Comparison of cerebrospinal fluid transport in fetal and adult sheep

R. MOLLANJI,1 C. PAPAICONOMOU,1 M. BOULTON,1 R. MIDHA,2 AND M. JOHNSTON1
1Trauma Research Program, Department of Laboratory Medicine and Pathobiology and
2Division of Neurosurgery, Sunnybrook and Women’s College Health Sciences Centre,
University of Toronto, Toronto, Ontario M4N 3M5, Canada

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Mollanji, R., C. Papaiconomou, M. Boulton, R. Midha, and M. Johnston. Comparison of cerebrospinal fluid transport in fetal and adult sheep. Am J Physiol Regulatory Integrative Comp Physiol 281: R1215–R1223, 2001.—We quantified cerebrospinal fluid (CSF) transport (conductance) and CSF outflow resistance in late-gestation fetal and adult sheep using two methods, a constant pressure infusion method and a bolus injection technique into the lateral ventricles. No significant differences in CSF conductance (fetus 0.013 ± 0.002, adult 0.014 ± 0.003 ml·min⁻¹·cmH₂O⁻¹) or CSF outflow resistance (fetus 83.7 ± 9.8, adult 84.7 ± 19.7 cmH₂O·ml⁻¹·min) were observed. To confirm CSF transport to plasma in fetal animals, 125I- or 131I-labeled human serum albumin (HSA) was injected into the lateral ventricles. The tracer entered fetal plasma with an average mass transport rate of 1.91 ± 0.47% injected/h (n = 9). In two fetuses, we monitored the tracer appearance in plasma and cervical and thoracic duct lymph after injection of radioactive HSA into the ventricular CSF. As was the case in adult animals, fetal tracer concentrations increased in all three compartments over time, with the highest concentrations measured in lymph collected from the cervical lymphatics. These results (1) indicate that global CSF transport parameters in the late-gestation fetus and adult sheep are similar and 2) suggest an important role for extracranial lymphatic vessels in CSF transport before birth.

arachnoid villi; lymphatic vessels; intracranial pressure; cerebrospinal fluid pressure; fetal cerebrospinal fluid outflow resistance; fetal cerebrospinal fluid conductance; cribriform plate; hydrocephalus

The concept that cerebrospinal fluid (CSF) is formed largely in the choroid plexus and is absorbed primarily through arachnoid villi and granulations provides the cornerstone of our understanding of CSF physiology and hydrocephalus. The microscopic arachnoid villi and macroscopic granulations are herniations of the arachnoid membrane into the venous sinuses of the brain and, as such, seem strategically placed to represent the primary sites where CSF absorption occurs. On the basis of this conceptual framework, pediatric hydrocephalus would be due to either pathophysiological changes that impair CSF flow from its formation in the choroid plexus to these absorption sites (noncommunicating type) or interference with CSF transport through these structures (communicating type).

CSF transport in the fetus has received very little attention, but there are several observations suggesting that further investigation of fetal CSF dynamics may be warranted. First, arachnoid villi may not even exist prenatally. In two microscopic studies of autopsy specimens from individuals up to 56 days old (33) and from 18 wk gestation to 80 years (18), no arachnoid villi or granulations were observed before birth. Even in adult animals, the evidence that supports a role for arachnoid villi/granulations in CSF transport is not entirely convincing (22). Second, in adult animals, there is increasing evidence that a significant portion of total CSF transport occurs through the cribriform plate into extracranial lymphatic vessels (reviewed in Refs. 6, 22). Occlusion of the cribriform plate on the extracranial side reduced CSF transport significantly (32). Given that extracranial lymphatics in the adult have an important role in CSF drainage and that arachnoid villi may be absent or poorly developed in the fetus, it is possible that a significant portion of CSF clearance before birth is a function of the lymphatic system.

As a first step in determining whether CSF transport in the fetus is fundamentally different or similar to that in adult animals, in this report we compared the global CSF transport characteristics in fetal and adult sheep. Second, we examined the possibility that extracranial lymphatics play a role in CSF absorption before birth.

MATERIALS AND METHODS

The animals were fed hay, pellets, and water ad libitum, but were fasted 24 h before surgery. Experiments were approved by the ethics committee at Sunnybrook and Women’s College Health Sciences Center and conformed to the guidelines set by the Canadian Council on Animal Care and the Animals for Research Act of Ontario.

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Experimental Design: Infusion Studies

Two different methods were used to assess CSF conductance and outflow resistance. In one method, a hydrostatic pressure was established in the ventricles via a reservoir filled with artificial CSF. The reservoir height was varied to alter intracranial pressure (ICP) incrementally, and the corresponding flow rate was measured (constant-pressure procedure). In another method, a bolus of artificial CSF was injected into the CSF compartment at a defined flow rate using a syringe pump, and the ICP was monitored (bolus infusion technique).

Fetal Surgical Preparation

For the fetal investigations, randomly bred pregnant ewes were used with fetal gestations of 106–119 days. The pregnant sheep were anesthetized initially by intravenous infusion of 5% pentothal sodium solution. After this, the sheep were intubated, and surgical anesthesia was maintained using halothane administered through a ventilator (Narkomed 2). An intravenous line was introduced into the maternal cephalic vein and perfused with saline and dextrose 5% solution. The radial artery was cannulated with an 18-gauge Novalon intravenous catheter (Becton Dickinson, Sandy, UT), which was subsequently connected to a pressure transducer (Cobe CDX disposable) to monitor maternal blood pressure. A midline incision was made in the lower abdomen of the ewe to expose the uterus. The fetal head was located within the uterus, and a cesarean section was performed. In most of the cases, the fetus was partly exteriorized, and in two cases the fetus was completely exteriorized. In the partly exteriorized fetuses, a 0 silk suture was used to suture the skin of the fetus onto the edge of the incision in the uterine wall to exteriorize the fetus, and a 0 silk suture was used to suture the skin of the fetus onto the edge of the incision in the uterine wall to exteriorize the fetus. In the partly exteriorized fetuses, the incised uterine wall was clamped to prevent leakage of amniotic fluid. In the cases of completely exteriorized fetus, the incised uterine wall was clamped to prevent amniotic fluid leakage. The fetal radial artery was cannulated with a 20-gauge Novalon intravenous catheter (Becton Dickinson), which was subsequently connected to a pressure transducer (Cobe CDX disposable). Maternal and fetal systemic arterial pressures were monitored continuously during the course of the experiments to provide an overall measure of the cardiovascular status of the animals. In all successful preparations, systemic arterial pressures were relatively constant over the duration of the experiment.

A midsagittal incision was made in the scalp of the fetus to reveal the junction of the sagittal and lambdoid sutures. Two ½-in. burr holes were made bilaterally 1 cm anterior and 0.5 cm lateral to the lambda, at an angle of 10° from the sagittal plane. A single catheter guide screw was inserted in each hole. A 16-gauge Novalon intravenous catheter (Becton Dickinson) was then attached to a column of artificial CSF (filter sterilized) and fed through the guide screw. The catheter from one lateral ventricle was connected to a pressure transducer (Cobe CDX disposable). Data were recorded on a computer-based data-acquisition system.

Constant Pressure Studies (Fetal and Adult)

Artificial CSF was delivered to a lateral ventricle using a modification of a method described by Davson's group (14). A reservoir filled with artificial CSF was placed on a balance (Setra, BL-410S, Laborc, Concord, Ontario) connected to a printer. The height of the reservoir and balance were elevated relative to the head of the animal to initiate a CSF inflow rate. The balance was set to register a weight every 60 s. The flow of artificial CSF into the ventricle was deduced from the rate of reservoir weight reduction. ICP was recorded from the contralateral ventricle continuously from the data-acquisition system. With this method, a steady-state ICP was achieved, usually within 1–2 min, and steady-state flow rates were attained within 5–10 min. The steady-state flow and ICP were measured at a minimum of three different reservoir heights.

Data analysis. Although the reservoir heights were standardized between experiments, the cranial CSF pressures varied between animals. Therefore, to facilitate comparisons, we normalized the data. To achieve this, we assumed that the CSF pressure-flow relationships were linear and obtained the equation of the best fit line for each experiment. We then recalculated flow rates for CSF pressures between 15 and 35 cmH2O at 5-cmH2O increments. Inasmuch as steady-state flow into the ventricles would equal CSF absorption, we plotted CSF pressure vs. flow rate, and the slope of the relationship was taken as CSF outflow resistance (Rout; cmH2O·ml⁻¹·min⁻¹). The reciprocal of Rout equals CSF conductance (ml·min⁻¹·cmH2O⁻¹). In our study, we used both diastolic and mean CSF pressures to calculate resistance and conductance.

Bolus Injection Studies (Fetal and Adult)

Artificial CSF was infused via a syringe pump (Kd Scientific, model 260) into a lateral ventricle after passage through a sterile syringe filter (Corning, 0.20 μm). In fetal sheep, a 1-ml volume at a rate 2.0 ml/min was injected. In adult animals, a 2.5-ml volume was injected at a rate of 5.0 ml/min. ICP was measured from the contralateral ventricle. Experience with the bolus injection method indicated that the CSF system needed to be primed to ensure consistent pressure patterns after subsequent injections. This is likely due to the fact that some CSF is lost inevitably when catheters are inserted into the ventricular system. The first bolus injection replenished the CSF compartment, resulting in reliable pressure patterns with subsequent injections (similar peak pressures and time to return to baselines and baseline pressures). With this consideration in mind, two bolus injections were given in each experiment, with bolus 1 representing the priming injection. CSF conductance and outflow resistance were assessed from the second bolus.

Data analysis. The CSF outflow resistance in the bolus injection experiments was calculated using the equations of Marmarou et al. (30). With a bolus injection into the CSF compartment, ICP increases rapidly. The rise in pressure enhances the absorption of CSF from the system, and ICP falls gradually to the preinjection level. Three values are taken from the response: the initial diastolic pressure (P₀),
the peak diastolic pressure (Pp), and a recovery diastolic pressure (P2) recorded at a time t2 min after injection. For each infusion, a pressure-volume index (PVI) was calculated from equation 1 and this value was inserted into equation 2 for estimates of the CSF outflow resistance. For each bolus infusion after the priming infusion, CSF outflow resistance was estimated at five different t2 points postinjection (100, 150, 200, 250, and 300 s) and then averaged. We compared the value of the outflow resistance measurements from the postpriming bolus in both fetal and adult sheep.

\[
PVI = \frac{\Delta V}{\log_{10} \frac{P_p}{P_0}}
\]

where \(\Delta V\) is the volume injected into the CSF space.

\[
R_{out} = \frac{P_0}{\frac{t_2 \cdot P_0}{PVI \cdot \log \left( \frac{P_2 \cdot P_0 - P_2}{P_p \cdot P_2 - P_p} \right)}
\]

**Experimental Design: Studies With Radioactive CSF Protein Tracers**

Two groups of fetal studies were performed with radioactive protein tracers in partially exteriorized fetuses. In the first, we injected I- or I-labeled human serum albumin (HSA) into the lateral ventricles and monitored tracer recoveries in the fetal plasma. This was to confirm CSF transport into the venous system. Second, in a limited number of fetuses, we cannulated one or both cervical lymphatic vessels, the thoracic duct, and the jugular vein. Lymph was collected continuously, and plasma was sampled after injecting the radiolabeled albumin into the CSF (lateral ventricle). The lymph and plasma compartments were monitored for radioactivity.

**Plasma Transport of the CSF Tracer**

Randomly bred pregnant ewes were used in the radioactive tracer studies with fetal gestations of 115–139 days. After access to the fetus as described earlier, access to the fetal venous system was accomplished with a catheter inserted in the jugular vein. Either 50 (0.5 mg) or 100 µl of I- or I-labeled HSA (1.0 mg) was injected into each lateral ventricle. At the same time, a second tracer (Evans blue-sheep albumin complex) was injected into the venous circulation to permit calculation of the plasma volume (VP) and determination of a coefficient of elimination from the plasma (Kexp). Plasma samples were taken over 3–6 h. Radioactivity was determined using a multichannel gamma spectrometer (Compugamma; LKB Wallac, Turku, Finland) with appropriate window settings and background subtraction.

**Data analysis.** As the CSF tracer enters the plasma, some of this protein will filter out of the vascular compartment with the result that plasma recoveries will be underestimated. To correct the data for the loss of filtered protein, Kexp was defined and entered into the following mass balance equation (3). Bin equals the time-averaged rate of mass transport of radioactive albumin into the plasma.

\[
\text{Mass Transport (Bin)} = \frac{[C_0(t_2) \exp(K_{exp}t_2) - C_0^{exp}]}{\exp(K_{exp}) - 1}
\]

The plasma disappearance curve for the Evans blue-sheep albumin complex (measured spectrophotometrically) was used to determine the Kexp for albumin and to calculate the volume of distribution of albumin (VP). With the assumption that VP was a constant throughout the experiment, the log values of the hourly tracer concentration (CP) were plotted, and the anti-log of the slope of this linear relationship minus 1 gave Kexp. Additionally, the tracer concentrations in plasma (CP) were plotted over 30 min, the linear relationship extrapolated to t = 0 and a tracer dilution method was used to calculate VP. All values are a function of time. Values for Bin were averaged from the time of injection of labeled HSA (t = 0) to the end of the experiment (time final or t). The values for Bin (cpm/h) were divided by the total radioactivity injected to give %injected/h.

**Transport of the CSF Tracer to Cervical and Thoracic Duct Lymph and Plasma**

In three fetuses, the thoracic duct at its junction with the venous system at the base of the neck, multiple cervical lymphatics, and a jugular vein were cannulated with plastic catheters of appropriate size (Crichtley, Silverwater, Australia), adapting methods employed by our group to achieve similar goals in adult animals (3, 4). Any cervical vessels that were too small to cannulate were ligated to prevent transport of the CSF tracer to plasma. The lymph was collected into separate heparinized test tubes. In two fetuses, 50 µl (0.5 mg) of I- or I-HSA was injected into each lateral ventricle. In the third animal, 250 µl (2.5 mg) was injected in one ventricle. Radioactivity was monitored in CSF, plasma, and lymph over 3–6 h (cpm/ml).

**Tracers and Solutions**

I-HSA-0.93 MBq/ml (10 mg/ml) and I-HSA-37 MBq/ml (10 mg/ml) were obtained from Drax Image (Kirkland, Quebec, Canada). Evans blue dye was purchased from Sigma. All radioactive tracer solutions were purified before use by passage through a Centricon centrifugal concentrator (10,000 molecular wt cut-off) to remove free I or I before infusion. In addition, precipitation of the protein tracers with 10% trichloroacetic acid in representative plasma and lymph samples in each experiment revealed that free or non-protein-associated I or I represented <1% of the total radioactivity.

**Statistical Analysis**

The data were analyzed using ANOVA.

**RESULTS**

**Constant Pressure Infusion Studies**

Eight fetuses and six adults were used in this series of experiments. Three fetal sheep were excluded from data analysis due to technical problems (intraventricular bleeding, a CSF leak, and very low blood pressure). As well, one adult animal was excluded due to intraventricular bleeding. The remaining ten animals, five adult and five fetal sheep, form the basis for data analysis.

In the successful fetal and adult animal preparations, equilibrium conditions were achieved as the height of the reservoir was elevated incrementally. Figure 1 illustrates one example from the fetal group. Every attempt was made to keep the maximum ICP <30 cmH2O in the infusion series to limit the possibility of pathophysiological changes. Our main concern was to prevent any changes in the dural venous sinus
pressure that could alter the CSF-venous pressure relationship and affect CSF conductance and outflow resistance estimates. This was especially true in the fetus, as the immature dura may not offer the protection of the adult counterpart. Due to the technical challenges, we were able to monitor superior sagittal venous sinus pressure in only one fetus (Fig. 2). In this animal, the dural venous pressure was relatively unaffected by elevation of ICP over the range of pressures used in this study.

Figure 3 illustrates the diastolic ICP vs. flow relationship in fetal and adult animals. The flow data were calculated from the steady-state infusion rates. As can be seen in Fig. 3 and Table 1, CSF conductance and outflow resistance in fetuses and adults were very similar (no significant differences). Additionally, there were no significant differences in either parameter whether diastolic or mean ICP was used for calculation. With diastolic pressure-based values, the average CSF conductances were 0.013 ± 0.002 and 0.014 ± 0.003 ml·min⁻¹·cmH₂O⁻¹ in fetal and adult animals, respectively. CSF outflow resistances were 83.7 ± 9.8 (fetus) and 84.7 ± 19.7 cmH₂O·ml⁻¹·min (adult).

The most important elements of the analysis (the conductance and outflow resistance estimates) are not dependent on CSF formation. However, the true flow would be equivalent to the infusion rate plus the CSF formation rate. We did not measure CSF formation directly. Nonetheless, assuming the conductance expression (ml·min⁻¹·cmH₂O⁻¹) represents the flow characteristics of a given CSF system in the presence or absence of additional volume infusions, we can estimate CSF formation using the baseline ICP in the animal. Baseline diastolic ICP multiplied by conductance is equal to flow (absorption), which in turn provides an estimate of CSF formation under equilibrium conditions at the resting ICP. With this approach, the CSF formation rate in fetal lambs at ~112 days gestation was 6.3 ± 0.6 ml/h, with an average resting ICP of 10.4 ± 0.8 cmH₂O. In the adult sheep, CSF formation was 7.8 ± 0.7 ml/h at an average resting pressure of 11.9 ± 1.4 cmH₂O.
CSF formation is relatively constant over a wide range of CSF pressures (reviewed in Ref. 15). In goats for example, Heisey et al. (21) observed that CSF formation did not change substantially over a range of CSF pressures between −10 and 30 cm H2O. Nonetheless, there is some evidence that CSF formation declines at high levels of ICP probably due to a reduction in perfusion of the choroid plexus. In our studies, CSF pressures were kept <30 cm H2O, and CSF formation rates were likely constant under these conditions. Consequently, a single estimated value for CSF formation in each animal was added to all measured flow numbers to more accurately represent the total volumetric transport (dotted lines in Fig. 3).

Bolus Injection Experiments

In this series of experiments, 23 animals were used (13 fetuses and 10 adults). All of the adult preparations were successful. In the fetal studies, six animals were excluded (intraventricular bleeding, animal deaths, CSF leakage, and umbilical cord separation), leaving seven for data analysis.

As artificial CSF was injected, ICP rose to a peak (Pp) and then declined over time. An example from the fetal group is illustrated in Fig. 4. In our experiments, the increases in diastolic pressure above resting pressure associated with bolus injections were approximately six- and threefold in fetal and adult animals, respectively. The average Pp observed in fetal and adult animals was 26.2 ± 3.9 and 44.7 ± 3.6 cm H2O, respectively. Because the increased ICP was transient in nature, there was less concern about the pathophysiological effects associated with these volumes.

As was the case in the constant-pressure infusion studies, we did not observe any significant differences in CSF conductance or outflow resistance when fetal and adult sheep were compared. A summary of the data is presented in Table 2. CSF conductances were estimated to be 0.022 ± 0.002 and 0.017 ± 0.002 ml·min⁻¹·cm H2O⁻¹ in fetuses and adults, respectively. CSF outflow resistances were 47.9 ± 4.6 (fetal) and 69.4 ± 10.6 cm H2O·ml⁻¹·min⁻¹ (adult). CSF outflow resistances measured with the bolus injection method were lower (and conductances higher) than those estimated with the constant-pressure infusion technique, but these differences were not statistically significant. Comparisons between bolus and infusion techniques in humans demonstrate a similar phenomenon (29).

Studies with Radioactive Protein Tracers in the Fetus

In this group of experiments, we tested whether a CSF tracer instilled into one or both lateral ventricles of the fetus transported to plasma. Ten animals were used. In one fetus, high concentrations of the CSF tracer were observed in the first blood sample taken immediately after injection of the tracer into the ventricular system (t = 0). We assumed that a portion of the infusate had entered blood directly due to a problem with the ventricular catheter. This animal was excluded from data analysis. In the remaining nine fetuses, 125I- or 131I-HSA entered plasma, with the CSF tracer concentrations increasing over time (Fig. 5). These data confirmed that CSF was actually transporting to plasma in the fetus. The average mass transport to plasma (Bin) was 1.91 ± 0.47% injected/h.

In three animals, we were able to monitor concentrations of the CSF tracer in plasma and cervical and thoracic duct lymph. In one fetus, the cervical lymph was contaminated with blood and coagulation caused

Table 1. Summary of data from fetal and adult constant pressure infusion study

<table>
<thead>
<tr>
<th></th>
<th>Rout, cm H2O·ml⁻¹·min⁻¹</th>
<th>Conductance, ml·min⁻¹·cm H2O⁻¹</th>
<th>Baseline ICP, cm H2O</th>
<th>CSFFR, ml/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal Sheep</td>
<td>83.7 ± 9.8</td>
<td>0.013 ± 0.002</td>
<td>10.4 ± 0.8</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td>Adult Sheep</td>
<td>84.7 ± 19.7</td>
<td>0.014 ± 0.003</td>
<td>11.9 ± 1.4</td>
<td>7.8 ± 0.7</td>
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Measurements using diastolic ICP

<table>
<thead>
<tr>
<th></th>
<th>Rout, cm H2O·ml⁻¹·min⁻¹</th>
<th>Conductance, ml·min⁻¹·cm H2O⁻¹</th>
<th>Baseline ICP, cm H2O</th>
<th>CSFFR, ml/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal Sheep</td>
<td>88.9 ± 11.3</td>
<td>0.012 ± 0.002</td>
<td>10.4 ± 0.8</td>
<td>7.4 ± 0.9</td>
</tr>
<tr>
<td>Adult Sheep</td>
<td>91.0 ± 23.2</td>
<td>0.013 ± 0.002</td>
<td>11.9 ± 1.4</td>
<td>8.7 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. Rout, outflow resistance; ICP, intracranial pressure; CSFFR, cerebrospinal fluid formation rate.

Fig. 4. Example of the intracranial pressure response to the bolus injection of artificial CSF in 1 fetus. P0, initial diastolic pressure before injection; Pp, peak diastolic pressure attained; Pd, diastolic pressure measured at time t after injection.

Table 2. Summary of data from fetal and adult bolus injection study

<table>
<thead>
<tr>
<th></th>
<th>Rout, cm H2O·ml⁻¹·min⁻¹</th>
<th>Conductance, ml·min⁻¹·cm H2O⁻¹</th>
<th>Baseline ICP, cm H2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal Sheep</td>
<td>47.9 ± 4.6</td>
<td>0.022 ± 0.002</td>
<td>9.8 ± 1.7</td>
</tr>
<tr>
<td>Adult Sheep</td>
<td>69.4 ± 10.6</td>
<td>0.017 ± 0.002</td>
<td>12.6 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE.
the flow to cease. In the two successful preparations, tracer concentrations increased in all three compartments over time, with the highest concentrations measured in lymph collected from the cervical lymphatics (Fig. 6).

DISCUSSION

In this report we obtained evidence that the global CSF transport parameters in the late-gestation sheep fetus were very similar to those observed in adult animals. In addition, we acquired preliminary data suggesting that CSF can enter extracranial cervical lymphatic vessels in the fetus. To put these data into perspective, several issues need to be considered.

CSF Formation in Fetal and Adult Animals

The choroid plexus begins producing CSF early in embryonic life (36). Early in development of the subarachnoid compartment in rats, CSF does not appear to move out of the ventricles (26), but in humans, fluid can be visualized by ultrasonography within the subarachnoid compartment at 15 wk gestation (31).

CSF formation rates have been reported to be between 2.8 and 4.6 ml/h in late gestation sheep fetuses (1, 17). These estimates were obtained using a ventriculocisternal perfusion technique with the dilution of an appropriate tracer used to calculate CSF formation rates. In the study reported here, we did not assess CSF formation directly, but used the average conductance and baseline ICP to estimate slightly higher formation rates between 6.3 and 7.4 ml/h (Table 1). Without further investigation, we cannot determine which method provides the most accurate formation rate, but what is of greater interest is the fact that the reported range of CSF formation rates in the fetus is similar to that published for adult animals. CSF formation rates in adult sheep (25–40 kg) have been estimated to be between ~4 and 7 ml/h (4, 10, 17, 35). However, this type of comparison is somewhat misleading. The fetuses are ~1/10 of the weight of adult sheep (3–4 kg) and, therefore, CSF formation rates in the fetus are ~10-fold higher expressed as milliliter per hour per kilogram body weight.

In addition, the CSF volume of the near-term fetal sheep brain has been estimated to be 7.28 ml (17) and, therefore, CSF turns over in <2 h. Assuming that adult sheep have similar CSF volumes to those reported for goats (25 ml; Ref. 34), CSF in the adult sheep would turnover approximately every 4–5 h. Similarly, a higher CSF turnover rate in fetal sheep compared with adult animals has been described by other investigators (17). Overall, the relatively high CSF formation and turnover rates in the fetus imply that effective CSF drainage mechanisms must exist before birth.

CSF Conductance and Outflow Resistance

Based on two different methods to assess CSF conductance and outflow resistance, a constant-pressure perfusion technique and a procedure employing bolus injections of artificial CSF, our analysis indicated that CSF conductance and outflow resistance were very similar in fetal and adult sheep. In addition, the pressures that initiate CSF absorption (opening pressures derived by extrapolation of lines to the y-axis) were within a few centimeters H2O of each other.

Fig. 5. Fetal plasma concentrations of 125I- or 131I-labeled human serum albumin (HSA; % injected/ml) vs. time after injection of the tracer into 1 or both of the lateral ventricles. Main graph illustrates data from individual animals, and the inset represents the averaged data from all animals (means ± SE; 0–3 h, n = 9; 0–4 h n = 5; 0–6 h, n = 4).

Fig. 6. Compartmental CSF tracer concentrations vs. time in lymph and plasma in 2 fetuses. A: ○, 1 cervical lymphatic; ●, plasma; ▼, thoracic duct. B: ○ and ▼, bilateral cervical lymphatics; △, thoracic duct; ●, plasma.
To the best of our knowledge, the only report with which to compare these results relates to studies performed in rats using a constant-rate infusion method (24). Resistance to absorption in the rat fetus was between 10.8 and 16.3 mmH₂O·min⁻¹·µL⁻¹. Resistance increased sharply after birth (39.2 mmH₂O·min⁻¹·µL⁻¹) but fell steadily with age such that outflow resistance at 30 days and in adults approached 6.8 and 7.9 mmH₂O·min⁻¹·µL⁻¹, respectively. Similary in mice, the resistance to drainage of CSF was much higher in newborns than in older animals (23).

Several factors may contribute to these changes in CSF outflow resistance. In normal rats, CSF and dural venous pressures are not significantly different from one another in animals <3 wk old. A positive pressure gradient was not observed until 20 days after birth (25). If CSF transport occurs via arachnoid villi in neonatal rats, the lack of a suitable pressure gradient may contribute to the high outflow resistance observed immediately after birth. However, if this is true, one must argue that a pressure gradient favoring absorption exists at sites other than at the arachnoid villi–dural sinus interface, because these animals did not express hydrocephalus. Alternatively, the decline in outflow resistance after birth could be due to maturation of absorption sites as the animal ages or due to increases in the number of absorption sites (24).

Whatever the explanation for the elevation of CSF outflow resistance immediately after birth, measurements of CSF outflow resistance in the fetal and adult rats were not that dissimilar. Certainly, our data in sheep indicate no significant differences in fetal and adult global CSF transport characteristics. These results suggest that either CSF transport occurs through the same absorption pathways in the fetus and adult or the CSF drainage routes are different but exhibit the same resistance characteristics. Our data would favor the first explanation as is discussed below.

Potential CSF Transport Pathways

As noted earlier, there is evidence to question the role of arachnoid villi in CSF transport in adult animals and, moreover, these elements may not even exist before birth (22). In contrast, considerable anatomic and quantitative data exist to support a role for extracranial lymphatics in CSF transport. Although the central nervous system parenchyma does not contain lymphatic vessels, protein tracers injected into the brain interstitium or CSF exit the cranium and enter extracranial lymph. The injected molecules pass out of the cranium along the prolongations of the subarachnoid space associated with several nerves. The most important pathway is along the arachnoid sheaths of the olfactory nerves that penetrate the cribiform plate (5–9, 13, 16, 20, 28). There is also evidence in the rat that lymphatics play an important role in CSF transport at least in adult animals (2, 28). Experiments designed to quantify CSF lymph clearance have revealed that nearly one-half of the total CSF drained from the cranial system occurred through the cribiform plate in adult sheep (3, 4) and rats (2). These studies may have underestimated the lymphatic contribution to CSF transport, because some small cervical lymphatic vessels may have escaped detection.

More recently, we observed that occlusion of the cribiform plate on the extracranial side reduced CSF transport significantly in adult animals (32). We were able to deduce that the majority of CSF appeared to drain through the cribiform plate at low ICP. This observation may fit with what is known about the flow and distribution of CSF within the cranial vault. Studies with CSF markers generally illustrate the predominantly basal movement of CSF with the convexities being reached later. For example, autoradiographic studies using [¹⁴C]sucrose demonstrated that CSF flow throughout the ventricular system and into various subarachnoid velae and cisterns was rapid but rather limited over the cerebral and cerebellar cortices (19). Following its formation in the choroid plexus, it is possible that CSF gains its first access to absorption sites as it moves toward the base of the cranium. As time progresses or with higher ICPs, CSF may convect to the subarachnoid spaces over the convexities of the brain where it would be in proximity to potential absorption sites associated with the cranial venous sinuses.

If arachnoid villi or functional precursors do not exist before birth, one might postulate that CSF may be cleared from the subarachnoid compartment primarily by lymphatics in the fetus. Certainly, the late-gestation fetus has a comprehensive lymphatic circulatory system. The lymphatics first appear in the 10- to 11-mm-long embryo pig as primitive buds from the venous system. A plexus of lymphatics is formed from the primitive buds, and these ultimately grow and coalesce to form the jugular lymph sac in the base of the neck. From the jugular lymph sac, a number of lymphatics develop, including the nasal, retropharyngeal, and cervical vessels (11), all of which have been implicated in CSF transport in the adult. Especially conspicuous are the lymphatics developing from the cervical plexus that are associated with the external jugular veins. In this report, we obtained evidence that the cervical lymphatic vessels in the fetus contained high concentrations of a CSF tracer, a result that is similar to that observed in adult sheep. Therefore, preliminary data suggest that CSF transport occurs through the olfactory–cribiform nasal lymphatic pathway in the late-gestation fetus. Inasmuch as a major portion of CSF clearance occurs through this route in adult sheep (32), this suggests that CSF transport pathways in the fetus and adult are the same. If true, this could explain the observation that CSF conductances and outflow resistances in the fetal and adult were similar in magnitude.

If arachnoid villi/granulations have a role in CSF transport, their function may increase after birth. Arachnoid granulations appear in greater numbers as children age, with structures visible clearly by ~2
years of age (12). Another study noted few granulations in newborn infants, but numerous arachnoid granulations were found in all specimens from adults, and these increased in size and number with increasing age (18). In this regard, there is evidence that the area of the foramina in the cribriform plate decreases with increasing age after birth (27). This implies that CSF transport into the cervical lymphatic vessels may also decline and could support an increasing role for arachnoid villi in the older individual.

In a previous study in adult sheep, we observed some residual CSF transport after clearance through the cribriform plate had been blocked (32). This transport pathway appeared to be recruited as ICP was elevated. Therefore, in adults, we postulate that a dual CSF transport system exists through anatomically distinct pathways. As the pressure that initiates CSF transport is achieved (opening pressure), CSF clearance appears to occur primarily through the cribriform plate into extracranial lymphatic vessels. As ICP increases, a second system begins to absorb CSF from the cranial subarachnoid compartment. Arachnoid projections may contribute to the latter clearance.

Perspectives

Hydrocephalus continues to be an important neurosurgical problem. Current views on CSF transport have existed for many years, but, unfortunately, they have not provided fertile ground for the development of new therapeutic approaches to hydrocephalus beyond the physical diversion of CSF with shunts or the endoscopic third ventriculostomy procedure. It will be difficult to conceptualize the development of new therapies based on molecular concepts without first having a clear understanding of the transport parameters and pathways associated with CSF absorption. If a significant portion of total CSF transport in the fetus occurs through the cribriform plate into extracranial lymphatic vessels, attention directed to the cribriform plate may reveal new insights into the cause of hydrocephalus and provide novel targets for intra- and extracranial therapeutic intervention. Additional approaches to treat hydrocephalus might include, for example, the use of molecular regulators to stimulate lymphangiogenesis in the nasal mucosa or modulate pumping activity in the lymphatic collecting vessels.

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