxygenation could reasonably account for the left vs. right lobe fetal hepatic extraction that we reported (Mihaly et al., 1982) in vivo in the pregnant sheep model. We would add, however, that in the study of Bristow et al. (1983), the liver apparently was divided into left and right lobes along a line joining the midpoint of the umbilical vein as it enters the liver and the midpoint of the IVC at the superior margin of the liver. In doing so, a substantial portion of left lobe may have been included in the right lobe, influencing the calculation of fetal liver lobe oxygenation. Thus, left vs. right fetal liver lobe oxygenation might not be equal as reported by Bristow et al. (1983). In our present study, we divided the fetal liver into left and right lobes along Cantlie’s line as recommended by Bismuth et al. (1991). There also are differences between the lobes with respect to metabolic activity. For example, levels of malondialdehyde, an index of lipid peroxidation, oxygen consumption and cytochrome P450, are higher in the left lobe than the right lobe of the fetal sheep liver, whereas there are no differences in these levels between the lobes of the adult sheep liver (Bristow et al., 1983; Germain et al., 1987; Rudolph, 1983). Moreover, in fetal mice, the levels of cytochrome P450b and P450e mRNAs are higher in the left than in the right fetal liver lobe (Chianale et al., 1988). This lobar heterogeneity of expression disappears as the pattern of adult liver circulation is attained (Chianale et al., 1988; Larrieu and Galtier, 1988; Watkins et al., 1990).

**Discussion**

There is in vivo evidence in the pregnant sheep indicating that the right and left lobes of the fetal liver differ in the efficiency with which they eliminate maternally administered PROP from the fetal circulation. The fetal hepatic extraction ratio for PROP in an acute fetal sheep preparation was 0.35, representing drug extraction by the left lobe of the fetal liver (Mihaly et al., 1982). In contrast, extraction of PROP by the right lobe of the fetal liver was negligible. In a recent study in the isolated fetal sheep liver preparation perfused in situ via the umbilical vein, overall PROP extraction was 0.30 when corrected for shunting of blood via the ductus venosus (Ring et al., 1995). By selectively cannulating the right and left hepatic veins, we were able to measure directly PROP extraction by the right and left lobes of the isolated perfused fetal sheep liver without making corrections for blood flow through the ductus venosus shunt, as was previously necessary. Under these conditions, we measured mean PROP extraction by both lobes to be equal at 0.25 (table 5).

There is considerable evidence to suggest that the fetal liver operates as two distinct subunits. For example, hemopoiesis is greater in the right lobe, there is a tendency to greater vacuolation of hepatocytes in the right lobe and the cells of the left lobe receive more highly oxygenated blood than those of the right lobe (Edelstone et al., 1978; Emery, 1963; Reuss and Rudolph, 1981; Rudolph and Heymann, 1970). The right lobe is darker in appearance and to the naked eye obviously less well oxygenated. In another study (Bristow et al., 1981; 1983), it was reported that there is little difference between left and right lobes with respect to hepatic oxygen delivery and consumption. This brings into question whether differences in oxygenation could reasonably account for the left vs. right lobe fetal hepatic extraction that we reported (Mihaly et al., 1982) in vivo in the pregnant sheep model. We would add, however, that in the study of Bristow et al. (1983), the liver apparently was divided into left and right lobes along a line joining the midpoint of the umbilical vein as it enters the liver and the midpoint of the IVC at the superior margin of the liver. In doing so, a substantial portion of left lobe may have been included in the right lobe, influencing the calculation of fetal liver lobe oxygenation. Thus, left vs. right fetal liver lobe oxygenation might not be equal as reported by Bristow et al. (1983). In our present study, we divided the fetal liver into left and right lobes along Cantlie’s line as recommended by Bismuth et al. (1991). There also are differences between the lobes with respect to metabolic activity. For example, levels of malondialdehyde, an index of lipid peroxidation, oxygen consumption and cytochrome P450, are higher in the left lobe than the right lobe of the fetal sheep liver, whereas there are no differences in these levels between the lobes of the adult sheep liver (Bristow et al., 1983; Germain et al., 1987; Rudolph, 1983). Moreover, in fetal mice, the levels of cytochrome P450b and P450e mRNAs are higher in the left than in the right fetal liver lobe (Chianale et al., 1988). This lobar heterogeneity of expression disappears as the pattern of adult liver circulation is attained (Chianale et al., 1988; Larrieu and Galtier, 1988; Watkins et al., 1990).

PROP is metabolized by the fetal sheep liver primarily by oxidative pathways both in vivo and in vitro (Mihaly et al., 1982; Ring et al., 1995), probably by isozymes of the cytochrome P450 family of drug metabolizing enzymes. The difference between the fetal liver lobes in propranolol extraction, observed in the in vivo study of Mihaly et al. (1982), therefore may have been due to a difference in expression or activity of the relevant drug metabolizing enzymes. If this were the case, a difference in extraction would have been expected in the isolated perfused organ. However, this was not the case (table 5). Measurement of the content in the two lobes of the isozymes responsible for PROP metabolism in the fetal sheep liver would reveal whether the difference in in vivo extraction was due to differences in enzyme expression,
but this was not possible because the isozymes responsible have not yet been identified.

PROP metabolism is impaired by even mild hypoxia in the adult rat liver \textit{(vide infra)} \cite{Elliott et al., 1993a; Jones et al., 1984}, and therefore the difference in PROP extraction between the lobes \textit{in vivo} may have been due to the fact that the right lobe is less well oxygenated than the left lobe \cite{Bristow et al., 1983; Emery, 1963; Rudolph, 1983}. In the present study, we took the approach that if the difference in oxygenation was the cause, then under conditions of equal oxygenation of the lobes the extraction of PROP would be the same. In our \textit{in vitro} isolated perfused fetal sheep liver experiments, oxygen delivery to the left and right lobes of the liver was the same because the two lobes received oxygenated perfusate from the same source (table 4). Oxygen consumption by the two lobes also was equal, as seen previously in fetal sheep \textit{in vivo} \cite{Bristow et al., 1983}. Under these conditions, PROP extraction by the lobes was the same (table 5). This indicates that low oxygenation of the right lobe probably was the cause of the difference in PROP extraction \textit{in vivo}.

In our detailed study of the effect of hepatic oxygenation on PROP metabolism in the single-pass isolated perfused rat liver, we observed that PROP extraction is very sensitive to hepatic oxygen supply; impairment of PROP clearance is apparent below a threshold oxygen delivery at the lower end of the normal physiological range of 5 to 7 \textmu mol/g/min \cite{Elliott et al., 1993a}. Both PROP clearance and oxygen consumption in the adult rat liver were directly related to oxygen delivered. Whether PROP clearance is directly related to oxygen supply and at what oxygen delivery threshold PROP clearance shows impairment are unknown in the fetal liver. We achieved oxygen deliveries of 6.9 \pm 3.3 \textmu mol/g/min (right lobe) and 7.4 \pm 4.0 \textmu mol/g/min (left lobe; table 4) and found no correlation between PROP extraction ratio and oxygen delivery at these levels, suggesting that oxygenation was not rate limiting for PROP elimination. These values compare with measurements of oxygen delivery in fetal sheep liver \textit{in vivo} ranging from 4.5 to 22 \textmu mol/g/min \cite{Bristow et al., 1983; Emery, 1963; Rudolph, 1983}. Characterizing the relationship between PROP extraction and oxygen content, \PO_2, or percent oxygen saturation, in this model would clarify the relationship between the rate of PROP elimination and hepatic oxygen supply.

The advantage of the isolated perfused fetal sheep liver preparation is that it allows us to study fetal hepatic function in the intact organ and in the absence of other fetal elimination processes. The purpose is not to attempt to precisely reproduce the \textit{in vivo} situation or milieu but rather to control variables in the preparation that can confound \textit{in vivo} studies. It should be borne in mind that factors associated with isolated perfusion of the liver, such as the use of adult human red cells, low hematocrit, perfusate \PO_2, exteriorization of the fetus and equality of perfusate flow to the left and right liver lobes, could potentially affect PROP elimination by the fetal liver. Precisely how perturbation of each of these factors (type of red cells, hematocrit, \PO_2, exteriorization of the fetus, perfusate flow) may affect fetal hepatic disposition of PROP is unknown, but we think that the difference in \textit{in vivo} hepatic oxygenation is the most likely explanation for our findings.

The range in ductus venosus shunt (6.8–71.3\%) was wider than previously reported \textit{in vivo} \cite{Edelstone et al., 1978} and may be due to the acute nature of our preparation.

The fetal liver is the most important organ for drug metabolism \textit{in vivo} \cite{Krauer and Dayer, 1991; Moldeus, 1987}. The extent to which it carries out drug detoxification or drug activation is as yet undefined, although an increasingly wide range of catalytic activities is being identified \cite{Juchau et al., 1980; Krauer and Dayer, 1991; Raucy and Carpenter, 1993}. Our findings demonstrate that there is significant elimination of PROP by both lobes of the isolated perfused fetal sheep liver and that elimination is of equal efficiency under conditions of equal oxygenation in this model. These findings provide a plausible mechanism for the slower elimination of PROP by the right lobe of the fetal liver \textit{in vivo}.

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