Cerebrospinal fluid formation in ventricles and spinal subarachnoid space of the rhesus monkey

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The authors perfused rhesus monkeys from lateral ventricles to lumbar sac with an artificial cerebrospinal fluid (CSF) containing a Blue Dextran 2000 marker. Analysis of marker dilution at steady state showed ventricular CSF formation occurring at a rate of 28.3 ± 2.5 µl/min. No significant CSF formation was found in the spinal subarachnoid space.

Key Words • cerebrospinal fluid formation • choroid plexus • spinal cord • spinal subarachnoid space • bulk flow

The choroid plexus has for some years been considered a significant source of the cerebrospinal fluid (CSF). However, in recent years the possibility of CSF production from extrachoroidal sources has been debated. A parallel and related debate has been waged over the concept of bulk flow of fluid through the extracellular space of the brain.

No direct measurement of such bulk flow has ever been reported. However, recent reports by Sato, et al., purport to measure directly the extrachoroidal production of CSF by both brain and spinal cord using a perfusion technique with an inulin marker. The examination of the spinal cord as a possible site of CSF production is particularly interesting because of its total lack of choroid plexus. Investigators prior to Sato, et al., had been unable to detect any CSF formation by the spinal cord.

The use of inulin as a marker in perfusion experiments to measure CSF formation rates has been common for several years. Its use is based on the concept that it is removed from the CSF compartment virtually exclusively by bulk absorption of CSF and not by diffusion, or at least any significant diffusion, into central nervous system (CNS) parenchyma. Thus, dilution of the inulin marker concentration should be a direct indication of the formation of new CSF. However, Davson, et al., using ventriculocisternal perfusion but determining CSF production gravimetrically, found that the inulin production rate was falsely elevated. He also found inulin production rates to be spuriously high when compared to those measured by a Blue Dextran 2000 marker. In addition, Hochwald and Wallenstein continued to detect some inulin clearance at perfusion pressures of -15 cm H2O, at which point albumin clearance was zero. Finally, Curran, et al., did a simultaneous inulin and albumin perfusion study that yielded inulin clearance values.
significantly higher than those for albumin, a finding which they attributed to the diffusional loss of inulin from the CSF compartment.

Since the surface area for diffusional loss of a marker into the spinal cord is extensive, there is some value in using a marker that is more clearly free from diffusional loss than inulin in reexamining the spinal cord as a possible CSF-producing organ. Blue Dextran 2000, with a molecular weight of $2 \times 10^6$, seems to be most suitable.

Materials and Methods

Adult rhesus monkeys weighing 2 to 4 kg were anesthetized with pentobarbital and were perfused from one lateral ventricle to the lumbar sac with an artificial CSF containing approximately 50 mg of Blue Dextran 2000* per 100 cc of perfusion fluid. The inflow needle was stereotaxically placed in the lateral ventricle through a burr hole. Its position was verified by the prompt appearance of blue dextran in the cisternal fluid following the introduction of a small amount of perfusion fluid into the ventricle. The outflow needle was placed in the lumbar sac by percutaneous lumbar puncture, and the height of the outflow adjusted to the interaural line. All animals were perfused for 4 hours at approximately 0.15 ml/min with a Harvard pump† which was calibrated for each experiment. Outflow samples were collected hourly for the first 3 hours and then every 15 minutes for the last hour. At the end of the 4-hour perfusion period a sample of the cisternal fluid was taken. Values for dextran concentrations in the inflow, outflow, and cisternal samples were determined by measuring absorbence at 554 nm on a Gilford 300-N spectrophotometer‡. All animals achieved and maintained a steady state for the entire fourth hour of the perfusion as indicated by the constancy of the dextran absorbence values for all outflow samples throughout that period. The steady-state outflow concentration of dextran ($C_o$) was therefore taken to be the arithmetic average of the values for all of the outflow samples during the fourth hour of perfusion.

If it is assumed that dilution of the blue dextran at a steady state occurs exclusively as a result of the formation of new CSF, the following equations can be written.

For that portion of the CSF system between inflow and outflow (that is, the ventricular system plus the spinal subarachnoid space),

$$V_f = \frac{V_i(C_i - C_o)}{C_o}, \quad (1)$$

where $V_f$ = total steady-state CSF formation rate in the system, $V_i$ = rate of inflow from the calibrated Harvard pump, $C_i$ = concentration of blue dextran in the inflow, and $C_o$ = concentration of blue dextran in the steady-state outflow.

For that portion of the CSF system between the inflow and the site of the cisternal sample (i.e., the ventricular system),

$$V_f(vent) = \frac{V_i(C_i - C_c)}{C_c}, \quad (2)$$

where $V_f(vent)$ = steady-state ventricular CSF formation rate, and $C_c$ = concentration of blue dextran in the steady-state cisternal sample.

For that portion of the CSF system between the site of the cisternal sample and the outflow (that is, the spinal subarachnoid space),

$$V_f(cord) = V_f - V_f(vent), \quad (3)$$

where $V_f(cord)$ = steady-state CSF formation rate in the spinal subarachnoid space.

It should be noted that this method has the advantage of not requiring an artificial anatomical or physiological isolation of the spinal cord subarachnoid space, since once the steady-state CSF formation rate for the whole system is known, the formation rate for any portion of that system can be determined by simply sampling the fluid at the appropriate demarcation point.

Results

Table 1 shows the tabulated results of all experiments in this series. The mean $V_f$ and the mean $V_f(vent)$ are virtually identical, and no significant production of CSF in the spinal subarachnoid space is shown. Indeed, the blue
TABLE 1

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Production of CSF</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Ventricular</td>
<td>Subarachnoid</td>
</tr>
<tr>
<td>1</td>
<td>22.6</td>
<td>22.6</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>42.6</td>
<td>42.6</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>26.7</td>
<td>23.9</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>33.4</td>
<td>32.6</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>28.7</td>
<td>29.9</td>
<td>-1.2</td>
</tr>
<tr>
<td>6</td>
<td>27.7</td>
<td>28.5</td>
<td>-0.8</td>
</tr>
<tr>
<td>7</td>
<td>22.6</td>
<td>20.4</td>
<td>2.2</td>
</tr>
<tr>
<td>8</td>
<td>24.4</td>
<td>26.0</td>
<td>-1.6</td>
</tr>
<tr>
<td>mean ± SE</td>
<td>28.6 ± 2.4</td>
<td>28.3 ± 2.5</td>
<td>0.3 ± 0.5</td>
</tr>
</tbody>
</table>

* All values are in µl/min.

dextran absorbence value for the steady-state cisternal sample (Cc) was identical to that for the steady-state outflow (Co) in two experiments, was slightly greater than Co in three experiments, and was slightly less than Co in three experiments. This finding is at variance with the findings of Sato, et al., 16,18 in dogs, and its significance will be discussed later.

The mean ventricular CSF formation rate [Vr_vent.] for these rhesus monkeys is 28.3 ± 2.5 µl/min. This agrees well with Curran’s rhesus monkey values of 30.1 and 31.7 µl/min in two separate series of experiments both using 131I-albumin as a marker.3

Perfusion of artificial CSF containing trypan blue dye through the system with subsequent gross postmortem examination of the CNS in some of these animals following the completion of the dextran experiments showed good, uniform staining throughout the ventricular system and the spinal subarachnoid space.

Discussion

It has certainly been well established that the composition of the CSF can be altered at points far distant from the choroid plexus. The relative increase in the protein content of lumbar CSF as opposed to ventricular CSF is an example. In addition, studies such as those of Sweet and Locksley19 with 42K, 36Cl, and D2O, show the passage of labeled materials into the CSF compartment below the site of a complete CSF block. However, molecular or ionic exchange of material, whether by diffusion or active processes, between CNS parenchyma and CSF should not be confused with bulk flow of fluid as in net CSF formation. Simple exchange of molecules and ions can readily occur in the absence of net movement of fluid as Graziani, et al., 8 have shown for calcium in experiments using 45Ca. Net CSF formation at sites distant from the choroid plexus is quite another matter, however, and is a crucial issue in formulating any comprehensive concept of total CSF dynamics.

The present study in rhesus monkeys shows no net CSF formation at such an extrachoroidal site, namely the spinal cord. Previous studies by Sato, et al.,16,18 have shown some apparent CSF formation by the spinal cord of dogs. While this could be a species variation, it is important to note that Sato’s work was done with an inulin marker. The accuracy of measurements using such a marker has been severely questioned by several recent investigators as has been noted earlier.3,6,7,11 The authors themselves, in studies of the effect of dexamethasone on their observed spinal cord CSF formation rate,18 noted that in some animals this rate approached zero after steroids and after 5 hours of perfusion. They suggested that deterioration of the preparation after such a long perfusion could account for such an observation. However, the complete or partial filling of the spinal cord extracellular space of such animals with inulin with a resulting decrease or total cessation of diffusional inulin loss could also account for such findings. This latter explanation would indeed be more consistent with the increasing evidence for significant amounts of diffusional loss of inulin from the CSF compartment. Since the studies of Sato, et al.,16,18 were not controlled with regard to measurements of CNS parenchymal inulin content, no certain conclusion can be drawn at present. However, the results of any such perfusion studies which show a net dilution of a marker and which are not controlled for diffusional loss of that marker must remain open to question.

Other investigators have perfused the spinal cord subarachnoid space and attempted to measure CSF production there as well. These include Coben and Smith’s studies in the dog in which a limited area of
CSF formation in the rhesus monkey cord isolated by ligatures was perfused. No CSF formation was found, but the disruption of blood flow caused by the ligatures makes the system relatively unphysiological. In the studies on the cat of Hammerstad, et al., and Lorenzo, et al., the animals were perfused in a cephalad to caudad direction using Blue Dextran 2000 and/or ¹²⁵I-albumin markers. The perfusions were either ventriculocisternal, ventriculolumbar, or cisternolumbar. From an analysis of the data from the different types of perfusions, the authors concluded that none of the observed CSF formation occurred in the spinal subarachnoid space.

In the present study, as has been noted earlier, no artificial spinal cord isolation was used. Moreover, with regard to the spinal cord portion of the perfusion, the technique is open to very little in the way of artifactual alteration. The concentration of dextran in the cisternal sample is affected by choroidal CSF production and could also be affected by any hypothetical CSF production from brain parenchyma above the site of the cisternal sample. However, once in the cistern, further dilution of the dextran before reaching the lumbar outflow needle could only occur by something happening in the spinal subarachnoid space, and such dilution was, of course, not seen in this case.

Thus, the conclusion is that there is no net CSF production by the spinal cord. While this does not rule out the possibility of bulk flow locally within the spinal cord tissue itself, it does indicate that at some point a diffusional component is necessary for the clearance of material from the spinal cord parenchyma via the CSF route.

The conclusions of this study also emphasize the need for more detailed examination of the relative roles of the choroid plexus and the brain parenchyma in the formation of intracranial CSF with careful controls over the marker substances used. Fundamental to our understanding of CSF physiology is that we determine to what extent the choroid plexus is responsible for total net CSF formation in the normal state.

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References


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