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HEPATIC UPTAKE AND EXCRETION OF [^{14}C]SODIUM TAUROCHOLATE BY THE ISOLATED PERFUSED FETAL SHEEP LIVER

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Abstract—We have developed an *in situ* isolated perfused fetal sheep liver preparation to study fetal hepatic function free from the confounding influences of the mother and other fetal organs, and we have used the preparation to study the fetal hepatic clearance and biliary excretion of sodium taurocholate (TC). The viability and stability of this model were established by monitoring perfusion pressure, oxygen consumption, perfusate enzymes and electrolytes, the perfusate concentration ratio of lactate to pyruvate, bile flow, and liver histology. Perfusate delivery was 300 mL/min with a mean value of 3.94 mL/min/g liver (range: 2.46–6.72 mL/min/g liver). Gadolinium radiolabeled 15 μm microspheres were used to quantify the ductus venosus shunt through the liver and to determine relative flow rates between right and left hepatic lobes. TC was added to the reservoir either as a [^{14}C]TC tracer bolus dose (2 μCi, N = 5) followed by a constant infusion of unlabeled TC, or as an initial bolus of [^{14}C]TC (54 μmol) followed by a [^{14}C]TC constant infusion (30 μmol/hr, specific activity 30 μCi/mmol; N = 3). Perfusate samples were taken from the reservoir every 15 min and bile was collected in 30 min aliquots. Perfusion pressure (7.9 ± 0.30 mmHg), perfusate potassium and oxygen consumption (0.9 ± 0.07 μmol/min/g liver) were constant throughout, and the perfusate lactate/pyruvate concentration ratio was low (<20). Liver histology showed no hypoxic changes. Bile flow fell slightly over the 150 min experiment time from 0.6 to 0.5 μL/min/g liver. These data indicate preparation viability and stability. The extent of the ductus venosus shunt was 16–66% (mean 35 ± 6%) of umbilical vein flow, which correlated inversely with fetal gestational age ($r = 0.94$, $P < 0.001$). Relative flow to right and left lobes of liver was 1:1.4. In bolus dose experiments, TC $t_{1/2}$ was 81.6 ± 26 min, clearance (Cl) was 35.0 ± 22.6 mL/min, shunt corrected extraction (E^*) was 0.29 ± 0.17 and biliary clearance (Cl_B) was 35.5 ± 19.5 mL/min. In constant infusion experiments the corresponding results were Cl: 34.7 ± 18.2, E^*: 0.23 ± 0.16, and Cl_B 32.7 ± 17.7. The cumulative biliary excretion of [^{14}C]TC in bolus dose experiments was 86.5 ± 8.7% of the dose, and in constant infusion experiments, concentration of TC in bile was on average over 800 times that in plasma. We conclude that the isolated perfused fetal sheep liver is a suitable model for studying fetal hepatic function, which remains viable for at least 2–3 hr. The fetal liver, while able to concentratively transport TC from perfusate to bile, is only able to clear TC from perfusate relatively slowly, in contrast to the adult liver. This suggests that immaturity of fetal hepatic TC transport reflects a reduced capacity of the transport system which thereby reduces hepatic TC clearance.

Key words: bile salt elimination; fetal bile; fetal liver; liver metabolism; liver perfusion; taurocholate

Little is known of the functional capacity of the intact fetal liver in terms of processes such as substrate uptake from the circulation, transport, metabolism and excretion into bile [1]. Assessment of fetal hepatic function is complicated by the presence of an extra input vessel, the umbilical vein, in addition to portal vein and hepatic artery, and by the substantial shunting of blood through the liver via the ductus venosus [2]. *In vivo* studies of fetal physiology and pharmacology have largely used the acute anesthetized or chronically, cannulated unanesthetized pregnant sheep preparation [3, 4]. These preparations have been of limited value in studying fetal hepatic function, because catheter placement and maintenance in hepatic vessels is unreliable, and maternal, placental and other fetal elimination processes confound the interpretation of the data. We have developed an isolated fetal sheep liver preparation which allows the study of fetal hepatic function in the intact organ, under controlled experimental conditions. In this preparation there is a single, controlled hepatic input, and the various outputs, including left and right hepatic veins and bile ducts, can be reliably sampled. We have used this preparation to study the biliary excretion of the bile salt TC‖ by the near term fetal sheep liver.

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‖ Abbreviations: TC, sodium taurocholate; i.d., internal diameter; o.d., outside diameter; AUC, area under the curve; E, extraction ratio; Q, perfusion flow rate; S, fraction of perfusate shunted; Vd, volume of distribution; Cl_B, biliary clearance; [^{14}C], carbon-14 radiolabel; $t_{1/2}$, half-life; Cl, clearance; E*, shunt-corrected extraction ratio; [^{153}Gd], gadolinium 153 radiolabel.

MATERIALS AND METHODS

Materials

TC was obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.), [^{14}C]TC (specific activity 57.7 mCi/mmol) was purchased from Amersham International (Sydney, NSW, Australia), and gadolinium 153 radiolabeled 15 μm latex microspheres from DuPont NEN (North Ryde, NSW, Australia). D-glucose was purchased from BDH Chemicals (Melbourne, Victoria, Australia), and bovine serum albumin from the Commonwealth Serum Laboratories (Melbourne, Victoria, Australia).

Animals and surgical procedure

Experiments were conducted on eight fetal sheep of gestational age 120–145 days (term = 147 days). Age was determined from joining dates and confirmed by crown rump length at operation. All animals were obtained from Tulka Bros, Australia, and were worm free. The experiments were approved by the Austin Hospital Animal Welfare Committee.

With the mother and fetus under general anesthesia using halothane and pentobarbitone [4] a cesarean section was performed. The fetus was delivered onto a warmed platform (37°) and the fetal head draped with a saline swab. Following a paramedian abdominal incision the fetal liver was exposed and the portal vein, artery, and bile duct located. The gallbladder was isolated by ligating the cystic duct. The bile duct was then cannulated and left to drain freely into a collection tube. The hepatic artery and portal vein were ligated. A midline thoracotomy was performed to expose the supra-hepatic inferior vena cava. Loose ties were applied to the aorta, the supra-hepatic vena cava, and the infra-hepatic vena cava above the renal veins. A Teflon inflow cannula (i.d. 3.5 mm, o.d. 6 mm), connected to a small glass bubble trap with a 30 cm length of circuit tubing, was then primed with warm Hartman's solution containing sodium heparin (5000 U/L), in readiness for umbilical vein cannulation.

The umbilical vessels were ligated distally and an incision made in the umbilical vein proximally which was then cannulated with the inflow cannula. The supra hepatic inferior vena cava was incised and the outflow Teflon cannula (i.d. 3.5 mm, o.d. 5 mm), connected to 45 cm of circuit tubing (Tygon® R3603 i.d. 1/4", o.d. 3/8", Cole-Parmer Instrument Co., Chicago, IL, U.S.A.), was inserted and let drain to waste. The liver was then flushed slowly with the heparinized Hartman's solution. Finally, the liver was fully isolated by ligation of the supra-renal inferior vena cava. Perfusion was commenced by connecting the primed perfusion circuit to the liver inflow catheter bubble trap and the distal end of the outflow cannula tubing was placed into the perfusate reservoir to complete the recirculating system. The liver was draped with warm, saline-soaked gauze and the fetus was culled by ligation of the ascending aorta followed by an intracardiac injection of potassium chloride.

Perfusion circuit

Figure 1 illustrates the perfusion circuit. The perfusate volume was 1000 mL. Perfusate composition was Krebs–Henseleit buffer with 10% human red blood cells (v/v), bovine serum albumin (1% w/v) and D-glucose (0.1% w/v). The reservoir was a 2 L conical flask, which was kept well-mixed by a magnetic stirrer. Perfusate was recirculated through an in-line coarse filter, large glass bubble trap with built-in pH probe, temperature probe and manometer port, a water-jacketed glass heat exchanger, a small pre-liver bubble trap and then the liver via the umbilical vein cannula. Hepatic venous effluent returned via the supra-hepatic inferior vena cava outflow cannula, back into the reservoir. A cannula was placed in the reservoir to sample hepatic inflow perfusate, and hepatic outflow perfusate was sampled from the outflow canula (Fig. 1).

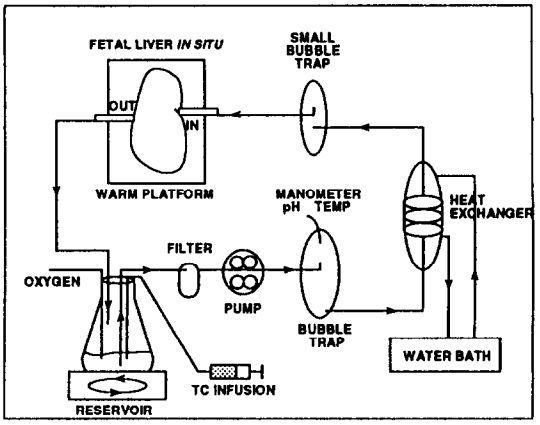

Fig. 1. Diagram of the isolated fetal liver perfusion circuit.

A peristaltic pump (Masterflex No. 7521-25 Cole-Parmer Instrument Co., Chicago, IL, U.S.A.) provided a flow rate of 300 mL/min. This was based on an expected term fetal liver weight of approximately 100 g, requiring a perfusion rate of 3 mL/min/g liver to reflect the *in vivo* fetal hepatic blood flow rate [5]. The perfusate was heated to 37° by recirculating warm water through a glass heat exchanger from a 5 L bath. Humidified oxygen was delivered at a rate of 2 L/min by bubbling it through the perfusate in the reservoir. Perfusate pH was maintained at 7.40 by mixing oxygen/carbon dioxide (95/5) in addition to the oxygen via a T-piece, as required at a rate of 1–2 L/min. TC was delivered at a rate of 4 mL/hr (7.5 mM) into the reservoir using a syringe pump (Sage instruments No. 355 Cambridge, MS, U.S.A.).

Viability and stability

The duration of each liver perfusion was 150 min following an initial 15 min equilibration period. Perfusate temperature and circuit pressure were measured at 15 min intervals. The "perfusion" pressure of the liver was determined by subtracting the circuit pressure obtained without the liver connected from the pressure reading with the liver in place. Oxygen delivery and consumption were calculated at 30 min intervals from 1 mL samples of

liver inflow and outflow perfusate [6] using a blood gas and pH analyser (Instrumentation Laboratory System 1302, Lexington, MA, U.S.A.). Perfusate concentrations of the liver enzymes γ-glutamyl transferase, alanine aminotransferase and lactate dehydrogenase, and aspartate aminotransferase, potassium and sodium concentrations were determined using 4 mL of perfusate taken at 0, 45, 75, 105 and 150 min. The perfusate lactate to pyruvate concentration ratio was determined on 2 mL samples of perfusate collected at 0, 45, 75, 105 and 150 min. Samples (N = 6) were collected into 4 mL of ice-cold 8% perchloric acid solution and stored at 3° until assayed. Pyruvate and lactate concentrations were determined by spectrophotometry using commercially available kits (kit Nos 826-A and 726, Sigma Chemical Co., St Louis, MO, U.S.A.). At the end of each experiment samples of right and left lobe of liver were taken and fixed in 10% formalin solution for histologic examination.

Experimental design

[^{14}C]*TC tracer bolus dose experiments* (N = 5). Standard tracer kinetic measurement techniques were used [7, 8]. After the initial 15 min stabilization period a 40 μL, 2 μCi tracer bolus dose of [^{14}C]TC was added to the reservoir. There was also a background infusion of unlabeled TC into the reservoir at a rate of 30 μmol/hr (4 mL/hr of a 7.5 mM solution). Perfusate samples (1 mL) were taken from the reservoir at 0, 2, 4, 6, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 135 and 150 min. The reservoir volume was maintained by replacing sampled perfusate with an equal volume of pre-warmed taurocholate-free perfusate. All bile was collected via the common bile duct cannula into pre-weighed tubes in 30 min aliquots, and the volume of bile collected was calculated by weight, assuming a specific gravity of 1.0. Perfusate and bile radioactivity was determined by liquid scintillation counting on a Packard 1900CA fluid scintillation analyser [9].

[^{14}C]*TC constant infusion experiments* (N = 3). At the end of the initial 15 min stabilization period a bolus loading dose of 54 μmol (7.2 mL of 7.5 mM [^{14}C]TC, specific activity 30 μCi/mmol) was added to the reservoir. This was followed by a constant infusion of the [^{14}C]TC solution into the reservoir for the next 150 min at 30 μmol/hr (4 mL/hr of 7.5 mM solution). Perfusate and bile samples were collected as in the tracer bolus dose experiments.

Hepatic distribution of umbilical perfusate flow

The proportion of ductus venosus flow shunting through the liver, the relative flows to left and right liver lobes, and the evenness of liver perfusion were studied using 15 μm gadolinium-labeled latex microspheres, which have been extensively used in the fetal lamb for the determination of liver blood flow and distribution [5, 10]. A stock solution of [^{153}Gd]-15 μm microspheres was prepared by diluting 1 mL of original product in 4 mL of saline containing 0.01% Tween 80. At the end of each liver perfusion experiment, 200 μL of this stock solution 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 with perfusate before entering the liver. The perfusate was collected from the outflow cannula, in 50 mL aliquots for 1 min, since all of the microspheres have eluted by this time. The liver was then removed from the carcass, weighed and divided into right, left and caudate lobes, the right and left being further divided into four quadrants each. After weighing, each segment was homogenized and 1 mL aliquots counted using an LKB Wallac 1260 Multigamma counter at an energy level of 85–115 keV. The total counts per lobe was determined from the counts per gram in each sample and the weight of each quadrant. Relative perfusate flow per lobe was assumed equal to relative total counts per lobe. The proportion of perfusate shunted through the ductus venosus was taken as the ratio of total counts in outflow perfusate to the sum of total counts in outflow perfusate and liver tissue. In a single experiment, to assess the evenness of perfusion, the liver was dissected into 1 cm cubes. After weighing, each cube was counted and the counts standardised against weight so that a direct comparison of counts and therefore blood flows, among segments of liver could be made.

Calculations and statistics

In the [^{14}C]TC tracer bolus dose experiments, the half-life of taurocholate disappearance from perfusate ($t_{1/2}$) was calculated from the terminal linear portion of the semilogarithmic perfusate radioactivity (in disintegrations per minute) versus time curve. The area under the perfusate radioactivity versus time curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity [11]. Volume of distribution was calculated as (Dose $\times$ $t_{\frac{1}{2}}$)/(0.693 $\times$ $AUC_{0\to\infty}$) and total clearance (CL) from perfusate as Dose/$AUC_{0\to\infty}$ [11]. Biliary clearance of [^{14}C]TC was calculated as the ratio of cumulative [^{14}C]TC excreted in bile/AUC for the 0–150 min interval. The hepatic extraction ratio (E) was calculated as:

$$E = CL/Q \quad (1)$$

where Q is perfusion flow rate. A corrected E, defined as E^*, was also calculated to take into account shunting of perfusate through the ductus venosus:

$$E^* = CL/[(1 - S)Q] \quad (2)$$

where S is the fraction of perfusate shunted. In the constant infusion experiments, total clearance of [^{14}C]TC from perfusate was calculated as a ratio of the [^{14}C]TC infusion rate/mean steady-state perfusate [^{14}C]TC concentration. Biliary clearance at steady-state was calculated as the ratio of mean biliary excretion rate/mean steady-state perfusate taurocholate concentration. E and E^* were calculated according to equations (1) and (2).

Data are presented as mean and standard deviation throughout unless otherwise specified. Correlations between variables were examined by linear regression analysis. A probability less than 0.05 was considered statistically significant.

RESULTS

Liver viability and stability

Liver appearance was satisfactory in that it

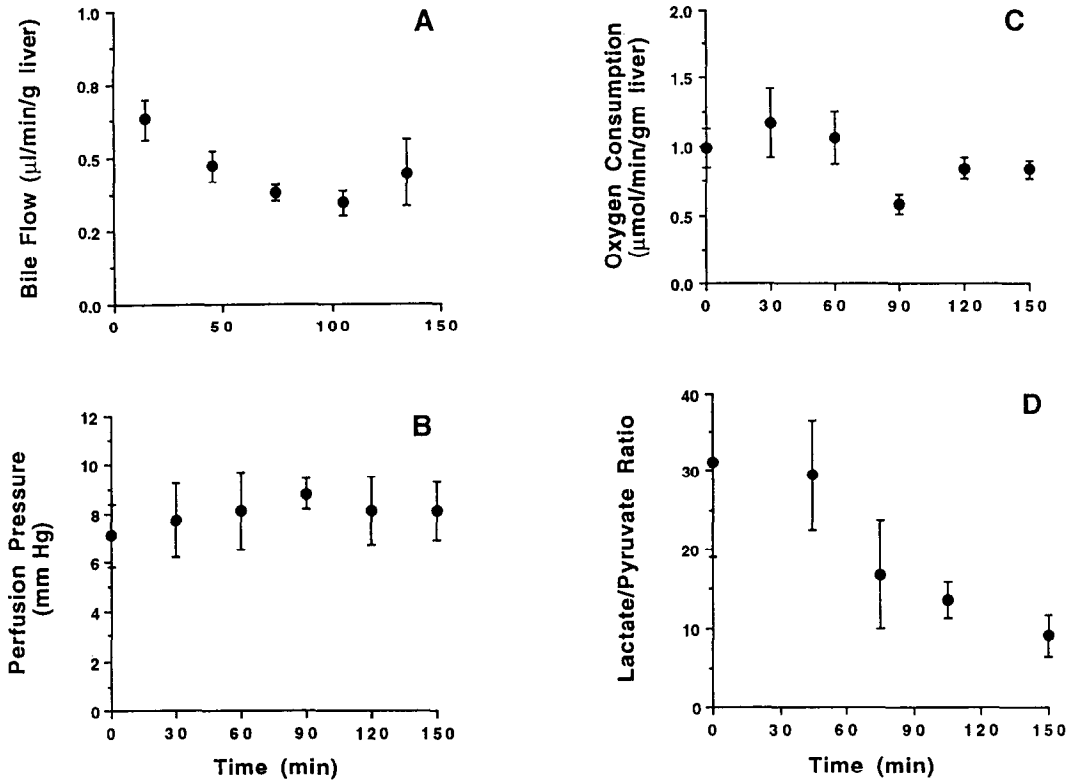

Fig. 2. Mean bile flow rate (A), perfusion pressure (B), hepatic oxygen consumption (C), and perfusate lactate/pyruvate ratio (D), throughout the experimental period in 8 experiments. Mean and standard error shown.

remained a uniform red-brown color, and there was no evidence of hepatic capsule distension or exudation. Light microscopy examination of samples from the perfused livers showed no evidence of hypoxic change; in particular, there was no hepatocyte swelling or vacuolization [12]. The perfusion was shown to be even as indicated by the homogeneous distribution of microspheres between dorsal, middle and ventral portions of liver segments from each lobe. Bile flow decreased slightly from an initial rate of 0.63 ± 0.07 μL/min/g liver to a final rate of 0.50 ± 0.12 μL/g liver/min [$r = 0.37$, $P = 0.03$; Fig. 2(A)]. Mean perfusion pressure was 7.9 ± 0.30 mmHg and did not change significantly throughout the experimental period [$r = 0.11$, $P = 0.5$; Fig. 2(B)]. Mean oxygen delivery was 6.33 ± 2.6 μmol/min/g liver and mean oxygen consumption was 0.86 ± 0.40 μmol/min/g liver. This also did not fall significantly over the experimental period [$r = 0.27$, $P = 0.09$; Fig 2(C)]. Perfusate concentrations of aspartate aminotransferase, alkaline phosphatase and γ-glutamyl transferase, were undetectable and perfusate potassium (4.0 ± 0.5 mmol/L) was low and did not rise during the experiments. The mean ratio of lactate to pyruvate in perfusate was low (19.5 ± 3.0), and did not rise over the perfusion time [$r = -0.36$, $P = 0.05$; Fig. 2(D)], indicating that oxygenation was satisfactory.

Hepatic distribution of umbilical perfusate flow

Recovery of ^{153}Gd-labeled microspheres in the hepatic venous effluent amounted to $35 \pm 18\%$ of microspheres injected in the umbilical vein. This represents the proportion of umbilical blood flow that was shunted through the ductus venosus (Table 1). There was a strong correlation between the size of the ductus venosus shunt and fetal gestational age ($r = 0.94$, $P < 0.001$; Fig. 3), although not between ductus venosus shunt and fetal liver weight. The relative perfusate flows between left and right lobes of the liver were $59 \pm 9\%$ to the left lobe and $41 \pm 9\%$ to the right lobe (Table 1).

Taurocholate elimination

[^{14}C]*TC tracer bolus dose experiments* (N = 5). The disappearance of [^{14}C]TC from perfusate was biphasic in each of the experiments [Fig. 4(A)]. Half-life of the terminal phase was 81.6 ± 25.8 min and volume of distribution was 3.90 ± 2.45 L (Table 2). Clearance of [^{14}C]TC from perfusate was 35 ± 22.6 mL/min (Table 2). This corresponded to an extraction ratio of 0.11 ± 0.06; when calculated to take account of shunted perfusate flow the corrected extraction ratio was 0.29 ± 0.18 (Table 2). The cumulative excretion of [^{14}C]TC into bile is shown in Fig. 4(B). By 150 min, $86.5 \pm 8.72\%$ of the [^{14}C]TC dose had been excreted into bile (Table

Table 1. Distribution of umbilical perfusate flow in fetal liver

Experiment No.	Gestational age (days)	Liver weight (g)			Shunt (%)	Liver flow (%)	
		Total	Left	Right		Left	Right
1	131	46	28	18	49	73	27
2	130	92	47	45	43	61	39
3	135	82	47	35	27	70	30
4	120	75	52	23	66	56	44
5	140	76	45	31	23	49	51
6	140	75	37	38	36	52	48
7	145	78	49	29	16	62	38
8	145	119	61	58	16	52	48
Mean ± SD	136 ± 9	80 ± 20	46 ± 10	35 ± 13	35 ± 18	59 ± 9	41 ± 9

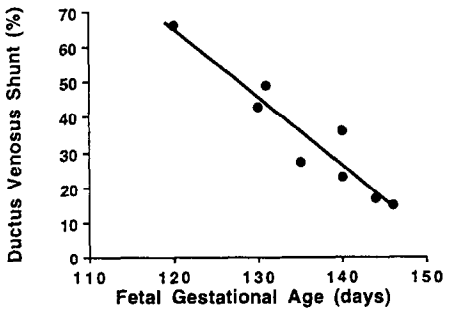

Fig. 3. Correlation between per cent of umbilical perfusate shunted through the ductus venosus and fetal gestational age ($r = 0.94$, $P < 0.001$).

2). Clearance of [^{14}C]TC into bile was 35 ± 19 mL/min, that is, virtually all of the [^{14}C]TC cleared from perfusate by the liver appeared in bile.

[^{14}C]*TC constant infusion experiments* (N = 3). Perfusate and biliary concentrations of [^{14}C]TC during the constant infusion of [^{14}C]TC into the reservoir are shown in Fig. 4(C). Steady-state perfusate concentrations were attained by 30 min in each experiment. Total perfusate clearance, biliary clearance, extraction ratio and shunt corrected extraction ratio were similar to values obtained with bolus experiments (Table 2). The mean ratio of taurocholate concentration in bile to that in perfusate was 856 ± 298.

DISCUSSION

Investigation of fetal hepatic function using *in vivo* animal preparations presents many difficulties. It may not be possible to control or measure key physiological variables such as hepatic blood flow or oxygenation, and interpretation of results is often confounded by the presence of placental transfer and the synthetic or elimination processes in the mother. Effects of other fetal organs may also need to be taken into account. The use of an isolated liver preparation in species such as the rat and rabbit has yielded much useful information about the functions

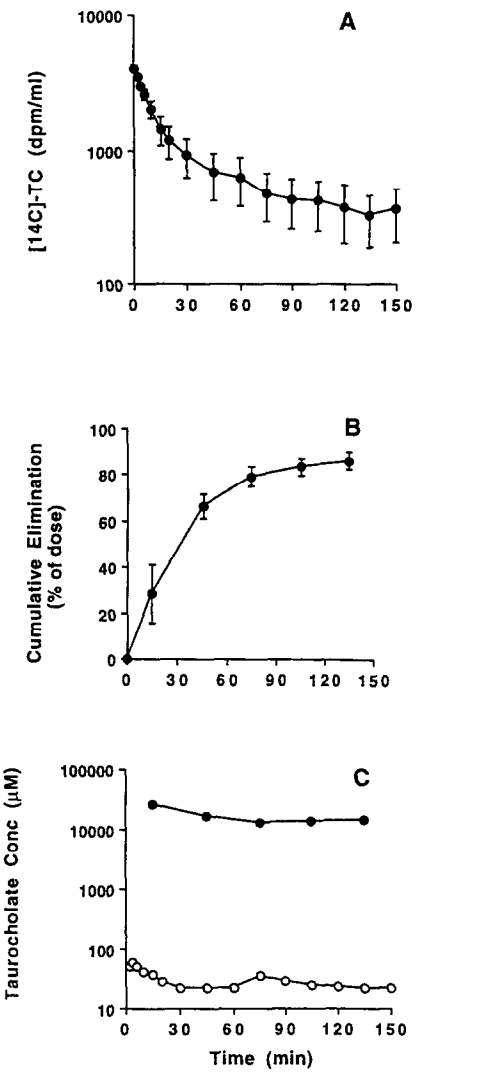

Fig. 4. Mean perfusate [^{14}C]TC d.p.m./mL vs time curve (A) and cumulative [^{14}C]TC excretion into bile (B) in bolus [^{14}C]TC tracer dose experiments, and mean perfusate (○) and biliary (●) taurocholate concentrations in [^{14}C]TC constant infusion experiments (C). Mean and standard error shown.

Table 2. Kinetics of taurocholate elimination

Experiment No.	Half-life (min)	Volume of distribution (L)	Clearance (mL/min)	Clearance into bile (mL/min)	Cumulative biliary excretion at 150 min	E	E^*
Bolus experiments							
1	106.0	1.95	17.7	16.7	94.2	0.042	0.086
2	85.1	4.11	33.5	40.0	84.4	0.112	0.260
3	37.7	1.17	21.5	15.4	72.3	0.071	0.263
4	90.4	7.30	67.4	61.4	90.1	0.189	0.286
5	88.5	4.98	48.7	44.0	91.6	0.132	0.574
Mean ± SD	81.6 ± 25.8	3.90 ± 2.45	35.0 ± 22.6	35.5 ± 19.5	86.5 ± 8.72	0.109 ± 0.056	0.294 ± 0.176
Infusion experiments							
6	NA	NA	11.6	9.80	NA	0.0388	0.0606
7	NA	NA	35.7	28.9	NA	0.119	0.142
8	NA	NA	41.6	42.1	NA	0.139	0.165
Mean ± SD	NA	NA	29.6 ± 15.9	26.9 ± 16.2	NA	0.0989 ± 0.0530	0.122 ± 0.0548
Overall Mean ± SD	NA	NA	34.7 ± 18.2	32.7 ± 17.7	NA	0.105 ± 0.0517	0.229 ± 0.163

NA, not applicable.

of the adult liver [13]. We have therefore developed an isolated perfused fetal sheep liver preparation which overcomes many of the difficulties associated with *in vivo* fetal studies. We chose the sheep because of the convenient size of the fetal sheep liver, the close anatomical and vascular similarity between sheep and human fetal livers [3, 5], and the substantial database that exists concerning hepatic physiology and pharmacology in pregnant sheep from previous *in vivo* studies in our laboratory and elsewhere [4, 5].

We used a perfusion flow rate of 300 mL/min on the basis that the fetal liver weight near term would be 100 g on average. This would yield a flow rate of 3 mL/min/g liver which is comparable to umbilical vein blood flow *in vivo* [5]. In fact the flow rates achieved were between 2.46 and 6.72 mL/min/g liver (mean value = 3.94 mL/min/g liver), which are within the range of values seen *in vivo* [5]. Perfusion pressure remained stable throughout the experiments [Fig. 2(B)], and the mean perfusion pressure of 7.9 ± 0.3 mmHg was comparable to values of fetal hepatic perfusion pressure of 7 ± 4 mmHg reported for sheep *in vivo* [5]. Maintenance of physiologic perfusion pressures in our preparation is important because artificially elevated perfusion pressures lead to increased sinusoidal permeability and could cause abnormally increased substrate access to the space of Disse and subsequent abnormal hepatic uptake of test substrate [14]. Additionally, high perfusion pressures damage the liver through barotrauma. A rising pressure can also indicate hepatic injury, as hypoxic hepatocytes swell and occlude the vascular spaces [13].

Mean hepatic oxygen delivery of 6.33 ± 2.6 μmol/min/g liver was well in excess of mean oxygen consumption of 0.86 ± 0.07 μmol/min/g liver, indicating that oxygen delivery was not rate-limiting in this preparation. This rate of oxygen consumption is somewhat lower than the 1.7 μmol/min/g liver observed in fetal sheep *in vivo* [3, 15], but is quite close to values obtained from adult isolated perfused sheep liver [16] of 1.2 μmol/min/g liver, and did remain stable throughout the experiments [Fig. 2(C)]. Previous work by us has demonstrated that the lactate/pyruvate concentration ratio in perfusate is a sensitive indicator of hepatic tissue hypoxia. In the isolated perfused adult rat liver preparation a two-thirds reduction in oxygen consumption induced by hypoxia caused a 10-fold increase in lactate/pyruvate ratio from a baseline value of 13.6 ± 9.2 [6]. The mean value in the present study was 20.1 ± 17.1 and fell from an initial ratio of 31.1 ± 12.1 to a final value of 9.1 ± 2.7 [Fig. 2(D)], and indicates that oxygenation of the fetal liver was adequate in this current study. The initial higher ratio was due to increased lactate levels present after flushing the liver with Hartman's compound lactate solution during surgery while the isolated preparation was being established.

Mean bile flow rate (0.48 ± 0.10 μmol/min/g liver) was comparable to values reported previously for the adult sheep *in vivo* [17] and in an isolated perfused adult sheep liver model [16], and remained stable throughout the experiments. Potassium was stable throughout, indicating that there was minimal cell damage. Perfusate γ-glutamyl transferase, aspartate aminotransferase and lactate dehydrogenase were undetectable; however, they may be as insensitive a marker of hepatic damage in the fetal sheep, as alanine aminotransferase is in the neonatal rat where hepatic levels are very low [18]. Overall, the indices of viability and stability measured support the acceptability of this experimental preparation for the study of fetal hepatic function.

The distribution of umbilical perfusate to right

and left hepatic lobes was very variable between experiments (0.7–2.4 mL/min/g liver left lobe; 0.6–1.5 mL/min/g liver right lobe). The lobular distribution of flow did not correlate with relative lobular weights, although on average the relative lobular distribution of umbilical flow was similar to relative lobular weights (Table 1). This is not an important consideration in the current experiments but demonstrates the need to measure the perfusate distribution between right and left liver lobes with microspheres in all experiments when investigating for differences in extraction of compounds between liver lobes, rather than assuming perfusate flow distribution based on relative lobe weights only.

Shunting of umbilical perfusate through the ductus venosus was highly variable, ranging from 16 to 66% (Table 1). Similarly wide ranges have been reported previously in acute *in vivo* experiments in fetal sheep (34–91%) [19] and in the human fetus (8–92%) [20]. In our experiments, in which gestational age ranged from 120 to 145 days, there was a strong negative correlation between ductus venosus shunting and gestational age (Fig. 3). In contrast to our findings, a previous study using fetal lambs *in utero* found no correlation between ductus venosus flow and gestational age [5]. This difference may relate to the difficulties of using microspheres to calculate organ blood flow rates *in vivo*, whereas in the isolated organ the calculation of liver blood flow using microspheres is simple and direct. The decrease in shunting that we observed with increasing gestational age (Fig. 3) is not likely to be due to acute changes caused by "extra-hepatic" variables. In the isolated perfused preparation such variables are either controlled (e.g. hepatic oxygen delivery) or eliminated (e.g. circulating vasoactive substances, autonomic innervation). The age-dependent change in function is more likely due to diminishing ductus venosus size or to changes in hepatic vascular resistance in the maturing fetus.

The role of the ductus venosus in controlling umbilical blood flow and distribution of umbilical blood *in vivo*, although not well understood, is thought to be minimal. Investigators have suggested that the ductus venosus is an important determinant of umbilical venous return and liver perfusion through its function as a sphincter, either under neurologic or hormonal control, acting to regulate blood flow in response to changing fetal requirements [21]. Subsequent work failed to support this hypothesis, and the ductus venosus was thought to react passively, secondary to changes in umbilical blood flow [5]. Recent *in vitro* work using ductus venosus tissue has shown that given acutely, prostaglandin synthesis inhibitors and prostaglandins PGE_2 and PGI_2 act to decrease and increase ductus venosus diameter, respectively. The relevance *in vivo* of these acute changes in ductus venosus diameter on umbilical venous return, hepatic perfusion and ductus venosus blood flow has not been determined. Currently it is thought that the major site of vascular resistance to umbilical venous return *in vivo* is in the umbilical vein and not in the ductus venosus [21].

In the [^{14}C]TC tracer bolus dose experiments approximately 86% of the administered dose of [^{14}C]TC had been excreted in bile by 150 min (Table 2). This is virtually identical to the biliary recovery of radiolabeled TC in the fetal sheep *in vivo*, where 88% of the dose was excreted in the same time [22]. In the [^{14}C]TC constant infusion experiments there was a large gradient of over 800 to 1 between TC concentration in bile and perfusate [Fig. 4(C)]. This is similar to the bile/plasma concentration ratio of taurocholate in the adult human *in vivo* where the ratio has been observed to range from 130:1 to 2000:1 while in the rat a ratio range of 160:1 to 600:1 has been found [23]. To our knowledge no similar data are available for the fetal or adult sheep *in vivo*. Thus the mechanisms for the concentrative excretion of bile salts into bile appear to be well developed in the near term fetal sheep as they are in the near term fetal dog [24, 25], but not in the near term fetal primate [26].

To characterise further fetal hepatic TC transport, clearance data are required. Fetal hepatic clearance data for bile salts have not previously been reported, due probably to difficulty gaining access to fetal hepatic blood vessels *in vivo*. In our perfused fetal liver preparation we were able to measure TC clearance directly and obtained a mean value for all experiments of 34.7 ± 18.2 mL/min (Table 2). This corresponds to a hepatic extraction ratio of 0.11 ± 0.05 which, corrected for perfusate shunted through the ductus venosus, yields a value of 0.23 ± 0.16 (Table 2). The hepatic extraction ratio of TC by the adult sheep is not known but in adults of all other mammalian species studied the hepatic extraction ratio is high (e.g. >0.85 in the rat, [27, 28]). Therefore, although the sheep fetal liver can effectively concentrate TC in bile, the low extraction of TC from perfusate indicates a transport process of low efficiency by comparison with that seen in the adult, and in that sense the fetal liver can be considered to be relatively immature.

In conclusion, we have developed a viable and physiologically stable isolated perfused fetal sheep liver preparation, which demonstrates a number of advantages over *in vivo* models. With this preparation we have been able to show that although the fetal liver can concentrate TC in bile, the efficiency of biliary TC uptake and elimination is appreciably less than in the adult.

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