Intracranial pressure accommodation is impaired by blocking pathways leading to extracranial lymphatics

R. MOLLANJI, R. BOZANOVIĆ-SOSIC, I. SILVER, B. LI, C. KIM, R. MIDHA, AND M. JOHNSTON

Trauma Research Program, Department of Laboratory Medicine and Pathobiology and Division of Neurosurgery, Sunnybrook and Women’s College Health Sciences Centre, University of Toronto, Toronto, Ontario M4N 3M5, Canada

Received 29 December 1999; accepted in final form 16 January 2001

Several factors have contributed to skepticism regarding the potential role of extracranial lymphatic vessels in the clearance of cerebrospinal fluid (CSF) from the cranial vault. First, lymphatic vessels are not found within the central nervous system parenchyma. Moreover, arachnoid villi and granulations seem strategically placed to drain CSF from the cranial vault, and these structures have been assumed to be the primary site for CSF absorption. Second, the quantitative data supporting a role for the lymphatic system in CSF clearance have not been entirely convincing. Third, ligation of those lymphatic vessels believed to be the most important for CSF transport produced inconsistent effects on intracranial physiology.

With regard to the first point raised in the foregoing, there is considerable anatomical evidence that the CSF and extracranial lymph compartments are connected (8, 15). An especially important pathway is CSF transport along the olfactory bulbs, with movement through the cribriform plate into the nasal submucosa. At this point, either lymphatics absorb CSF that has been mixed with interstitial fluid within the nasal submucosa or the CSF and lymph compartments are linked directly (15).

In relation to the quantitative evidence, work from Bradbury and Cserr (8) and other laboratories (reviewed in Ref. 8) have demonstrated that a significant portion of protein tracers injected into the CSF compartment can be recovered in cervical lymph. However, any tracer that had transported into the plasma by the arachnoid villi route would filter back into the lymphatic compartment, resulting in an overestimate of the lymphatic contribution to CSF clearance. Without appropriate correction for this factor, it is difficult to determine the portion of CSF tracer that enters the plasma by lymphatic and nonlymphatic (arachnoid villi) pathways.

In recent studies, however, the quantitative significance of lymphatics has been elucidated in animals. In adult sheep (4) and rats (6), extracranial lymphatic vessels transport approximately one-half of a protein tracer from the CSF compartment into plasma. The other half gains access to plasma by a nonlymphatic route, presumably through arachnoid villi. In a follow-up to these studies, a mathematical model was developed that permitted estimates of volumetric CSF absorption into lymphatics by use of tracer recovery data. An important element in the design of the model was the ability to correct the recovery data for errors.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
introduced by filtration (5). The data illustrated that 40–48% of all CSF removed from the cranial compartment in sheep was cleared by lymphatics. Furthermore, elevations of intracranial pressure (ICP) resulted in enhanced CSF clearance from the cranial vault, not only through arachnoid villi but also through cervical lymphatic vessels (3). Cervical lymphatic pressures and lymph flow rates increased as ICP was elevated with flows at 70 cmH2O ICP observed to be about fourfold higher than those at 10 cmH2O ICP (20). We estimated that ~77% of the total lymph in cervical vessels had its origins from CSF at the highest intracranial pressure tested.

The recent data point to an important role for cervical lymphatic vessels in CSF transport, but from what is available in the literature, there is no clear indication that obstruction of cervical lymph pathways has a consistent effect on CSF dynamics. There is some evidence in dogs that ligation of the lymph vessels or removal of lymph nodes in the cervical region produces a lymphostatic encephalopathy associated with elevated ICP and cerebral edema (reviewed in Ref. 11). However, others have failed to observe any significant changes after interruption of this pathway (8, 11). In the rat, the removal of the cervical lymph nodes and ligation of the relevant ducts had little effect on brain water and ionic content (8).

It is, perhaps, not surprising that this issue continues to be problematic. The complexity of lymphatic networks and their ability to regenerate rapidly (19) make it difficult to interrupt lymph flow for any period. An alternative method to stopping CSF transport into cervical lymphatics is to block the lymphatic pathway at a more upstream location, the cribriform plate. Bradbury and Westrop (9) achieved this in rabbits by removing the olfactory bulbs and sealing the cribriform plate with cyanoacrylate glue. In this way, the plasma recovery of a CSF protein tracer was reduced significantly, but no attempts were made to determine the impact of this procedure on intracranial CSF dynamics. In the present study, we tested the hypothesis that sealing the cribriform plate on the extracranial (nasal submucosa) side in sheep would have an adverse effect on the ability of the animals to accommodate infusions of artificial CSF into the CSF compartment.

MATERIALS AND METHODS

Animals

Randomly bred female sheep weighing 20–40 kg were used for this investigation. They 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 Centre and conformed to the guidelines set by the Canadian Council on Animal Care and the Animals for Research Act of Ontario.

Experimental Design

Two different methods of volume challenge into the CSF compartment were used in this study. In one method, artificial CSF was infused into the CSF compartment at defined flow rates via a syringe pump and the ICP was measured as the infusion rate was varied incrementally (constant-flow technique). In another method, a hydrostatic pressure was established in the ventricles via a reservoir filled with artificial CSF. The reservoir height was varied to alter ICP incrementally, and the corresponding flow rate was measured (constant-pressure procedure). ICP vs. flow rate relationships were determined before and after sealing of the cribriform plate in the same animal. With experience in using the various infusion protocols, we suspected that CSF transport into the spinal CSF compartment had a significant impact on the results. Because we were interested primarily in cranial CSF parameters, we also challenged the pressure accommodation within the cranial vault under conditions in which CSF flow into the spinal CSF compartment was prevented.

Surgery

Surgical procedures. The sheep were anesthetized initially by intravenous infusion of 5% pentotal sodium solution. After this, the animals were intubated, and surgical anesthesia was maintained using halothane administered through a respirator (Narkomed 2). A midsagittal incision was made in the sheep’s scalp to reveal the junction of the sagittal and lambdoid sutures. Two one-eighth-inch burr holes were made bilaterally 1 cm anterior and 1 cm lateral to the lambda at an angle of 10° from the sagittal plane. A single catheter guide screw was inserted into each hole. A 16-gauge Novalon intravenous catheter (Becton-Dickinson, Sandy, UT) was then attached to a column of artificial CSF (10) (filter sterilized) and fed through the guide screw. Entry of the catheters into the lateral ventricles was confirmed by a sudden drop in artificial CSF volume in the column. In some experiments, a laminectomy was performed on C1 to expose the cisterna magna, which was then cannulated with a 70-cm-long vinyl catheter filled with artificial CSF (Dural Clear Vinyl Tube, 1.00 mm ID; 1.50 mm OD). The catheter was secured to the dura, exteriorized, and connected to a pressure transducer (Cobe CDX disposable). Data were recorded on a computer-based data acquisition system (A-Tech Instruments, Toronto, Visual Designer software, Tucson, AZ).

Blocking CSF transport pathways to extracranial lymphatic vessels. To gain access to the extracranial side of the cribriform plate, a midsagittal incision ~5 cm in length was made longitudinally through the skin. The skin was retracted and the nasal bone exposed. Parallel transverse incisions ~3 cm in length were made through the nasal bone. The proximal incision started at the level of a line bisecting the medial canthi. The distal incision was made 3 cm distant from the proximal one. Two additional parallel incisions were made lateral to the midline to join the transverse incisions. An approximately 3 × 3 cm nasal bone was removed to expose the nasal mucosa. In the experimental group, 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 either bone wax or tissue glue (mixture of ethyl cyanoacrylate and polymethylmethacrylate, Surehold, Chicago, IL). We determined that both agents were effective at sealing the cribriform plate; however, the glue was easier to apply, and we adopted that technique for the constant pressure studies. At the end of the study, Evans blue dye was injected into the CSF compartment to check for CSF leaks.
Constant-Flow Studies

When we were considering the best approach to assess the responses of the ICP-regulating systems to constant-rate infusions, it was sometimes difficult to decide when steady-state ICPS had been achieved. At the commencement of the infusion (0.1 ml/min), it often took up to 30 min for an equilibrium ICP to be attained. At higher infusion rates, equilibrium pressures generally occurred more rapidly (~15 min). In roughly one-half of the animals (both sham and experimental groups), ICP declined a few centimeters of water at variable times after steady-state conditions had seemingly been achieved. This phenomenon occurred even at the lowest infusion rates tested. With this in mind, we assumed that steady-state ICP had been attained when the diastolic ICP remained constant for 250 s (a period characterized by consistently stable conditions).

Artificial CSF was infused continuously via a syringe pump (Kd Scientific, model #260) into a lateral ventricle after passage through a sterile syringe filter (Corning, 0.20 μm) and ICP was measured from the catheter in the cisterna magna. Infusion rates were set at 0.1, 0.2, and 0.3 ml/min. CSF formation in sheep ranges between 4 and 7 ml/h (6, 11). An infusion rate of 0.1 ml/min would essentially double the fluid formation rate in the cranial vault, and an infusion rate of 0.3 ml/min would represent a threefold increase in CSF formation. Once a steady state had been achieved, the flow rate was increased to the next higher level. When the data at an infusion rate of 0.3 ml/min was acquired, the infusion was stopped and the ICP was allowed to return to preinfusion levels. In the experimental group, the cribriform plate was scraped and sealed; in the sham animals, the nasal bone was removed, but the cribriform plate was not scraped or sealed. After this, the infusions were repeated in the same animal.

Constant-Pressure Studies

Artificial CSF was delivered to a lateral ventricle by use of a modification of a method described by Davson et al. (12). The surgical procedures were similar to those described earlier; however, ICP was measured from the contralateral ventricle in this group of animals. A reservoir filled with artificial CSF was placed on a balance (Setra, BL-410S, Abcor, Concord, ON, Canada) connected to a printer. The height of the reservoir and balance was elevated relative to the head of the animal to initiate a CSF inflow rate. The flow of artificial CSF into the ventricle was deduced from the rate of reservoir weight reduction. ICP was recorded continuously from the data acquisition system. By use of this method, steady-state ICPS were usually achieved within 1–2 min and steady-state flow rates attained within 5–10 min. The steady-state flow and ICP were measured at a minimum of three different reservoir heights. After this, the ICP was allowed to return to baseline levels, and either the cribriform plate was sealed with the glue or the animal was subjected to sham surgery. The experiment was then repeated, with data collected at the same reservoir heights. These studies were performed with CSF transport to the spinal CSF compartment blocked.

Blocking CSF transport pathways to the spinal CSF compartment. In the constant-pressure experiments, we prevented CSF transport into the spinal cord by performing a laminectomy at the C1 level. 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. In all animals, systemic arterial pressure increased immediately after the cord was ligated, but blood pressure returned to baseline or slightly lower and remained stable for the duration of the experiment.

Data Analysis

Constant-flow studies. Plots of the steady-state ICP (y-axis) vs. infusion rate (x-axis) gave a linear relationship, and with the assumption that CSF outflow equals inflow in the steady-state condition, the slope of the linear relationship in the constant-flow (CF) studies was taken as a measure of CSF outflow resistance (CF-Rout). We used both mean and diastolic ICP for data analysis and observed no differences (statistical or conceptual) in the outcome measures. Consequently, we settled on diastolic ICP for data presentation. Constant-pressure studies. Because steady-state flow into the ventricles would equal CSF absorption, we plotted ICP vs. flow rate, and the slope of the relationship in the constant-pressure experiments was taken as CSF outflow resistance (CP-Rout). To facilitate comparisons, we also normalized the data. The y-intercept extrapolated from linear regression of the phase 1 data (cribriform intact) represented the opening pressure at which cranial CSF drainage was initiated. This value became the reference point, and the equations of the best fit lines from pre- and postcribriform scrape (or sham) were used to recalculate flow rates at ICPS of 1–5, 10, 15, 20, and 25 cmH2O above opening pressure. The data were replotted with ICP above opening pressure on the y-axis (not the actual measured pressure) and flow rate on the x-axis. For comparisons between the CF and CP studies, we also normalized the former data using the same method.

Statistical Analysis

The data were analyzed using ANOVA. We interpreted P < 0.05 as significant.

RESULTS

Effects of Sealing Cribriform Plate

Constant-flow series. Sixteen animals were used in this series of experiments. Four were excluded because of technical problems (CSF leaks, animal death, unstable ICP). Twelve animals were available for data analysis, including five in the sham group and seven in which the cribriform plate had been sealed. In the sealed group, blockage of the cribriform plate shifted the ICP vs. infusion rate regression lines to the left. A two-way ANOVA indicated that this effect was significant. The mean data are illustrated in Fig. 1A. These results indicated that, for a given ICP, CSF clearance was reduced after sealing of the cribriform plate. Similarly, for a given flow rate, ICP was higher. The y-intercepts increased from 16.9 ± 3.5 in phase 1 of the experiment to 22.0 ± 1.9 cmH2O after pathways through the cribriform plate had been obstructed. There were no significant changes in the slopes of the ICP vs. flow relationships, suggesting that CSF outflow resistance did not increase when CSF transport through the cribriform plate was prevented (phase 1 CF-Rout = 99.3 ± 12.9 cmH2O·ml⁻¹·min⁻¹; phase 2 CF-Rout = 102.6 ± 8.2 cmH2O·ml⁻¹·min⁻¹).

Figure 1B illustrates the mean data from the five animals in which the cribriform plate was left intact. In sham experiments, there was a tendency for the ICP...
vs. infusion rate relationship to shift to the right, but this effect was not significant. The y-intercepts averaged 22.4 ± 1.0 and 20.5 ± 1.1 cmH₂O in phases 1 and 2, respectively. No significant changes in the slopes of the relationships (CF-Rout) were observed. CF-Rout values averaged 119.4 ± 17.1 cmH₂O·ml⁻¹·min before and 119.1 ± 18.0 cmH₂O·ml⁻¹·min after sham surgery.

Constant-pressure series. Twenty-one animals were used in this series of experiments. In contrast to the constant-flow studies, we encountered a greater number of technical problems in this series, no doubt due to the stress associated with the added surgical complexity of the preparation (sealing the cribriform plate and laminectomy, followed by ligation of the spinal cord). These included unstable systemic arterial pressure (2 animals), unstable ICP (2 animals), CSF leaks (2 animals), and hemorrhaging into the CSF compartment (1 animal). In three animals, we could confirm that the catheters were not positioned correctly in the ventricles; in two sheep, we could not establish any flow in phase 2 of the experiment (even at high levels of ICP). With these animals excluded, nine sheep were available for data analysis, including four in the sham group and five in which the cribriform plate had been sealed.

With CSF transport to the spinal compartment blocked, the effects of sealing the cribriform plate were qualitatively similar to those observed in the constant-flow experiments. The ICP vs. flow relationship was shifted to the left, indicating that the flow rates were markedly less after the plate had been obstructed. However, the slopes of the ICP vs. flow relationships were more variable. The raw data from four of the five experiments are illustrated in Fig. 2. In some of the animals, the linear regression lines pre- and postcribriform seal were relatively parallel to one another (example C in Fig. 2 and another result not illustrated).

In two sheep, the slope of the relationship after the
been sealed (calculated from the nonnormalized data, preseal CP-R_{out} = 122.0 \pm 26.0 \text{cm}^2\text{H}_2\text{O}\cdot\text{ml}^{-1}\cdot\text{min}; postseal CP-R_{out} = 291.7 \pm 98.5 \text{cm}^2\text{H}_2\text{O}\cdot\text{ml}^{-1}\cdot\text{min}). However, due to the variability discussed earlier, the slopes were not significantly different (with paired t-test applied to the nonnormalized data, P = 0.192; with ANOVA applied to the normalized data, P = 0.052).

In the sham surgery group, the ICP vs. flow relationship was shifted slightly to the right, but this effect was not significant. Moreover, we did not observe any significant effects of the sham procedure on the slopes of the relationships (presham CP-R_{out} = 97.5 \pm 20.0 \text{cm}^2\text{H}_2\text{O}\cdot\text{ml}^{-1}\cdot\text{min}; postsham CP-R_{out} = 72.1 \pm 12.0 \text{cm}^2\text{H}_2\text{O}\cdot\text{ml}^{-1}\cdot\text{min}).

For comparison, the data from the constant-flow experiments were normalized using the same approach and were plotted as inserts in Fig. 3. As was the case with the analysis of the raw data, the left shift of the ICP vs. flow relationship was significant (two-way ANOVA with Greenhouse-Geisser-adjusted P values). However, with the normalized data, the slopes of the regression lines (CF-R_{out}) increased significantly after the cribriform plate had been sealed. No significant differences in any parameter were observed in the sham group.

**Proportional CSF Transport Through the Cribiform Plate and Other Pathways**

The raw data (Fig. 2) and the normalized values (Fig. 3) from the constant pressure series demonstrated that obstruction of the cribriform plate reduced flow rates (CSF absorption) markedly. Because we determined the total flow rates with the cribiform plate intact (phase 1 of the experiment) and the residual flow rates after the plate had been sealed (noncribriform transport phase 2), we were able to calculate the CSF transport that occurred via the cribiform plate by subtracting phase 2 from phase 1 values. Transport rates through the cribiform plate and transport that occurred via other (undefined) pathways were plotted against the ICP above opening pressure (Fig. 4). At pressures close to the opening pressure, the majority of CSF transport occurred through the cribiform plate. To initiate CSF transport through noncribriform pathways a pressure of \(-5 \text{cm}^2\text{H}_2\text{O}\) higher was required.

Additionally, we calculated the proportion of CSF transport via cribiform and noncribriform pathways in both the constant-flow (Fig. 5A) and constant-pressure experiments (Fig. 5B) and plotted these data against the ICP above opening pressure. With both infusion protocols, it was apparent that the cribiform plate was the dominant location for CSF transport at low to moderate ICP levels. The estimated proportion of CSF transport that drained via other pathways did not approach 50% of the total transport until the ICP approached \(-10 \text{cm}^2\text{H}_2\text{O}\) above opening pressure with the spinal CSF compartment intact (Fig. 5A) and \(-25 \text{cm}^2\text{H}_2\text{O}\) above opening pressure with the spinal compartment blocked (Fig. 5B).

---

**Fig. 3. Normalized data illustrating the relationship between ICP and flow rate by use of the constant pressure method.** Cerebrospinal fluid (CSF) access to the spinal subarachnoid compartment was blocked. Mean (± SE) flow rates have been estimated from linear regression analysis of the raw data with values of ICP 1–5, 10, 15, 20, and 25 cm H2O above opening pressure, as described in MATERIALS AND METHODS. • represent data obtained before and ○, data collected after the cribiform plate had either been sealed (A; n = 5) or subjected to sham surgery (B; n = 4). A two-way ANOVA with Greenhouse-Geisser-adjusted P values revealed significant differences between the phases of the experiments outlined in A (pre- vs. postcribriform plate obstruction), but no significant differences were observed in the sham studies (B). There was an increase in the slope of the ICP vs. flow relationship after the cribiform plate had been sealed, but this was not significant. Constant-pressure outflow resistance (CP-R_{out}) values were observed to decline in the sham group, but this effect was not significant. *Insets:* graphs illustrate data derived from the constant flow experiments (from Fig. 1) normalized and replotted using methods identical to those used in the constant pressure series. A and B represent the data derived from the cribiform sealed group or from the sham animals, respectively.

plate was sealed was much higher (examples A and D). In A for example, CP-R_{out} increased 6.4-fold after the cribiform plate was sealed; in one animal, the slope declined slightly (~20%, example B).

The normalized data are illustrated in Fig. 3 (A, pre- and post-cribriform seal; B, pre- and postsham surgery). A two-way ANOVA with Greenhouse-Geisser-adjusted P values indicated a significant left shift in the ICP vs. flow relationships. On average, the slope of ICP vs. flow relationship was higher after the plate had
DISCUSSION

We investigated CSF dynamics in adult sheep under conditions in which CSF transport through the cribiform plate was interrupted. We demonstrated that blockage of the pathways leading to cervical lymphatic vessels had a significant impact on the ability of the animal to respond to volume infusions.

Technical Issues Associated with Choice of Method to Impair Lymphatic CSF Transport

In past attempts to test the relevance of the extracranial lymphatic pathways to CSF transport, investigators have either ligated the relevant cervical vessels or excised the cervical lymph nodes and monitored CSF and central nervous system parameters. The results from previous studies have been inconclusive. It is very difficult to identify all of the relevant lymphatic vessels if the objective is to obstruct this pathway. Additionally, if we were to assume that an intermediate compartment exists between CSF and extracranial lymph (nasal submucosa), the compliance characteristics of this tissue may have to be saturated before any effect of lymphatic obstruction on ICP could be detected. Furthermore, fluid could be lost at several locations along the lymphatic system, thus lessening the impact of downstream obstruction. In lymph nodes, protein-free fluid transports between blood and lymph as required to establish an equilibrium of Starling forces across the blood-lymph barrier (1). Any increase in lymph pressure could result in loss of fluid (containing CSF) from the nodes into capillaries or loss from the lymphatic vessels themselves via convection of liquid into the blood vessels present in the walls of the ducts (vasa vasorum). Additionally, on the basis of the data presented in this report, the full quantitative impact of lymphatic blockade on cranial CSF clearance may not be appreciated without first negating the influence of spinal CSF absorption. This factor has not been appreciated in the past.

With these issues in mind, we reasoned that the most effective way to test the impact of lymphatic obstruction on CSF transport was to seal the cribiform plate. Of course, total cessation of lymphatic CSF transport may not have been achieved in our studies, because other lymphatic vessels exiting the cranium may have carried some CSF extracranially as well. However, it is likely that the majority of CSF absorption into lymphatics was prevented.

There are several other factors related to the experimental design that deserve mention, because they impact on the interpretation of the results. First, we used two different infusion methods to assess the impact of cribiform blockade on CSF dynamics, and the data from both techniques indicated impaired CSF absorption. Second, data from experimental animals and humans indicate that sagittal sinus pressure is independent of CSF pressure unless very high ICPs are achieved (13). For example, in humans, sagittal sinus pressure remained relatively unchanged in most individuals up to ICPs of 75 mmHg (~100 cmH2O, Ref. 17). However, if ICP is allowed to approach very high levels during infusion into the subarachnoid compartment, there is the possibility that venous sinus pressure would be affected. Under these conditions, measure-
ments of CSF outflow resistance would be difficult to interpret. Although we did not measure dural sinus pressure in our experiments, average diastolic ICPs at the highest flow rates measured did not exceed ~60 and ~30 cmH2O in the constant-flow and constant-pressure experiments, respectively. Therefore, it is unlikely that the sagittal sinus pressure would be affected significantly by the infusion volumes used in this study. Finally, and perhaps the most remarkable element in considering the outcome of these experiments, was the fact that CSF transport was inhibited by surgically interrupting tissues externally.

Importance of CSF Transport Through the Cribiform Plate into Extracranial Lymphatics

In both the constant-flow and constant-pressure experiments, occlusion of the cribiform plate had a significant effect on the ICP vs. flow relationship. In both cases, the shift of the relationship to the left indicated that for a given ICP, CSF transport was inhibited, and for a given flow rate, a higher ICP was required. What was unexpected, however, was the magnitude of the inhibition in CSF absorption that we observed after the cribiform plate had been sealed and the proportion of CSF transport that occurred through the cribiform and noncribriform pathways (Figs. 4 and 5).

In our previous investigations, we used tracer recovery data and mass balance equations to estimate total CSF transport and the proportion cleared by lymphatic and nonlymphatic routes. We estimated that, on average, nearly one-half of total volumetric CSF removal from the cranial system occurred through the cribiform plate. It now appears that these values may have been underestimated. In the tracer experiments, we focused primarily on the two large bilateral cervical lymphatics and the thoracic duct. However, it is very difficult to identify all of the relevant lymphatic vessels in any given tissue compartment, as many of these are small and not easy to distinguish from surrounding tissues. In retrospect, it seems likely that some of the CSF transport we attributed to arachnoid villi (based on tracer recovery in plasma in lymph-diverted animals) was actually via other lymphatic vessels that are small and not easy to distinguish from surrounding tissues. In contrast, CSF transport to the spinal cord appeared to compensate somewhat for the deficiency in cranial CSF absorptive capacity that was lost when the cribiform plate was obstructed.

CSF Outflow Resistance

When we started these experiments, we expected that sealing the cribiform plate would increase CSF outflow resistance. We reasoned that there were a fixed number of CSF absorption sites and that removing a significant number of these would elevate outflow resistance. Although some of the data noted in Results suggested an increase in the slope of the ICP vs. flow relationships, overall the data did not demonstrate clearly a statistically significant increase in CSF outflow resistance when absorption through the cribiform plate was prevented. However, we may be able to rationalize this seeming incongruity by postulating that additional drainage sites were recruited during the infusion process as ICP was elevated.

Recruitment of Spinal CSF Absorption Sites

The spinal cord appears to play an important role in helping to maintain ICP at a constant level. Marmarou et al. (16) have estimated that about one-third of the total central nervous system compliance resides within the spinal axis. In addition, arachnoid villi are associated with spinal nerve roots (reviewed in Ref. 13), and there is evidence that CSF is absorbed into lymphatic vessels draining the spinal cord (7). An elevation in ICP would likely force more CSF into the spinal subarachnoid compartment, where increasing volumes of CSF could be removed by these drainage routes. Therefore, the recruitment of additional CSF drainage sites in the spinal compartment in the constant-flow experiments may have helped to offset the loss of drainage sites caused by cribiform plate sealing.

The importance of spinal pathways in CSF drainage is reflected by the data in Fig. 5. Even though the results illustrated in Fig. 5, A and B, represent data using two different infusion protocols, the fundamental principles behind the two methods are the same, and comparisons may be informative. In the constant-flow studies with an intact connection between the cranial and spinal CSF compartment, the majority of CSF transport occurred through the cribiform plate at low pressures, but as ICP was increased above opening pressure, the proportion of total CSF transport cleared by noncribriform pathways increased (Fig. 5A). When an ICP equivalent to 10 cmH2O above opening pressure was achieved, ~50% of total CSF clearance could be attributed to a noncribriform route. This is in contrast to the results from the constant-pressure studies, in which access to the spinal CSF compartment was blocked (Fig. 5B). In the absence of spinal absorption sites, the point at which CSF transport was equally distributed between cribiform and noncribriform pathways was not reached until pressures approached 25 cmH2O above opening pressure. Consequently, the proportional CSF clearance through noncribriform routes appeared to be greater when CSF had access to spinal absorption sites.

Recruitment of Additional Cranial CSF Absorption Sites

The data suggested that the major site for cranial CSF clearance was through the cribiform plate, but when this route was obstructed, some CSF continued to be removed from the cranial subarachnoid compartment, especially at higher ICP levels. This implied that additional cranial CSF drainage sites were recruited as
ICP was elevated. It would seem reasonable to suggest that the arachnoid villi were responsible for a major portion of the residual CSF drainage. Adult sheep are known to have arachnoid villi, and their anatomy in this species has been studied extensively (13). However, we cannot be certain that these elements accounted for this transport. The most important lymphatic CSF transport pathway is undoubtedly the olfactory route leading to cervical lymphatic vessels in the nasal submucosa, but there are other cranial nerves that may conduct CSF extracranially (8). Additionally, there is evidence that the dura contains lymphatics (2). It is theoretically possible that these vessels could have been responsible for a portion of the CSF drainage that we observed after the cribriform plate had been sealed. In any event, the recruitment of additional cranial drainage sites may have helped to reduce the impact of cribriform plate obstruction on CSF outflow resistance.

There is also some question of whether the recruitment of additional cranial CSF absorption sites represented transport through one pathway or through two different anatomical routes. In the individual examples illustrated in Fig. 2, we observed several patterns of ICP vs. flow relationships that may not have been due to variation between animals. Although we did not observe a nonlinear relationship between ICP and flow in any given animal, it is possible that our data collection captured inadvertently the opposite ends of a continuum in which CSF transport exhibited two distinct phases, a high CSF outflow resistance segment at pressures close to the opening pressure and a lower CSF outflow resistance portion at higher ICPs. The schematic in Fig. 6 illustrates this concept.

When CSF transport through the cribriform plate was prevented, some examples may represent CSF clearance through an initial high CSF outflow resistance pathway (examples A and D in Fig. 2 and illustrated by window A in Fig. 6). Other examples may depict CSF clearance through a lower CSF outflow resistance route (examples B and C in Fig. 2 and illustrated by window B in Fig. 6). Indeed, the two high CSF outflow resistance examples were generated at pressures that were relatively close to the opening pressure (and flow rates that were <0.02 ml/min), whereas those curves that were parallel to the phase 1 response were derived at higher pressures and exhibited larger flows. In the latter case, extrapolation of the dotted line in Fig. 6 may correspond to the average opening pressure for the recruitment of this higher pressure transport system. We estimate that this pressure would be ~5 cmH2O above the opening pressure that initiates clearance through the cribriform plate. Any nonlinearity in the ICP flow relationships could be due to the graded opening of arachnoid villi as ICP is elevated. Alternatively, two distinct CSF drainage pathways may be involved; some clearance (possibly the higher resistance portion) may occur along other (nonolfactory) cranial nerves, and some CSF may be transported through the arachnoid villi (possibly the lower CSF outflow resistance segment). These possibilities require further investigation.

In summary, we obstructed those pathways leading to the cervical lymphatics selectively at an extracranial location (the nasal mucosal side of the cribriform plate). When this route was interrupted, CSF clearance was impaired significantly. The data suggest that several anatomically distinct pathways exist for cranial CSF transport and indicate a prominent role for extracranial lymphatic vessels in global CSF transport.

**Perspectives**

Data presented in this report suggest that CSF transport pathways may be represented by a cribriform-lymphatic route that operates at relatively low ICPs and possibly an arachnoid villi transport system that operates at higher ICPs. In adults, therefore, arachnoid villi may be recruited only under conditions in which CSF transport through the cribriform plate is overwhelmed. In addition, in the fetal and neonatal periods, arachnoid villi may play no role in CSF drainage. These structures have been difficult to identify in the human fetus, whereas they are evident shortly after birth and increase in number with age (reviewed in Ref. 14). If arachnoid villi, granulations, or functional precursors do not exist in the fetus or exist in small numbers, CSF transport may occur almost exclusively through extracranial lymphatics before birth. In this regard, several observations regarding the pathogenesis of hydrocephalus are difficult to explain on the basis of the current concepts that give a prominent position to the arachnoid villus in CSF physiology (14, 18). When all of these factors are taken into

---

**Fig. 6. Schematic illustration of the proposed relationship between ICP and flow rate with CSF pathways through the cribriform plate obstructed and CSF access to the spinal subarachnoid compartment blocked.**
consideration, it may be appropriate to reconsider the role of arachnoid villi in cranial CSF transport.

The authors thank Dianna Armstrong for technical assistance and Marko Katic (Department of Research Design and Biostatistics) for help in the statistical analysis of the data.

This research was funded by the Medical Research Council of Canada.

REFERENCES


