Obstruction of Ductus Venosus Stimulates Cell Proliferation in Organs of Fetal Sheep

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In growth restricted fetuses, hepatic blood flow is reduced. This suggests the hypothesis that liver blood flow controls fetal growth. In 11 near term sheep the ductus venosus was blocked with an embolization coil in one fetus (experimental) and left patent in the twin (control). Arterial catheters were placed in both fetuses. After termination [mean (s.d.) 5 days (2) after surgery] the fetal body and organs were weighed. The cell proliferation rate (pKi-67) was determined in tissue samples of the liver, heart, skeletal muscle, kidneys and placenta (n=6). Blood flow through the umbilical vein measured by Doppler ultrasound did not differ in control and experimental fetuses [experimental: 600 (101) ml/min; control: 626 (89) ml/min]. In experimental fetuses, blood flow through the ductus venosus was negligible (colour Doppler), and thus hepatic blood flow was increased. Absolute and relative (percentage of body weight) liver weights were increased in experimental fetuses [liver weight: 119 (34) g versus 84 (17) g; relative liver weight: 4.3 (0.8) per cent versus 3.4 (0.8) per cent; P=0.002, n=11]. The cell proliferation rate was increased significantly (twofold) in heart muscle, skeletal muscle and kidneys, and sixfold in liver. It is concluded that increases of hepatic blood flow stimulate cell proliferation in major organs of the ovine fetus.

INTRODUCTION

Fetal growth is determined mainly by genetically programmed production of growth and differentiation factors, and by the availability of nutrients to the fetus. There is evidence that insulin-like growth factors and their binding proteins play a major role in fetal growth (Lok et al., 1996; Han, 1996; Somerset et al., 1997; van Kieffens et al., 1998), but the question of how fetal development is controlled and regulated remains largely unanswered. Whatever the mechanism, it will become challenged in cases of intrauterine growth restriction (IUGR) when fetal growth has to be matched to the restricted supply. In clinical observations we found (Tchirikov et al., 1998a) that hepatic blood flow rate is reduced in IUGR fetuses, and speculated that fetal growth restriction may be causally related to low liver perfusion. Reduction of hepatic flow is thus thought to be instrumental for the adaptation of fetal demands to impaired supply. In more general terms, the concept implies that, in the fetus, hepatic blood flow controls fetal growth. The unique parallel arrangement of feeding liver veins with the ductus venosus provides a mechanism for the control of liver blood flow: the perfusion of liver tissues depends on the relative resistances to blood flow of the ductus venosus and liver veins. The resistance ratio determines the respective blood flow through these vessels. It has been demonstrated that blood flow rates can indeed be regulated in this manner. In acute hypoxia, for example, the proportion of blood that returns from the placenta and passes through the ductus venosus is increased because the resistance increase following the rise of circulating catecholamine concentrations is larger in liver vessels than in the ductus (Paulick et al., 1991).

In this report we test the hypothesis that increased hepatic blood flow stimulates the proliferation of fetal tissues. We developed a technique to block the ductus venosus in fetal sheep for several days (Tchirikov and Schröder, 1998) and thereby divert all blood flow returning from the placenta to the liver. This increases hepatic blood flow (Rudolph et al., 1991), and the effects on fetal tissue proliferation can be studied.

MATERIALS AND METHODS

Procedures

Surgical and experimental procedures were approved by the board on animal studies of the ‘Behörde für Arbeit, Gesundheit und Soziales’ of the state of Hamburg, Germany. Studies were carried out in ewes at a gestational age of 125 (4) days (term 145 days). The ewes were sedated with xylazine i.m. (Rompun®; 0.25 mg/kg) and were then anaesthetized with an
intravenous thiopental–sodium (Trapanal®, 1.0 g) followed by ventilation with 1.0–1.5 per cent isoflurane in O₂/N₂O (2 : 1). Group I of experimental animals included six ewes (five twins, one triplet). The maternal abdomen was opened under aseptic conditions. An incision was made in the uterus and the head and neck of one fetus were delivered. A catheter (5 F HNB-5.0-38-45-PW-NS-MPA, Cook®) and guide wire were passed through a catheter port (RCFW, Cook®), inserted into the right external jugular vein of the fetus, and advanced through the right atrium and ductus venosus into the intrahepatic umbilical vein with ultrasound B-scan imaging control (ATL Apogee 800® or Acuson Aspen®). A Jackson embolization coil (MWCE 38-4-4, Cook®) was then placed so as to occlude the ductus venosus (experimental fetus). The decrease of ductal flow to near zero was confirmed by Doppler ultrasound, which was also used to measure umbilical vein blood flow rate during surgery (Tchirikov et al., 1998b). The insertion catheter was removed and replaced by a short chronic venous catheter. Additional catheters were placed in the right carotid artery and in the amniotic cavity. The fetal incisions were closed and the head was returned into the uterus which was then closed.

The head and neck of the twin fetus were exposed through a second uterotomy. Catheters were inserted into the right external jugular vein and the carotid artery as described above, while the ductus was left patent. The fetal wounds were closed, the head was returned to the uterine cavity and the uterine wall sutured. This fetus with unobstructed ductus served as a control. All catheters were led to the left flank of the ewe and stored in a pouch attached to the maternal skin.

Postoperatively catheters were flushed daily with heparinized saline (1000 U/ml). Antibiotics were given to the ewe (cefalozolin–sodium, Elzogram®, 1 g/day i.v.) and to the fetuses via the amniotic fluid (1 g/day). Blood gases were determined daily from carotid arterial blood samples (Radiometer Copenhagen ABL 30). Blood pressure, heart rate and Doppler ultrasound signals were measured at days 1 and 4, and later if possible. The experimental protocol specified a fetal survival time of 1 week after surgery (Tchirikov and Schröder, 1998), but when blood gas analysis indicated impending death of one fetus, the experiment was terminated. One experiment lasted until day 2, two until day 4 and three until days 6 and 7, respectively.

**Recording of haemodynamic variables**

On the morning of each experimental day, arterial blood pressure, amniotic fluid pressure and fetal heart rate were recorded (Ohmeda P23 XL transducers with Gould amplifiers) for 30 min with a polygraph system (Servogor® 462, Metrawatt). Using B-scan imaging, the position of the embolization coil was determined. Blood flow rates in the intra-abdominal umbilical vein of control and experimental fetuses were determined using Doppler ultrasound. It was verified using colour Doppler that blood flow through the ductus of experimental fetuses remained negligible.

**Termination of experiments**

The experiment was terminated by injection of a proprietary euthanasia solution T61® (Hoechst). Autopsies were performed on both twin fetuses in parallel. The fetuses were towel-dried and weighed. The weights of liver, heart and kidneys were determined. Tissue samples from the liver (left and right lobe), heart, kidneys, quadriceps femoris and from randomly selected cotyledons of the placenta were placed in 3.5 per cent formaldehyde for 24 h and then embedded in paraffin in preparation for immunohistochemistry.

**Immunohistochemistry of the proliferation marker pKi-67**

The antibody Mib-1 is directed against the nuclear antigen pKi-67 (Cattoretti et al., 1992) which is expressed in all phases of the cell cycle except G₀. Mib-1 can be stained in paraffin-embedded sections following microwave treatment (Shi et al., 1998). Thus cell nuclei involved in mitotic activities can be detected in histological sections.

Paraffin sections from all blocks were prepared for Mib-1 immunohistochemistry. Antibody binding was detected using a streptavidin–biotin–peroxidase sequence (Frank et al., 1994) with 3-amino-9-ethyl-carbazol as chromogen. Endogenous peroxidase was blocked with 1 per cent H₂O₂ in absolute methanol for 10 min at room temperature. For negative controls the first antibody was replaced by phosphate buffered saline supplemented with bovine serum albumin (pH 7.4). No control sections revealed any immunostaining.

**Detection of apoptotic cells**

The TUNEL assay was used in paraffin sections of liver tissues to detect apoptotic nuclei in which DNA fragmentation had occurred. Because the assay may result in false positive reactions (Yasuda et al., 1995), in a pilot study we compared TUNEL positivity with changes in nuclear morphology which are typical for apoptosis (nuclear shrinkage and annular chromatin condensation; results not shown). From a variety of kits tested, the results obtained with the ApopDETEK kit (Enzo Diagnostics, Farmingdale, NY, USA) matched the distribution of nuclei with apoptotic morphology. Samples were treated according to the manufacturer’s protocol.

**Quantitative evaluation**

As an index of proliferative activity the number of Mib-1 positive cell nuclei per square micron was determined. Measurements were performed automatically on a
Table 1. Proliferation index of fetal tissues. Density of nuclei (number of nuclei \( \mu m^2 \times 10^{-4} \)) stained with Mib-1 which marks proliferation associated antigen pKi-67. Ratio is ratio of nuclei density in experimental (ductus obstructed) fetuses divided by nuclei density in control fetus (ductus patent). Means (s.d.) of six (kidney: five) animals. Densities are significantly different between both groups (Mann–Whitney \( U \)-test) as indicated in column of \( P \)-values.

<table>
<thead>
<tr>
<th></th>
<th>Obstructed</th>
<th>Patent</th>
<th>( P )-value</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>59.9 (14.2)</td>
<td>10.1 (1.2)</td>
<td>0.004</td>
<td>5.9</td>
</tr>
<tr>
<td>Heart muscle</td>
<td>2.4 (1.3)</td>
<td>1.0 (0.6)</td>
<td>0.025</td>
<td>2.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.7 (1.0)</td>
<td>1.5 (0.3)</td>
<td>0.009</td>
<td>1.9</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.7 (0.3)</td>
<td>0.3 (0.2)</td>
<td>0.028</td>
<td>2.3</td>
</tr>
<tr>
<td>Placenta</td>
<td>1.1 (0.6)</td>
<td>2.4 (1.3)</td>
<td>0.016</td>
<td>0.4</td>
</tr>
</tbody>
</table>

C.A.S.T.-Grid system (Olympus, Denmark) which scanned a random set of microscope fields without human interference or bias and counted all stained nuclei. The calculations are based on data from the complete section area, from the area of each microscope field, the number of microscopes fields and the number of counted nuclei (Schmitz et al., 1999).

The rate of apoptosis was determined (TUNEL test) in tissues of the right and left liver lobe as number of apoptotic nuclei per square micron.

Nuclear volumes were determined in all sections, and cell volumes of liver, heart and skeletal muscles were estimated using the point sampled intercept method (Gundersen and Jensen, 1985).

Other experiments

In group II, five ewes (three twins, one triplet; one singleton) of the same gestational age as group I were instrumented as described above. The experiments were terminated 2–7 days after surgery, and from these animals only body and liver weights were determined.

Statistics

Data are presented as means (s.d.). The non-parametric Mann–Whitney \( U \)-test was used to determine the significance of differences between proliferation rates (Table 1). Otherwise, the unpaired \( t \)-test was applied to establish differences (e.g. liver weights) unless specified differently. Calculations were carried out using Statistics R software (Statsoft, Tulsa, USA). \( P<0.05 \) was considered statistically significant.

RESULTS

In both groups, the experiments were terminated after 2–7 days [5 (2) days]. Table 1 provides summary data on nuclear density (number of nuclei per square micron) in the different organs of experimental group I. The proliferation rate of the liver was markedly and significantly elevated when the ductus venosus was obstructed, as were the proliferation rates in heart and skeletal muscle, and in the kidneys. In contrast, proliferation of the placenta was significantly depressed in fetuses in which an embolization coil had been placed. Typical histological results are shown in Figure 1. The proliferating cells were hepatocytes and hematopoietic cells in the liver, striated muscle cells and endothelial cells in heart and skeletal muscle, and mainly tubular cells in kidneys. In the placenta, the fetal uninucleate cuboidal epithelial cells (trophoblast cells) were those affected. There were no signs of oedema or blood congestion detectable in any tissue, and there were no differences in proliferation rate between the left and right liver lobe.

The number of apoptotic nuclei per square micron in the liver was 6.6 (0.74) \( 10^{-5} \) (left lobe) and 6.7 (0.55) \( 10^{-5} \) (right lobe) in controls and 7.1 (0.44) \( 10^{-5} \) (left lobe) and 7.2 (0.26) \( 10^{-5} \) (right lobe) in experimental fetuses with no significant differences between the lobes or between the experimental and control fetuses.

The determination of nuclear density could depend to some extent on nuclear volumes because of the increased likelihood of finding a large nucleus in tissue sections. Nuclear volumes differed in the tissues of the various organs. Mean volumes in the liver, for example, were 281 (3) \( \mu m^3 \); whereas in the skeletal muscle they were 100 (2) \( \mu m^3 \). Nuclear volumes in a particular type of tissue, however, varied less than 4 per cent between control and experimental fetuses. Thus the difference in nuclear density between tissues of control and experimental animals is not influenced by variations in nuclear volumes. In contrast, mean cell volumes were significantly (\( t \)-test) reduced in the liver (by 4 per cent, hepatocytes), in the heart (by 19 per cent, cardiomyocytes) and skeletal muscle (by 14 per cent, myocytes) of fetuses with obstructed ductus venosus. In the liver, these changes indicate cellular hyperplasia because liver weight was increased also.

In groups I and II combined, absolute and relative liver weights were significantly increased in fetuses (\( n=11 \)) with obstructed ductus venosus [liver weight: 119 (34) g and 84 (17) g in controls, \( P=0.002 \); relative liver weight (percentage of body weight): 4.3 (0.8) per cent and 3.4 (0.8) per cent in controls, \( P=0.002 \); body weight: 2746 (528) g and 2507 (326) g, \( P=0.15 \)]. In group I (six animals), fetal body weights did not differ in experimental [2542 (437) g] and control [2542 (222) g] fetuses. The average absolute [103 (23) g and 88 (12) g] and relative liver weights [4.0 (0.46) per cent and 3.5 (0.67) per cent] were larger in fetuses with obstructed ductus venosus, but the differences did not reach statistical significance. Heart and kidney weights were not different.

When measured during surgery, umbilical venous blood flow averaged 618 (117) ml/min before occlusion of the ductus venosus. It decreased to 388 (64) ml/min (\( n=6 \)) immediately after occlusion of the ductus (paired \( t \)-test, \( P<0.005 \)), but then during the first postoperative day returned to baseline levels, as shown in Table 2. It remained at baseline levels until termination of the experiments. Ductus venosus flow, which in fetal sheep is normally (Tchirikov et al., 1998b) about 40 per cent of
Figure 1. Comparison of proliferative activity detected by the monoclonal antibody Mib-1 in tissue samples from experimental (left column) and control fetuses (right column). The sections shown were topographically matched. Control (LC, HC, MC, KC, PC) and experimental tissues (L: liver, H: heart, M: skeletal muscle, K: kidney, P: placenta) are each from one pair of twins. Liver and placenta sections are from the same experiment, the other tissues are from three different experiments. Tissue is counter-stained with light green, and photographed with a blue filter and Nemeski contrast. Bar is 50 μm.
remained unchanged in the 11 fetuses. These changes were not statistically significant as judged by colour Doppler ultrasound. The position of the coil (B-scan) was confirmed daily and remained unchanged throughout the experimental period as judged by ultrasound appearances.

Fetal umbilical venous blood flow, decreased to near zero immediately after deposition of the embolization coil. It remained negligible throughout the experimental period as judged by colour Doppler ultrasound. The position of the coil (B-scan) remained unchanged in the 11 fetuses. These flow measurements confirm that blood flow supplied from the placenta to the liver had nearly doubled. Portal blood flow could not be measured.

Table 2 provides average values for blood gases and haemodynamic findings for experimental days 1 and 4. The values did not differ significantly between experimental and control fetuses.

### DISCUSSION

Fetal blood returning from the placenta divides to take one of two alternate routes. It either passes through the substance of the liver, where it provides approximately 75 per cent of total liver inflow (Rudolph, 1983) with the portal vein and hepatic artery supplying the remainder, or bypasses hepatic tissues by shunting through the ductus venosus. The partitioning of blood between these routes is altered in human fetuses that are growth restricted (Tchirikov et al., 1998a) and in ovine fetuses with reduced oxygen supply (Reuss and Rudolph, 1980; Tchirikov et al., 1998b). In both these circumstances hepatic flow is diminished while ductal flow is maintained or increased. This circulatory rearrangement effectively permits more of the blood returning from the placenta to flow directly to the fetal heart and brain. This is likely to be a useful adaptation for fetal survival because oxygen supply to the myocardium and brain will be maintained. It is our hypothesis that over the longer term the reduction of liver blood flow limits liver growth and also the growth of other fetal organs. Because reduction of fetal mass will reduce fetal nutritional requirements, this downregulation of growth will help the fetus to survive when placental exchange is indeed insufficient. However, the adaptation results in IUGR and this is not without risk. Intrauterine growth restriction is still one of the leading causes of fetal perinatal morbidity and death in the developed countries (Brar and Rutherford, 1988). Except for premature termination of pregnancy which increases the risk for perinatal death (Allen, Donohue and Dusman, 1993) and for neurological, behavioural and cognitive disturbances later in life (Dammann et al., 1996; Stewart et al., 1999), in most cases no rationale for treatment is available.

We hypothesize that there may be forms of IUGR which are not exclusively generated by insufficient supply of the fetus. Growth restriction could rather be caused by inappropriate regulation, for example, of liver blood flow rate. The adaptation, once initiated to defend the fetus, may stay active even when the initial cause of IUGR has vanished (Mellor, 1983), or it may be inappropriately activated from the beginning in spite of sufficient fetal supply. Causal treatment of these IUGR fetuses should be possible by affecting the misadjusted regulatory mechanism though, admittedly, at present there is no way to detect this (hypothetical) form of IUGR.

The results of this study demonstrate that long-term blockage of the ductus venosus in fetal sheep significantly increases the proliferation rate in liver, heart, kidneys and skeletal muscle tissues and decreases the proliferation rate of cotyledons (placenta). Complete occlusion of the ductus was followed by an increase of liver weight by nearly 40 per cent compared to twin control fetuses with patent ductus. Because the rates of apoptosis between experimental and control fetuses were not different, changes of programmed cell death are not involved. It is unlikely that intracellular or extracellular oedema contributes to the increase of liver weight because cell volumes were not increased, and the number of cells per unit area was nearly identical in the liver of control and experimental fetuses (results not shown). The increase of liver weight was measured after 2–7 days and was thus quite rapid. Similar rapid gain of hepatic mass is seen in adults during regeneration following partial resection of the liver (Ankoma-Sey, 1999).

### Table 2. Fetal blood gas values and haemodynamics. Arterial blood gas values and haemodynamic variables of fetal sheep with obstructed (obstr.) and open (patent) ductus venosus at day 1 and 4 after surgery. Means (s.d.).

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 4</th>
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<tr>
<td></td>
<td>Obstr. (n=6)</td>
<td>Patent (n=6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.37 (0.02)</td>
<td>7.39 (0.02)</td>
</tr>
<tr>
<td>P(O2) (mmHg)</td>
<td>14.6 (6.9)</td>
<td>17.3 (6.6)</td>
</tr>
<tr>
<td>P(CO2) (mmHg)</td>
<td>46.5 (4.4)</td>
<td>44.9 (4.1)</td>
</tr>
<tr>
<td>BE (mmol/l)</td>
<td>1.2 (2.2)</td>
<td>2.0 (2.4)</td>
</tr>
<tr>
<td>FHR (bpm)</td>
<td>148 (35)</td>
<td>149 (37)</td>
</tr>
<tr>
<td>FAP (mmHg)</td>
<td>46.0 (7.9)</td>
<td>45.3 (8.3)</td>
</tr>
<tr>
<td>UV flow (ml/min)</td>
<td>536 (125)</td>
<td>583 (78)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Obstr. (n=4)</td>
<td>Patent (n=5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.31 (0.04)</td>
<td>7.35 (0.05)</td>
</tr>
<tr>
<td>P(O2) (mmHg)</td>
<td>14.6 (2.8)</td>
<td>16.2 (3.8)</td>
</tr>
<tr>
<td>P(CO2) (mmHg)</td>
<td>48.4 (4.5)</td>
<td>45.1 (4.5)</td>
</tr>
<tr>
<td>BE (mmol/l)</td>
<td>–2.2 (1.6)</td>
<td>–1.4 (1.4)</td>
</tr>
<tr>
<td>FHR (bpm)</td>
<td>198 (8)</td>
<td>176 (38)</td>
</tr>
<tr>
<td>FAP (mmHg)</td>
<td>48.8 (13)</td>
<td>44.8 (7.3)</td>
</tr>
<tr>
<td>UV flow (ml/min)</td>
<td>600 (101)</td>
<td>626 (89)</td>
</tr>
</tbody>
</table>
After occlusion of the ductus venosus with an embolization coil, the Doppler measurements indicated that flow through the ductus venosus decreased to negligible levels and remained near zero during the postoperative period. Umbilical venous flow remained stable at baseline levels during the experimental period. Because all blood flow returning from the placenta must pass through the ductus or through hepatic vessels, occlusion of the ductus must have effectively decreased hepatic flow from the umbilical vein by nearly twofold (Rudolph et al., 1999). These results permitted one to conclude that increases of liver blood flow directly stimulate proliferation and growth of the liver, and also stimulate proliferation of the fetal heart, kidneys and skeletal muscle.

Some of the pathways by which fetal growth (Han, 1996; Somerset et al., 1997; van Kleffens et al., 1998) may be stimulated are summarized in Figure 2, which serves as our present working model. Increased blood flow through the liver, acting via increased shear stress, stimulates the intrahepatic release of growth factors (e.g. insulin-like growth factor-I and -II). In addition, there may be alterations in the synthesis and secretion of growth factor binding proteins. Growth factors will act to stimulate proliferation in the liver, and they also will spill over into the circulation (Sjögren et al., 1999) to promote the proliferation observed in the heart, kidneys and muscle. Prominent among these factors are hepatic growth factor I (Lok et al., 1996) and epidermal growth factor (see below). A large variety of other mitogens, co-mitogens (for example norepinephrine) and growth-inhibitory factors may also be involved (Clemmons, 1998; Ankoma-Sey, 1999). As depicted in Figure 2, it is also conceivable that the liver metabolism of proteins, carbohydrates and lipids contributes to the substrate supply for growth of other fetal organs.

The failure of the heart, kidneys and skeletal muscle to gain weight despite clear increases in proliferation suggests that these organs could not respond sufficiently to growth stimuli. This inference is supported by the reduced cell volumes especially of cardiomyocytes and myocytes of experimental fetuses in conjunction with increased proliferation rates. After occlusion of the ductus the liver becomes totally privileged and other fetal organs receive only oxygen and metabolic fuel which hepatic cells do not remove. Whether increase of liver supply by increase of liver perfusion does indeed raise liver consumption, remains to be established.

Blocking the ductus venosus is a major experimental intervention which will have other effects besides increases of hepatic blood flow, and the unexpected high rate of experiments which did not last for the projected 7 days indicate the stress of twin surgery. Table 2 suggests that the fetuses were in a chronic hypoxic condition. However, oxygen levels and other measured variables were statistically indistinguishable in experimental and control fetuses. Previous reports have shown that chronic hypoxia in fetal sheep is more likely to reduce

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**Figure 2.** Working model of fetal growth stimulation following obstruction of the ductus venosus. This increases blood flow rate through the hepatic sinusoids, and it affects upstream tissues which are known to produce growth factors such as insulin-like growth factors (IGF) and their binding proteins (IGFBPs) as well as epithelial growth factor (EGF) and insulin. These factors enter liver tissues and influence hepatocytes. They may also pass to other fetal organs. Increased hepatic flow will increase shear stress in endothelial cells and stimulate generation of nitric oxide (NO) and prostaglandins (PGs). These substances affect some liver cells to increase hepatocyte growth factor (HGF) production which in turn stimulates hepatocytes to produce IGF and affect the production of IGF BPs. The growth factors may act in a paracrine or autocrine fashion inside the liver tissues, and they spill over into the sinusoidal blood stream to increase cell proliferation in other fetal organs. Their growth may be influenced also by changes of hepatic protein, lipid and carbohydrate metabolism. Norepinephrine (NE) is known as a co-mitogen.
DNA synthesis (Hooper et al., 1991; Gagnon et al., 1995; Asano et al., 1997) and cell proliferation than to increase it, and thus proliferation occurred in experimental fetuses despite chronic hypoxia.

Another event associated with ductal obstruction is a rise of venous pressure (1–2 mm Hg) upstream of the occlusion (Rudolph et al., 1991). This increase may reduce portal venous inflow into the liver and affect tissues that drain into the portal vein. Some of these tissues are known to excrete growth factors (Ankoma-Sey, 1999) (e.g. epithelial growth factor by Brunner’s glands in the duodenum), and thus their concentration in portal blood may have increased. These increases of venous pressure may also be associated with the decrease of proliferation rate in the placenta which at present evades explanation.

In conclusion, when blood flow through the ductus venosus of fetal sheep is obstructed, blood flow from the placenta to the liver tissues nearly doubles. Proliferation is stimulated in several organs, and the liver grows rapidly. These responses are consistent with the concept that liver blood flow controls fetal growth, and that intrauterine growth restriction is a regulated event which is not exclusively due to the lack of fetal oxygen and nutrient supply. Further, if indeed blood flow rate through the liver is a major determinant of fetal growth, a rationale for the treatment of some IUGR fetuses is provided.

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