The effects of raised ICP on lymph flow in the cervical lymphatic trunks in cats

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Lymph was collected from cervical lymphatic trunks of anesthetized cats under conditions of normal cerebrospinal fluid (CSF) pressure and again when the CSF pressure was elevated by infusing artificial CSF into the subarachnoid space at the cisterna magna. There was an immediate increase in lymph flow on initiation of the CSF infusion, but this increase was not maintained although the CSF infusion continued. Lymph protein concentrations fell when the CSF infusion started and remained depressed while the infusion of CSF continued. It is postulated that under steady-state conditions much of the CSF leaving the subarachnoid space via the cranial nerves enters the capillaries from the extravascular spaces, and that large molecules from the CSF, such as proteins, return to the blood via the lymphatic system.

KEY WORDS • cervical lymphatic system • intracranial hypertension • lymph flow • lymph protein • cerebrospinal fluid absorption

For more than a century the arachnoid villi of the cerebral venous sinuses have been thought to provide the preeminent route for the return of cerebrospinal fluid (CSF) to the blood. While other routes have been described, most have been dismissed as nonparticipatory. Although the central nervous system is devoid of lymphatics, the lymphatic vessels outside the neuraxis have received persistent attention as vehicles for the return of CSF to the blood. Recent studies in cats and rabbits have clearly demonstrated that tracers introduced into the CSF or brain interstitial fluid can be recovered from the cervical lymph trunks. These studies have suggested that up to 50% of the tracer may be recovered in lymph from the rabbit and up to 20% in the cat under conditions of normal CSF pressure. The olfactory and optic nerves have been identified as pathways along which CSF can travel from the subarachnoid space to reach the lymphatics.

We have investigated the effect of raising the CSF pressure on the flow and protein concentration of lymph collected from the cervical lymphatic trunks of cats.

Materials and Methods
Thirteen adult female cats, weighing 2.5 to 3.5 kg each, were anesthetized with sodium pentobarbital (30 mg/kg), endotracheally intubated, and ventilated to maintain normal blood gases. Cannulas were placed in the femoral artery and femoral vein for the purposes of measuring the pressure in these vessels, withdrawing blood, and adding drugs as required. A small polyethylene cannula (0.61 mm outside diameter) was placed in the sagittal sinus through a midline burr hole, and was directed toward the torcular. This was used to measure sagittal sinus pressure (SSP). Ringer's lactate solution, adjusted to a pH of 7.4 with sodium bicarbonate, was slowly infused into the femoral vein. The hematocrit was checked regularly as a guide to the hydration of the animal.

The retropharyngeal lymph node on one side was injected with 0.05 ml of blue ink to identify the lymph trunk(s) leaving the node. The largest trunk was cannulated with polyethylene tubing (0.28 mm inner diameter, 0.61 mm outer diameter, 8 to 10 cm length) and any remaining trunks were tied off. All lymph trunks from the retropharyngeal lymph node on the contralateral side were identified in a similar manner and were ligated.

* Ventilator, Model 607, manufactured by Harvard Apparatus Co., Inc., 150 Dover Road, Millis, Massachusetts.
† Polyethylene cannula (Intermedic PE-10) manufactured by Clay Adams, Division of Becton, Dickinson and Co., Parsippany, New Jersey.
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181 Mean Arterial Pressure

16

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CSFP

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-0.4- ~Lymph Flow

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Concentration I

0.0 a 0

TIME (hr)

FIG. 1. Results of measurements in one animal after infusion of cerebrospinal fluid (CSF) showing the relationship between the various parameters. Upper: Mean arterial pressure, CSF pressure (CSFP), and sagittal sinus pressure (SSP). Values are given in kiloPascals (kPa). Lower: Lymph flow rate and lymph protein concentration.

With the animal secured in a stereotaxic frame, a cannula was placed in the cisterna magna to measure the CSF pressure and to infuse CSF when appropriate. In two cats, a Swan-Ganz catheter† was placed extradurally over the left parietotemporal cortex through the midline burr hole, which allowed access to the sagittal sinus. The integrity of the cranium in all animals was restored with cyanoacrylate adhesive.

Vascular and CSF pressures were measured using Sanborn pressure transducers and were recorded on a Sanborn four-channel polygraph.§ Transducers were zeroed at the level of the intra-aural line. Artificial CSF was infused using a Harvard infusion pump.¶ Lymph was collected continuously in weighed vials which were changed every 15 minutes. The protein content of the lymph and blood samples was determined by the Lowry method. Blood gases were checked hourly. *

† Swan-Ganz catheter manufactured by Edwards Laboratories, 17221 Red Hill Avenue, Santa Ana, California.
§ Sanborn four-channel polygraph manufactured by Hewlett-Packard, Sanborn Division, Waltham, Massachusetts.
¶ Harvard infusion pump, Model 940, manufactured by Harvard Instrument Co., Inc., 150 Dover Road, Milis, Massachusetts.
* Blood gas monitor, Model 165, manufactured by Corning Scientific Instruments, Medfield, Massachusetts.

and body temperature was monitored with a rectal thermistor and was maintained by means of a heating pad.

When all cannulas were in position and functioning, control measurements were made until the lymph flow had stabilized. This required a minimum of 75 minutes, but usually took longer. At the end of the control period, infusion of artificial CSF commenced at a rate of 2.83 cu mm-sec⁻¹, and continued until all parameters became stable, particularly lymph flow and CSF pressure. This period lasted for approximately 4 hours. Six animals were examined in this manner. In two of these animals, dextran blue 2000 was added to the artificial CSF at the end of the test period and additional lymph samples were collected to determine if the dye appeared in the cervical lymph following its introduction into the subarachnoid space. In seven animals, the standard protocol was not followed completely. Two were prepared as for the standard protocol, but no infusion of CSF was carried out. Lymph was collected for 6 hours to test the stability of the preparation. In two animals, the CSF pressure was raised by inflating the balloon of the Swan-Ganz catheter rather than by infusing CSF. For two other animals, the standard protocol was followed, but the infusion rate was 1.15 cu mm-sec⁻¹. For the last animal, a cannula was placed in the major lymphatic trunk from each retropharyngeal lymph node, and the standard infusion protocol was followed.

Results

The mean lymph flow rate (± standard deviation) during the control period for the 13 cats was 0.181 ± 0.067 cu mm-sec⁻¹. There was no correlation between the lymph flow and the mean arterial pressure or the plasma protein concentrations. Lymph flow rates and protein concentrations were well maintained in the two animals that did not have a CSF infusion over a 6-hour period.

The infusion of CSF caused the CSF pressure, SSP, and lymph flow to increase immediately (Fig. 1). The peak lymph flow usually occurred later than the CSF pressure peak, and then declined to values close to the control values. In one animal, the lymph flow reached a peak and did not show any tendency to fall although the CSF pressure had fallen considerably. The relationships between the control CSF pressure and control lymph flow and the peak CSF pressure for eight animals are shown in Fig. 2 (six animals with a CSF infusion rate of 2.83 cu mm-sec⁻¹ and two with a rate of 1.15 cu mm-sec⁻¹). For six of eight animals, the lymph flow at peak CSF pressure was less than the peak lymph flow, the latter occurring after the CSF pressure had fallen from its peak.

Figure 3 shows the linear regression line through the individual points of Fig. 2, supplemented by points representing the control CSF pressure and lymph flow values of the remaining five animals. The slope of the
ICP and cervical lymph flow

The effect of the CSF infusion on lymph protein concentration was determined (Fig. 1). The mean lymph protein concentration just prior to the start of CSF infusion in 12 cats was \(21.48 \pm 6.30 \text{ mg ml}^{-1}\). A small reduction in protein concentration was seen in the first sample of lymph collected after the infusion started, but the lowest values for lymph protein concentration were usually found in the third or fourth sample; that is, 45 to 60 minutes after the infusion began. The mean low lymph protein concentration, determined from six cats, was \(6.71 \pm 3.42 \text{ mg ml}^{-1}\). Thereafter, there was a rise in the protein concentration concomitant with the falling flow rates of lymph. As long as the CSF infusion continued, the mean lymph protein concentration, as measured in six animals, remained below the control values, at \(10.70 \pm 3.81 \text{ mg ml}^{-1}\).

Dextran blue 2000 introduced into the subarachnoid space in the infused CSF appeared in the lymph within 15 to 30 minutes. Inflation of the Swan-Ganz balloon catheter caused an immediate rise in CSF pressure and lymph flow. However, it was difficult to maintain a steady CSF pressure with this technique, and so results of the collection of lymph were not considered very reliable. For the two animals perfused with CSF at 1.15 \(\text{ cu mm} \cdot \text{sec}^{-1}\), the results were qualitatively similar to those in animals perfused at the higher rate, and they have been included in Figs. 2 and 3. In the single animal with two lymphatic canulas, the results were similar to those obtained from animals in which one lymphatic cannula was employed.

The effect of infusing artificial CSF in the subarachnoid space on the CSF pressure and SSP may be seen in Fig. 1 and Table 1. The SSP rose more slowly than the CSF pressure, resulting in a larger pressure gradient between the two during this dynamic phase compared with that during the stable states.

**Discussion**

Although only one cervical lymph trunk was cannulated in the present study, the control lymph flows were greater than those reported by Bradbury, et al.,\(^2\) for the cat. Those authors cannulated a major lymphatic trunk from each retropharyngeal lymph node, but they did not ligate any remaining lymphatics and the canulas were smaller in diameter than those used in this study. In our study, while it was possible to identify the major

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**TABLE 1**

*Effect of CSF infusion on CSF pressure and SSP*

<table>
<thead>
<tr>
<th>Parameter Measured</th>
<th>Pressure at Infusion</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control Pressure (no infusion)</td>
</tr>
<tr>
<td>CSFP</td>
<td>0.88 (6.6)</td>
</tr>
<tr>
<td>SSP</td>
<td>0.56 (4.2)</td>
</tr>
<tr>
<td>CSFP − SSP</td>
<td>0.31 (2.3)</td>
</tr>
</tbody>
</table>

* CSF = cerebrospinal fluid; CSFP = CSF pressure; SSP = sagittal sinus pressure. Values are mean pressures given in kiloPascals (and mm Hg in parentheses). The data were determined from six animals that underwent infusing at 2.83 \(\text{ cu mm} \cdot \text{sec}^{-1}\).† Mean SSP at peak CSFP.
lymphatic vessel from the retropharyngeal lymph node as a glistening vessel filled with clear fluid, secondary lymphatics were rarely identified until the node had been injected with ink. The number of subsidiary lymphatics ranged from zero to three and varied between animals and even from side to side in individual animals.

The protein concentrations in the first lymph collections were higher than those obtained later in the control period. It is possible that injury to blood vessels in the lymph node during injection of ink led to a protein leak which resolved over the control period. The protein concentration stabilized at a mean value of 21.48 mg·ml⁻¹, which is lower than that reported by Bradbury and Cole² (33 mg·ml⁻¹).

When artificial CSF was infused at 2.83 cu mm·sec⁻¹, the CSF pressure rose to a peak and then fell to a stable value well below the peak. This has been reported previously in association with a decrease in CSF outflow resistance, and it was suggested that the institution of alternative pathways may have contributed to the change in outflow resistance. Under the conditions of the present study, it is probable that CSF outflow was facilitated by a decrease in outflow resistance.

We found that the SSP increased when the CSF pressure increased, and that the gradient between CSF pressure and SSP increased. Such a change has been noted previously in many species, including man. Nevertheless, it is customary to assume, when CSF outflow resistance is being calculated, that SSP is not influenced by CSF pressure. The gradient between CSF pressure and SSP in the present study increased by 81%, even though CSF absorption increased from 0.33 cu mm·sec⁻¹ (normal intrinsic production) to 2.83 cu mm·sec⁻¹ or more, an increase of 850%. Either the movement of CSF into the venous sinuses of the cat is of little importance at these CSF pressures or there is a very large decrease in outflow resistance across the sinuses.

Lymph flow generally reflects the balance of fluid moving out of and into capillaries at their arterial and venous ends, respectively. This fluid movement depends on the Starling forces; that is, the relationship between hydrostatic and osmotic forces within the vessels and in the extravascular spaces. However, lymph collected from the outflow pathways of the retropharyngeal lymph nodes may contain fluid that has reached the extravascular spaces from the subarachnoid space, namely CSF. Such fluid is low in protein compared with the extracellular fluid originating from capillaries. When CSF was infused into the subarachnoid space, the lymph flow increased, presumably as an increased volume of CSF reached the extravascular space drained by the retropharyngeal lymph node. The effect of the addition of this low-protein fluid to the extravascular space would be primarily to reduce the extravascular osmotic forces. There may also have been some increase in tissue hydrostatic pressure. These changes in Starling forces would promote the return of fluid to the capillaries and reduce the production of extravascular fluid from them. Since CSF is composed primarily of water and electrolytes that can pass easily across the wall of the capillary endothelium, it is hypothesized that, in a state of equilibrium, most of the CSF reaching the extravascular spaces is returned to the blood across the capillary wall and that a very small volume, with large molecules, returns to the blood via the lymphatic system.

These hypothesized modifications of the Starling forces took up to 4 hours to complete. The lymph flow in the first samples collected after the CSF pressure peak was achieved probably provides a close approximation of the effect of CSF pressure on cervical lymph flow before absorptive modifications have occurred. The slope of the line in Fig. 3 is 0.0817 cu mm·sec⁻¹·kPa⁻¹. Thus, as shown in these experiments, a 4-kPa increase in CSF pressure would allow the removal of all intrinsically produced CSF (0.33 cu mm·sec⁻¹) by this route.

The peak lymph flow in six of eight animals occurred well after the CSF pressure had peaked. In the remaining animals, the collection interval may have been so long as to hide a discrepancy between the CSF pressure peak and the peak lymph flow. This finding suggests that the outflow resistance for CSF leaving the subarachnoid space decreased, assuming that the pressure gradient from the subarachnoid space to the extravascular spaces was the driving force.

The pattern of changes in lymph protein concentration may be explained by considering that the lymph came from two different sites, one where CSF contributed to the extracellular fluid and the other where it did not. It is assumed that lymph from the latter did not change during the course of the experiment either in quantity or quality. The lymph containing excess CSF, however, would contain very much less than the normal amount of protein during the CSF infusion. Initially, with the increased lymph flow, the total lymph protein level would be greatly depressed, while later, when lymph flow returned to normal values, the total lymph protein would rise. At this time, lymph from CSF-contaminated areas would still be low in protein due to the diluting effect of the CSF, but the contribution of lymph from this area would now be normal. The continuously depressed protein concentrations that occurred while the CSF infusion was being given, together with the finding of the dextran blue 2000 in the lymph, point to the continued addition of CSF to the extravascular spaces despite the return to the normal lymph flows.

These results do not give a clear indication of the volume of CSF leaving the subarachnoid space by the cranial nerve routes. However, using the maximum increase in lymph flow as a guideline, between 6% and 27% of CSF reached the lymph cannulas. This is in the same range as estimated by Bradbury, et al.² These values are likely to be underestimated since we do not know how much fluid may be absorbed into capillaries.
ICP and cervical lymph flow

either in the extravascular spaces or at the retropharyngeal lymph nodes.

We conclude that raising the CSF pressure results in a temporary increase in cervical lymph flow associated with an increased volume of CSF reaching the extravascular spaces drained by the retropharyngeal lymph nodes.

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References


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