Does neonatal cerebrospinal fluid absorption occur via arachnoid projections or extracranial lymphatics?

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Papaiconomou, C., R. Bozanovic-Sosic, A. Zakharov, and M. Johnston. Does neonatal cerebrospinal fluid absorption occur via arachnoid projections or extracranial lymphatics? Am J Physiol Regul Integr Comp Physiol 283: R869–R876, 2002. First published June 27, 2002; 10.1152/ajpregu.00173.2002.—Arachnoid villi and granulations are thought to represent the primary sites where cerebrospinal fluid (CSF) is absorbed. However, these structures do not appear to exist in the fetus but begin to develop around the time of birth and increase in number with age. With the use of a constant pressure-perfusion system in 2- to 6-day-old lambs, we observed that global CSF transport (0.012 ± 0.003 ml·min⁻¹·cmH₂O⁻¹) and CSF outflow resistance (96.5 ± 17.8 cmH₂O·ml⁻¹·min) were very similar to comparable measures in adult animals despite the relative paucity of arachnoid villi at this stage of development. In the neonate, the recovery patterns of a radioactive protein CSF tracer in various lymph nodes and tissues indicated that CSF transport occurred through multiple lymphatic pathways. An especially important route was transport through the cribriform plate into extracranial lymphatics located in the nasal submucosa. To investigate the importance of the cribriform route in cranial CSF clearance, the cranial CSF compartment was isolated surgically from its spinal counterpart. When the cribriform plate was sealed extracranially under these conditions, CSF transport was impaired significantly. These data demonstrate an essential function for lymphatics in neonatal CSF transport and imply that arachnoid projections may play a limited role earlier in development.

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THE MICROSCOPIC ARACHNOID villi and macroscopic granulations are herniations of the arachnoid membrane into the dural venous sinuses of the brain. Based primarily on anatomic studies of adult human or animal specimens, it has been generally assumed that these structures represent the primary locations where cerebrospinal fluid (CSF) absorption occurs. However, earlier in development, this conventional view would not seem to apply. Several studies failed to observe arachnoid villi/granulations in the human fetus. In two microscopic studies of autopsy specimens from individuals up to 56 days old (38) and from 18 wk gestation to 80 yr (21), no arachnoid villi or granulations were observed before birth. At or around the time of birth, arachnoid projections start to become visible in the dura (24) and some of these appear to be associated with veins (23). As the infant ages, the villi and granulations increase in number, and in the adult, they exist in abundance (16). The role of the arachnoid projections in the neonate is important because reduced CSF transport to these absorption sites or impaired clearance through these structures is believed to constitute the principal defect in hydrocephalus (26).

If arachnoid projections do not exist (or exist in very small numbers or in an immature form), we are left with the question of how CSF is drained in the neonatal period. We believe that current evidence favors a role for the lymphatic circulation. The central nervous system parenchyma does not contain lymphatic vessels. However, protein tracers injected into the brain interstitium or CSF exit the cranium and enter extracranial lymph (8). The injected molecules passed out of the cranium along the prolongations of the subarachnoid space associated with several nerves. There is also the suggestion that CSF may transport through the adventitia of cerebral blood vessels with final absorption into the cervical lymphatics in the neck (20). The most important pathway seems to be along the arachnoid sheaths of the olfactory nerves that penetrate the cribriform plate (7, 9, 10, 12, 25, 30, 33). Although the available evidence has been derived from studies on animals, there are circumstantial data that cribriform-lymphatic CSF transport occurs in humans and nonhuman primates as well (14, 17, 32, 34, 40, 44). These results have led to some discussion of the immunological implications of the transport of brain antigens to extracranial lymphoid tissues (18) with speculation that this process may have pathological significance. It has been suggested that the cervical lymph nodes may play a role in the induction of encephalomyelitis as the lymphatic drainage of brain antigens may facilitate the priming of T lymphocytes that could subsequently target the brain. In support of
this concept, cervical lymphadenectomy reduced the level of cerebral pathology in an animal model of autoimmune encephalomyelitis (39). Additionally, there is evidence that human immunodeficiency virus (HIV) antigens within the CSF can be carried to the cervical lymph nodes via this pathway (13), suggesting a mechanism by which a brain reservoir of HIV might reinflect peripheral lymphoid tissues.

However, it has become clear recently that in several animal species, clearance of CSF into extracranial lymphatics plays an important role in CSF volume and pressure regulation. The development of mathematical and tracer methods to estimate lymphatic CSF transport (2–5), the assessment of the relationship between intracranial pressure and cervical lymph flow (1, 42), and the results of intervention experiments in which the lymphatic pathways have been physically obstructed (35, 41) have led to the conclusion that a significant volume of CSF transport occurs into extracranial lymphatic vessels in the adult. Indeed, under some conditions, there is evidence that the cribiform-lymphatic route may be the dominant pathway for CSF clearance (35). If the cribiform route is obstructed, resting intracranial pressures increase significantly (36).

Given the prominence of lymphatic CSF transport in the adult (when abundant arachnoid projections are known to exist), it is not unreasonable to assume that lymphatics may play an even more important role in the neonatal period (a period during which arachnoid projections are beginning to develop). In the studies reported here, we quantified the global CSF transport characteristics in the neonate, investigated the neonatal CSF lymphatic drainage pathways, and examined the impact of surgically blocking the neonatal olfactory/cribriform plate route on CSF absorption. These data support an important role for extracranial lymphatic vessels in neonatal CSF transport.

MATERIALS AND METHODS

Randomly bred 2- to 6-day-old lambs weighing 3.1–6.5 kg were used for this investigation. They were bottle-fed formula (Lamb Replacement, Grober Cambridge, Ontario) until surgery. Experiments were approved by the ethics committee at Sunnybrook and Women’s College Health Sciences Centre and conformed to the guidelines set by the Canadian Council on Animal Care and the Animals for Research Act of Ontario.

The sheep were anesthetized initially by mask administration of incremental concentrations of halothane from 0.5 to 3%. Subsequently, 1–2% halothane was delivered through an endotracheal tube via an A. D. S. 1000 respirator for surgical maintenance. For continuous monitoring of systemic arterial pressure, femoral artery cannulation was performed. The general oxygen status of the animal was monitored by a pulse oximeter (Nonin 8600V, Benson Medical, Markham, Ontario) attached to an ear.

Constant pressure infusion experiments. A midsagittal incision was made in the scalp of the neonate to reveal the junction of the sagittal and coronal sutures. Two 1/8-in. burr holes were made bilaterally 1 cm anterior and 1 cm lateral to the junction of the aforementioned sutures, at an angle of ~10° from the sagittal plane. A single catheter guide screw was inserted into each hole. An 18-gauge Novalon intraventricular catheter (Becton Dickinson, Sandy, UT) was attached to a column of artificial CSF (filter sterilized) and fed through the guide screw. The contralateral ventricular catheter was connected to a pressure transducer (Cobe CDX disposable).

Data were recorded on a computer-based data-acquisition system (A-Tech Instruments, Toronto, Canada and Visual Designer software, Tucson, AZ).

Artificial CSF was delivered to a lateral ventricle using a modification of a method described by Davson’s group (19). Artificial CSF contained (in mM) 125 NaCl, 2.8 KCl, 1.2 CaCl_2, 0.9 MgCl_2, 25 NaHCO_3, and 0.5 Na_2HPO_4/KH_2PO_4 (15). A reservoir filled with artificial CSF was placed on a balance (Setra, BL-410S, Labor, 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. Intracranial pressure was recorded from the contralateral ventricle continuously to the data-acquisition system. With the use of this method, steady-state flow rates were achieved usually within 1–2 min and steady-state flow rates were attained within 5–10 min. The steady-state flow and intracranial pressure were measured at a minimum of three different reservoir heights.

Even though the reservoir heights were standardized between experiments, the cranial CSF pressures varied among animals. Therefore, to facilitate comparisons, we normalized the data (35). To achieve this, we assumed that the CSF pressure/flow relationships were linear and obtained the linear regression equation for each experiment. We then recalculated flow rates for CSF pressures between 0 and 21 cmH_2O at 3-cmH_2O increments. Because steady-state flow into the ventricles would equal CSF absorption, we plotted CSF flow vs. intracranial pressure, and the slope of the relationship was taken as CSF conductance (ml·min^{-1}·cmH_2O\(^{-1}\)). The reciprocal of this value is equal to CSF outflow resistance (cmH_2O·ml\(^{-1}·\)min). In our study, diastolic CSF pressures were used to calculate resistance and conductance.

It is important to note that the fluid we infused to derive the CSF flow vs. intracranial pressure relationship was not the total new volume added to the CSF system because CSF formation was ongoing during the experiment. To more accurately represent the system dynamics, we estimated CSF formation, assumed it was constant over the range of pressures generated during infusion, and added this constant value to each point on the CSF vs. flow curve. We did not measure CSF formation directly. Nonetheless, assuming the conductance expression (ml·min\(^{-1}·cmH_2O\(^{-1}\)) represents the flow characteristics of the CSF system in the presence or absence of additional volume infusions, we can estimate CSF formation using the baseline intracranial pressure in the animal. Baseline diastolic intracranial pressure multiplied by conductance is equal to flow (absorption), which, in turn, provides an estimate of CSF formation under equilibrium conditions at the baseline intracranial pressure.

Analysis of lymphatic pathways that transport cranial CSF. Approximately 10–12 µCi of \(^{125}\)I- or \(^{131}\)I-human serum albumin (HSA) was injected into the cisterna magna. Three hours later, the animal was euthanized. Various lymph nodes and selected other tissues present within the limbs, body cavities, and neck of the animal were collected, weighed, and counted for radioactivity. These included tissues: spleen, tongue, thymus, skin, skeletal muscle, lung, liver, kidney, heart, adrenal gland, adipose tissue, plasma, CSF, nasal mucosa, nasal conchi, and nasal septum; nerves: optic, vagus,
and sciatic; and lymph nodes: cervical, preauricular, retropharyngeal, submandibular, thymic, tracheal, axillary, car- diac, caudal mediastinal, mesenteric, hepatic, iliac, para- aortic, pleural, prefrontal, and precapillary. In cases where nodes were bilateral, an average value was obtained. The radioactivity per gram tissue weight was expressed as a percentage of the radioactivity present in 1 ml of CSF.

Obstructing CSF transport through the cribriform plate. The cribriform plate was obstructed using a method developed in our laboratory (35). To gain access to the extracranial side of the cribriform plate, the skin over the frontal-nasal area was reflected to reveal the frontal and nasal bones. An ~2 × 3-cm section of nasal bone was removed to expose the nasal mucosa with the upper edge around the level of a line bisecting the medial canthi. An ethmoidectomy was performed; the nasal mucosa, olfactory nerves, and all soft tissue on the extracranial surface of the cribriform plate were scraped away with a curette, and the bone surface was sealed with glue (mixture of ethyl cyanoacrylate and polymethylmethacrylate, Surehold, Chicago, IL). At the end of each experiment, an Evans blue dye protein complex was injected into the CSF compartment to check for possible CSF leaks around the cribriform plate area.

To facilitate data comparisons, we normalized the data. The y-intercept extrapolated from linear regression of the phase 1 data (cribriform plate intact) represented the estimated opening pressure at which cranial CSF drainage was initiated. This value became the reference point and the linear regression equations from pre- and postcribriform plate obstruction data were used to recalculate flow rates at intracranial pressures between 0 and 21 cmH2O at 3-cmH2O increments above opening pressure. The data were replotted with intracranial pressure above opening pressure on the y-axis and flow rate on the x-axis.

Blocking CSF transport pathways to the spinal CSF compartment. In some experiments, we were interested in cranial CSF dynamics alone. Therefore, we isolated the cranial CSF system from its spinal counterpart. To achieve this, we prevented CSF transport into the spinal cord by performing a C1–C2 laminectomy. A 0 silk ligature was passed around the thecal sac between C1 and C2 and tied tightly to compress the meninges and spinal cord, thereby separating the cranial and spinal subarachnoid compartments (35). In all animals, systemic arterial pressure increased immediately after the cord was ligated but stabilized at a level lower than baseline for the duration of the experiment. The cisterna magna was cannulated with an angiocatheter connected to a vinyl cannula filled with artificial CSF. The catheter was secured to the dura with glue and exteriorized. The CSF and arterial catheters were connected to Cobe CDX disposable pressure transducers.

Estimation of the proportion of cranial CSF transport through the cribriform plate. Because we determined the total flow rates (CSF absorption) with the cribriform plate intact (phase 1 of the experiment) and the residual flow rates after the plate had been sealed (phase 2), we were able to calculate the CSF transport that occurred via the cribriform plate by subtracting phase 2 from phase 1 values. The proportion of CSF transport through cribriform and noncribriform pathways would be affected not only by the relative positions of the pre- and postcribriform obstruction curves with respect to each other but also by the positions of the curves in relation to the absolute values for flow. For this reason, estimates of CSF formation rates were added to the flow values before the proportional flow estimates were calculated.

From this analysis, we focused on cranial transport and used the data collected from animals in which the spinal cord was ligated. We calculated the proportion of CSF transport that occurred through the cribriform plate and through other pathways and plotted these data against the intracranial pressure above opening pressure.

Statistical analysis. These data were analyzed using ANOVA. We interpreted P < 0.05 as significant.

RESULTS

CSF transport in the neonatal sheep. Artificial CSF was infused into the cisterna magna using a constant pressure system. A hydrostatic pressure was established in the ventricles via infusion from a reservoir filled with saline. The reservoir height was varied to alter intracranial pressure incrementally, and the corresponding inflow rate was measured. Once equilibrium was established, the flow into the CSF compartment must equal the flow out (absorption). The pressure-flow relationship is illustrated in Fig. 1. The slope of this relationship equals the conductance of the absorption system and averaged 0.012 ± 0.003 ml·min⁻¹·cmH2O⁻¹. The inverse of conductance is CSF outflow resistance, which averaged 96.5 ± 17.8 cmH2O·ml⁻¹·min. We estimated the CSF formation rate to be 4.1 ± 1.2 ml/h at an average baseline pressure of 6.6 ± 1.1 cmH2O. These CSF parameters were very similar to those published earlier by our group for fetal (~0.8 gestation) and adult sheep (Table 1).

The dotted line (○) represents the flow-pressure relationship with estimates of CSF formation added to each value. This relationship likely reflects more accurately the total system characteristics (see MATERIALS AND METHODS). From this analysis, it is evident that the

![Flow Rate vs ICP](http://ajpregu.physiology.org/graphics/fig1.png)

Fig. 1. Normalized data illustrating the relationship between the flow rate [cerebrospinal fluid (CSF) absorption] and intracranial pressure (ICP) in the neonatal lamb (●, n = 7). The mean values ± SE have been estimated from linear regression analysis of the raw data using incremental ICP values as described in MATERIALS AND METHODS. The slope of the relationship (conductance) was 0.012 ± 0.003 ml·min⁻¹·cmH2O⁻¹ and the reciprocal of the slope (CSF outflow resistance) equaled 96.5 ± 17.8 cmH2O·ml⁻¹·min. These values are very similar to those obtained in a previous study in fetal and adult sheep (comparisons provided in Table 1). The dotted line with ○ represents the conductance curve with values for CSF formation rate added (see MATERIALS AND METHODS). Arrow illustrates the baseline ICP and the corresponding flow (CSF absorption – CSF formation rate) at this pressure.
opening pressure for CSF absorption in the neonate (x-intercept) is ~1 cmH2O. Additionally, the arrow represents the average baseline pressure and corresponding CSF absorption rate. Assuming that CSF absorption and formation are equal under equilibrium conditions, this value is expected to represent the average rate of CSF formation (4.1 ± 1.2 ml/h or 0.068 ml/min; Table 1).

Evidence for cranial CSF absorption into multiple extracranial lymphatic networks. As part of their physiological function, lymphatic vessels absorb extravascular protein and after passage through various lymph nodes, return it to the vasculature. Taking advantage of this function, 125I-HSA or 131I-HSA was injected as a bolus into the cisterna magna, and the radioactivity in various lymph nodes and other tissues was assessed. This method provides only a rough guide to the potential pathways for CSF absorption. Many variables affect the outcome such as the time taken for the tracer to enter the various lymphatic networks. In some cases, the protein may have already passed through the nodes in question, and in other cases, the tracer may not have reached a given location at its peak concentration. In any event, increased radioactivity would indicate the presence of the CSF tracer in transit through the lymphatic channels in the nodes and provide a map of drainage routes. Additionally, one would expect to see the protein tracer in the nodal blood, as some of the tracer would have passed through the lymphatic system (and possibly immature arachnoid projections) into the plasma. For example, expressed as a percentage of the concentration in 1 ml of CSF, the highly vascular spleen contained 0.02% (Fig. 2). Lymph nodes that one would not expect to drain CSF (for example, popliteal and prefemoral nodes) contained similar levels of radioactivity to those measured in the spleen.

Although there was considerable variability in the levels of tracer due to the reasons outlined above, all of the nodes tested in the head and neck region had elevated radioactivity ranging from 0.41 to 1.66% (deep and superficial cervical, preauricular, retropharyngeal, submandibular, and thymic nodes). The nasal mucosa, nasal conch, and nasal septum demonstrated accumulation of the CSF tracer, supporting further the role of the cribriform lymphatic pathway in CSF transport. We did not attempt to perform a systematic analysis of all cranial nerves. However, increased radioactivity in the optic and vagus nerves suggested that CSF and extracranial lymph compartments were linked at other locations as well.

Impact of cribriform plate obstruction on CSF absorption. As noted earlier, several groups emphasized the importance of CSF transport along the olfactory nerves as they penetrate the cribriform plate. We assessed the intracranial pressure vs. flow (absorption) relationship before and after the cribriform plate had been sealed extracranially in the same neonatal animal. This was achieved by removing the tissues on the extracranial surface of the bone and sealing the area with glue. In one group of five animals, CSF was prevented from entering the spinal subarachnoid compartment to restrict the analysis to the cranial system. In four of five preparations, obstruction of the cribriform plate pathway shifted the intracranial pressure vs. flow relationship to the left. An example is illustrated in Fig. 3. It is clear that obstruction of the cribriform plate (1) reduced cranial CSF absorption for a given intracranial pressure and (2) increased intra-

![Image](http://ajpregu.physiology.org/Downloadedfrom)

Table 1. CSF dynamics in fetal, neonatal, and adult sheep

<table>
<thead>
<tr>
<th>Condition</th>
<th>R_{out}, cmH2O·ml⁻¹·min⁻¹</th>
<th>Conductance, ml·min⁻¹·cmH2O⁻¹</th>
<th>Baseline ICP, cmH2O</th>
<th>CSF_{FR}, ml/h</th>
</tr>
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<tbody>
<tr>
<td>Fetal sheep 106–119 days gestation</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>Neonatal sheep 2–6 days postbirth</td>
<td>96.5 ± 17.8</td>
<td>0.012 ± 0.003</td>
<td>6.6 ± 1.1</td>
<td>4.1 ± 1.2</td>
</tr>
<tr>
<td>Adult sheep</td>
<td>84.7 ± 13.7*</td>
<td>0.014 ± 0.005*</td>
<td>11.9 ± 1.4*</td>
<td>7.8 ± 0.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE. R_{out}, outflow resistance; ICP, intracranial pressure; CSF_{FR}, cerebrospinal fluid formation rate. * Data from Ref. 37.
cranial pressure at any given cranial flow rate. The averaged normalized data are illustrated in Fig. 4.

In a second group of seven animals, the cranial and spinal CSF compartments were left in communication with one another. Similar to the situation just described, prevention of CSF transport through the cribriform plate shifted the intracranial pressure vs. flow relationship to the left, although, on average, the displacement was not as great as that observed when the spinal CSF compartment was negated (Fig. 5). This was likely due to the recruitment of additional spinal CSF absorption sites.

**Significance of the olfactory/cribriform plate route for cranial CSF absorption.** With the cranial and spinal CSF systems isolated from one another, we were able to focus on cranial absorption parameters without the additional complexities of transport mechanisms associated with the spinal cord. On the basis of the data in Fig. 4, we estimated the proportion of cranial CSF transport that occurred through the cribriform plate and through other pathways. Excluding the animal in which technical problems were encountered, the data suggest that the majority of CSF transport occurs through the cribriform pathway (Fig. 6). Extrapolating the cribriform curve to the y-axis.

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**Fig. 3.** Individual example indicating that extracranial obstruction of the cribriform plate shifted the ICP vs. flow (CSF absorption) relationship to the left. CSF access to the spinal subarachnoid compartment was prevented in this experiment. ● Represents data obtained with the cribriform plate intact; ○ represents values taken after the cribriform plate was obstructed. Estimates of CSF formation rates have not been added to the data in this graph.

**Fig. 4.** Normalized average data indicating that extracranial obstruction of the cribriform plate shifted the ICP vs. flow (CSF absorption) relationship to the left. Estimates of CSF formation rates have not been added to the data in this graph. CSF access to the spinal subarachnoid compartment was blocked. The average values for flow rates were estimated from linear regression analysis of the raw data using incremental ICP above opening pressure as described in MATERIALS AND METHODS. ● Represents data obtained before, and ○ represents data collected after the cribriform plate was sealed (n = 5). In one of the experiments, some technical problems made it very difficult to establish equilibrium conditions, and data for this sheep were quite different from those observed in the other 4 animals. Inset: data with this example removed. A 2-factor ANOVA (between-factor intact vs. cribriform seal and within-factor pressure) revealed significant differences between the pre- and postcribriform plate obstruction phases of the experiment (P = 0.029, n = 5 and P = 0.005, n = 4).

**Fig. 5.** With the cranial and spinal CSF compartments in communication with one another, obstruction of the cribriform plate also tended to shift the ICP vs. flow relationship to the left. ● Represents data obtained before, and ○ represents data collected after the cribriform plate was sealed. Estimates of CSF formation rates have not been added to the data in this graph. A 2-factor ANOVA did not reveal significant differences between the pre- and postcribriform plate obstruction phases of the experiment (P = 0.290, n = 7).

**Fig. 6.** Proportion of cranial CSF transport that occurred through cribriform and noncribriform pathways. A ligature around the spinal cord prevented CSF access to the spinal subarachnoid compartment. The estimates in this figure are based on the data illustrated in Fig. 4, inset (n = 4). ○ Represents estimated values for cribriform plate transport, and ● represents estimates of CSF clearance that occurred through noncribriform plate routes.
indicates that ~80% of CSF clearance occurred through this route at opening pressures. If we include the additional animal, the y-intercept value drops to ~60% (data not illustrated). In either case, at relatively low pressures above the opening pressure, a significant portion of cranial CSF absorption proceeded through the cribriform plate. As pressures increased, other absorption sites were recruited. For example, at a pressure of ~15 cmH_2O above opening pressure in Fig. 6, other routes assumed the responsibility for ~40% of CSF clearance.

**DISCUSSION**

*Extracranial lymphatics play an important role in CSF transport in the neonate.* Data reported here support a role for extracranial lymphatic vessels in CSF transport in newborn animals. After administration of a protein tracer into the CSF compartment, the pattern of radioactivity external to the cranial vault indicated that transport through the cribriform plate represents an important mechanism for the clearance of CSF. These data also suggest that once delivered by the olfactory nerves into the nasal mucosa, CSF is absorbed by lymphatic vessels and transported back to plasma through a variety of lymph nodes in the neck. Additionally, CSF transports along other nerves as they exit the cranial vault (8, 18). This concept is supported in this study by elevated radioactivity in the optic and vagus nerves and increased tracer in the preauricular and submandibular nodes.

The olfactory-cribriform pathway appears to be the dominant location where extracranial lymphatics have access to CSF. Taking advantage of the fact that this is the only CSF-lymph transport pathway that can be disrupted conveniently, we investigated further the significance of the cribriform plate in volumetric neonatal CSF transport. We compared CSF transport parameters in the same animal before and after obstructing this pathway. An important element of the experimental design was the separation of the cranial and spinal subarachnoid compartments. With this method, we were able to assess cranial CSF absorption without the complexities of CSF transport mechanisms associated with the spinal cord.

We already established the importance of spinal CSF absorption in adult sheep (6). Under baseline conditions, we estimated that ~25% of global volumetric drainage in adult sheep occurred from the spinal subarachnoid compartment (6). The analysis illustrated in Fig. 5 suggests that CSF is absorbed from the spinal subarachnoid compartment in the neonate as well. Several groups provided evidence for spinal CSF transport into lymphatics (4–6, 11). In adults at least, arachnoid proliferations resembling the villi and granulations of the cranial system have also been described in spinal tissues (22, 31, 43), although in many cases, these structures were not associated with veins (43). In any case, the spinal ligation procedure unmasked the important role of the cribriform route in cranial volumetric drainage because the impact of sealing the cribriform plate was greater when CSF movement into the spinal subarachnoid space was negated. Presumably, CSF transport from the spinal subarachnoid compartment can compensate somewhat for the loss of cranial absorption sites.

With the cranial CSF system isolated from its spinal counterpart, several concepts emerge. First, the evidence in this paper and in a previous publication using adult animals (35) suggests that a major portion of cranial CSF transport occurs through this pathway at least at relatively low intracranial pressures. The observed impact of cribriform plate obstruction on cranial CSF dynamics is even more noteworthy considering that this location is only one of many sites where the CSF and lymphatic compartments are in continuity with one another.

Second, some other pathway transported CSF from the neonatal cranial vault when the cribriform plate was sealed (Figs. 3 and 4). A similar pattern was observed in adult sheep (35). In the latter case, we speculated that this transport might have occurred through the arachnoid projections. However, in the neonate, we are left to ponder the nature of this transport under conditions in which it is difficult to attribute this clearance to arachnoid projections. At this stage of development, it is possible that the additional CSF transport occurs along other nerves as they exit the cranial vault.

Finally, the recruitment of the noncribriform CSF transport just alluded to appears to occur only when higher intracranial pressures are attained. Therefore, extrapolation of the postcribriform seal line in Figs. 3 and 4 may correspond to the average opening pressure for the induction of this higher pressure transport system. We estimate that this pressure would be ~4–5 cmH_2O above the opening pressure that initiates clearance through the cribriform plate.

*Comparison of CSF transport in the fetus, neonate, and adult.* Another factor to consider is whether there are any significant differences in CSF dynamics in the neonatal and adult periods. Comparing the data reported here with previous studies from our group (37), we find that CSF dynamics in the fetus, neonate, and adult are remarkably alike (Table 1). The situation in rodents may be somewhat different. In rats and mice, CSF outflow resistance has been observed to be higher in newborns than in older animals (27, 28). For example, resistance to absorption in the rat fetus was between 10.8 and 16.3 mmH_2O·µL⁻¹·min. Resistance increased after birth (39.2) and fell steadily with age such that outflow resistance at 30 days and in adults approached 6.8 and 7.9 mmH_2O·µL⁻¹·min, respectively. Similarly in mice, the resistance to drainage of CSF was much higher in newborns than in older animals (27).

Although our data indicated that CSF outflow resistance was slightly higher in the sheep neonatal period compared with adult or fetal animals, the differences were quite small. It should be noted, however, that we averaged the resistance values in animals between 2 and 6 days old. In the rodent studies, CSF outflow
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resistance was assessed at more times with the highest outflow resistance measured at 1 day after birth (28). In the fetal or 5-day-old rat, CSF outflow resistances were considerably less. Therefore, we have no value in the newborn lamb that is comparable directly.

It is not clear why CSF outflow resistance would be elevated in rodents the day after birth. This could signal a change in absorption mechanisms but more likely reflects some altered physiological reality. For example, in normal rats, CSF and dural venous pressures were not significantly different from one another in animals less than 3 wk old. A positive pressure gradient was not observed until 20 days after birth (29). It is well established that CSF absorption is pressure driven. The lack of a suitable pressure gradient would be expected to provide some impediment to CSF absorption whether this occurs via immature arachnoid projections into the cranial venous sinuses or via lymphatic transport into the veins at the base of the neck. Pertaining to the latter possibility, earlier work from our group indicates an important role for lymphatics in CSF transport in the adult rat (4).

In summary, comparison of the newborn lamb studies reported here with those in the fetus and adult suggests the following. 1) Global CSF transport parameters (CSF conductance and outflow resistance) are very similar in the sheep fetus, newborn, and adult despite the relative scarcity of arachnoid projections in the fetus and neonate. 2) Multiple CSF drainage routes exist in the adult and neonate, and these can compensate to some extent for the loss of CSF clearance sites at a specific location. 3) Negation of CSF transport into the spinal subarachnoid compartment has revealed the importance of the olfactory nerve-cribriform plate pathway in volumetric cranial CSF transport in the adult and neonate. Once CSF convects into the nasal submucosa, it is absorbed by extracranial lymphatic vessels and transported to plasma. 4) At least at relatively low intracranial pressures, the olfactory nerve-cribriform pathway appears to be a dominant site for cranial CSF clearance at all levels of development. 5) When CSF transport into the spinal CSF system was prevented in both neonatal and adult animals, CSF continued to be cleared from the cranial vault even though the cribriform plate was obstructed. This transport occurred at higher intracranial pressures. Whether this clearance took place through arachnoid projections in the neonate is unclear because only the adult has these structures in abundance.

Perspectives

The fact that CSF transport parameters are similar in the fetus, neonate, and adult despite the fact that obvious arachnoid villi and granulations are present in significant numbers only in the adult suggests that the accepted views on CSF absorption may be in need of revision. It is clear that extracranial lymphatic vessels play an important role in CSF transport at each stage of development. Therefore, it seems feasible to propose that the cranial nerve-lymphatic connection represents the primary mechanism by which CSF is cleared from the cranium before and around the time of birth. We cannot of course say that the few arachnoid projections that exist in the neonate have no function. They may have a limited role in CSF transport. It is easier to assign a function to arachnoid villi and granulations as the individual ages. Nonetheless, everything considered, there seems to be considerably more quantitative data supporting a role for extracranial lymphatics in CSF transport than exists to support a function for arachnoid projections at any stage of development (26, 41).

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