Extraventricular origin of the cerebrospinal fluid: formation rate quantitatively measured in the spinal subarachnoid space of dogs

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The spinal subarachnoid space of the dog was perfused with an artificial cerebrospinal fluid containing inulin as a tracer. The experimental procedures were based upon the concept that the decrease in inulin concentration occurring during the perfusion was exclusively a function of the volume of newly formed cerebrospinal fluid in the system.

KEY WORDS cerebrospinal fluid surface area spinal cord subarachnoid space

The site of cerebrospinal fluid (CSF) production is generally considered to be the choroid plexus. The assumption that CSF is formed by the choroid plexuses is based upon Cushing’s observation of fluid coming from the plexus and upon Dandy’s experiments with hydrocephalus. Dandy removed the choroid plexus from one lateral ventricle of the dog, plugged the foramen of Monro of the same side, and then made the animal hydrocephalic by plugging the aqueduct of Sylvius. In Dandy’s experiment, only unilateral enlargement of the opposite side ventricle developed, the ventricle with the choroid plexus removed not enlarging. He considered this as a good evidence that the choroid plexus produced CSF.

According to Bering, however, this conclusion was based upon the erroneous presumption that if the foramen of Monro had been patent, there should have been a bilaterally symmetrical hydrocephalus. However, Dandy did not attempt this important control experiment, and this was done by Bering who in 1962 stated that in the absence of a choroid plexus the ventricle did not enlarge whether the foramen of Monro was open or closed.

Weed in his now classical series spoke of a dural origin of the CSF. There are now a number of reports suggesting or demonstrating an extraventricular CSF formation. In 1963 Bering and Sato proposed that there was evidence for intracranial extraventricular CSF formation as measured quantitatively in the dog by means of inulin perfusion. We are reporting a study done to see if any CSF is formed in the spinal subarachnoid space in the dog.

Method

Mongrel dogs weighing between 12 to 15 kg were anesthetized intraperitoneally with 30 mg of Pentobarbital per kilogram of body
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weight, and endotracheally intubated. They were then given 20 mg of succinylcholin intramuscularly to maintain an immobilized position throughout the experiment, and the endotracheal tubes were connected to a respirator. The whole perfusion system and technique used followed the method described by Pappenheimer, et al., but with some modifications to adapt it to this particular purpose. The dog was placed in a prone position with the head slightly elevated. Laminectomies were carried out over the area of the cauda equina and upper cervical region. The lumbosacral subarachnoid space was punctured initially with an Argyle Medicut cannula (0.70 mm i.d.) which was connected with an inflow tubing through which an artificial cerebrospinal fluid containing 25 mg of inulin was injected. Inflow fluid pumping rates were in the range of 0.30 to 0.36 mL/min, but constant for any experiment to ±0.002 mL/min. The upper cervical subarachnoid space was also carefully entered with an Argyle cannula, and this was connected with an outflow tubing whose height could be altered to change the perfusion pressure two or three times during the experiment. Meticulous care was taken not to allow any leakage of CSF in and around the puncture sites, and the possible tear of the meninges was sealed with Eastman 910 glue. Outflow samples, of which volume was determined gravimetrically, were obtained every 10 min after steady states were reached at different pressures, and these were analyzed by the Resorcinol recovery method for inulin concentration determination.

By the method of Heisey, et al., CSF formation rates were calculated combining the effects of hydrostatic pressure on outflow-inflow differences and on clearance of inulin which measured only bulk absorption. The notation used is as follows:

\[ \dot{V} = \text{rate of flow in mL/min} \]

\[ i, o = \text{subscripts referring to inflow and outflow} \]

\[ f, a = \text{subscripts referring to formation and bulk absorption of fluid} \]

\[ C = \text{concentration of quantity (inulin)} \]

\[ \bar{C} = \text{mean concentration in the perfusion system} \]

\[ = C_o + 0.37 (C_1 - C_o) \]

\[ \dot{N}_x = \text{steady-state transport of any substance (X) from the perfusion system of CSF to the blood} \]

\[ = \dot{V}_f C_1 - \dot{V}_o C_o \]

\[ C_{in} = \text{steady-state clearance in mL/min of inulin out of the perfusion system per unit concentration}. \]

The relation between inulin clearance and bulk absorption can be written as:

\[ \dot{V}_o - \dot{V}_i = \dot{V}_f - \dot{V}_a. \]

If \( \dot{V}_o - \dot{V}_i \) is substituted for \( O - I \) and \( \dot{V}_a \) for \( C_{in} \), from Equation 1 the formation rate can be calculated by the following equation:

\[ \dot{V}_f = (O - I) + \dot{V}_a. \]

We know from the equation the outflow rates are less than inflow as more fluid is absorbed than is produced. The outflow rate is increased as the outlet is lowered and decreased when it is raised.

**Results**

Perfusions were made in 42 normal dogs but in only 12 dogs (41 different pressures) were the data considered satisfactory with reference to good fit of the tubing system and distribution of the area perfused. In this series of experiments the formation rate of CSF in the space was expressed as follows:

\[ \dot{V}_f = 0.0201 (\pm 0.0024) - 0.288 \times 10^{-4} \text{ CSFP mL/min}. \]

The outflow-inflow difference and absorption rate were given as:

\[ O - I = 0.0206 (\pm 0.00165) - 0.348 \times 10^{-3} \text{ CSFP mL/min}, \]

and

\[ \dot{V}_a = 0.0005 (\pm 0.00005) + 0.320 \times 10^{-3} \text{ CSFP mL/min}. \]

CSFP stands for cerebrospinal fluid press-
Fig. 1. Graph plotting outflow-inflow difference rate of CSF in the spinal subarachnoid space. 

\( O - I = 0.0206 \pm 0.00165 - 0.348 \times 10^{-3} \text{CSFP mL/min.} \)

From Equation 4, the formation rate was calculated as 0.018 (±0.002) mL/min when the outflow was equal to the inflow rates at the pressure of 59 mm H₂O. The perfusion pressure ranged between -22 and +288 mm H₂O with the reference to the level of the spinal cord during the perfusion. The clearance of inulin was taken as a measure of absorption bulk. This absorption rate (Equation 6) increased as the perfusion pressure was elevated by raising the outflow tubing outlet, and there was a good linear correlation between the rate and the pressure altered.
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When the outflow was equal to the inflow, the absorption rate was equal to the rate of production of CSF.

The effect of hydrostatic pressure on the rate of formation of CSF was a slight negative slope in Equation 4, but the slope of regression was very gentle (Fig. 3). Therefore, within the range of hydrostatic pressure studied, pressure was proved to have only a little effect, if any, on the function of CSF production in either the spinal subarachnoid space or the intracranial cerebrospinal spaces.

Although Bering and Sato claimed that the difference between the venous pressure and the pressure of the CSF was a more precise measurement of the effect of the hydrostatic pressure in CSF perfusion experiments, no attempt was made to measure venous system pressure in these experiments because of technical problems raised in this particular site of perfusion.

Every perfusion experiment was followed by an autopsy to see if the perfusion was limited to the spinal subarachnoid space and had bathed the entire system. This was confirmed by perfusion of methylene blue under the same conditions immediately after the final sample had been collected.

In this method of cephalad perfusion it was recognized that there was no mixing of subarachnoid fluid between the intracranial and spinal subarachnoid spaces. In some experiments, it was noticed that the blue dye streaked over the dorsal surface of the spinal cord for the distance between the inflow and outflow tubing. This was conceived as good evidence of limited distribution of inulin containing artificial CSF in the space perfused.

Almost no CSF formation was detected, and the data were discarded.

Discussion

The formation of CSF has been investigated by a variety of methods in the past year, but the perfusion method of studying the CSF system developed by Pappenheimer, et al., and Heisey, et al. is the most satisfactory one. Although Bering and Sato claimed that the difference between the venous pressure and the pressure of the CSF was a more precise measurement of the effect of the hydrostatic pressure in CSF perfusion experiments, no attempt was made to measure venous system pressure in these experiments because of technical problems raised in this particular site of perfusion.

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pobormite, and salicylate appear in the CSF of dogs below a complete spinal block. The chemical studies of Wallace and Brodie showed that ions can cross from plasma into the CSF at points other than the choroid plexus. Boldrey, et al., and Tubiana, et al., both demonstrated that radiosodium passes into the CSF below a complete spinal block, while Sweet, et al., showed the same to be true of tagged serum albumin.

The work of Bito, et al., was one of the few studies in which the spinal subarachnoid space was perfused. Perfusions were circulated from the lumbar subarachnoid space to the cisterna magna of the rabbit, but no data concerning the rate of formation of the CSF in that compartment were given. Nor did these authors mention whether the perfusions were limited to the space.

Coben and Smith investigated iodine clearance in the spinal subarachnoid compartment by means of perfusion of the space with artificial CSF containing Na131I and Na125I as tracers. They found no CSF production in the spinal subarachnoid space in the dog; however, they isolated the cord by extradural ligation or transection, and the derangement of the blood supply of the isolated spinal cord could have been a factor. The dogs they used varied from 6 to 17 kg in weight, and the area perfused was short and limited, extending from T7–8 down to L6–7. Thus, the rate of formation, if any, of the CSF in the limited area of the spinal subarachnoid space of a 6-kg dog might be very small, and could be readily overlooked.

Hammerstad, et al., performed an experimental work in which the spinal subarachnoid space was perfused from cisterna magna to the lumbar CSF compartment of the cat. Blue dextran or human albumin were added as the indicators of bulk formation of CSF but the authors found that the indicators were not diluted by newly formed CSF arising from the compartment perfused, as the mean rate of formation during the cisternolumbar perfusions was 0.015 ± 0.001 mL/min, and this figure was equal to the rate of 0.015 ± 0.001 given during the ventriculolumbar perfusions. Lorenzo, et al., did ventriculocisternal perfusions in the cat and assumed that the rate of formation in cisternolumbar perfusions came from the intracranial part of the space flowing down to the area perfused; it is possible that this could happen in their caudal direction of the perfusions, but the extent of the perfusate is still unknown. It could be postulated that the rate of 0.015 ± 0.001 mL/min during the cisternolumbar perfusions might come, at least in part, from the area perfused if one estimates that in ventriculolumbar perfusions there could be poor mixing of the artificial CSF with the tracers in that relatively large and extensive cerebrospinal space.

In our study, the extent of the area perfused was confirmed by methylene blue pumped into the system under the same condition at the end of each experiment. No dye ascended beyond the level of the outflow tubing orifice when the compartment was perfused in the cephalad direction; actually, the use of a cephalad direction for the perfusion was adopted because of difficulty in inserting the inflow cannula into the high cervical spinal subarachnoid space when a caudally directed perfusion was attempted in preliminary experiments. Furthermore, while the spinal subarachnoid space was being perfused, methylene blue introduced into the lateral ventricles resulted in good staining of the whole ventricular system and the CSF space at the base of the skull; the dye emerged from the fourth ventricle at a certain interval of time after it was introduced but was not seen coming down into the spinal subarachnoid space. Therefore, in this cephalad perfusion method, adequate physiological isolation of the spinal subarachnoid space was readily achieved without ligating or dissecting the spinal cord.

As noted from Fig. 3, the rate of CSF production in the spinal subarachnoid space was revealed to be independent from the CSF pressure, as the slope of the regression is very gentle and not much different from zero. The result is in good agreement with the cases in other compartments of the CSF. Sahar, et al., stated that the production rate in the hydrocephalic ventricles of the cat was pressure-dependent, decreasing in higher pressures; they postulated that this was caused by an adverse effect of intraventricular pressure on the choroid plexus, ependyma, and adjacent brain tissue.

In previous papers Sato and Bering described that the rate of CSF formation in the
intracranial subarachnoid space of dogs weighing 15 to 20 kg was 0.014 mL/min by direct measurement, and 0.020 mL/min when the figure of 0.027 mL/min, which was the rate of CSF formed in the ventricular system, was subtracted from 0.047 mL/min, the rate produced in the entire intracranial spaces. Considering the surface area of the intracranial and spinal subarachnoid spaces, we obtained very interesting results. After fixing the brain and spinal cord with 10% formalin, we made serial sections 2 mm thick in the coronal plane. We calculated the surface areas by using a rotatory measuring device (Kelvimeter) on every consecutive section. Average surface areas of the intracranial and spinal subarachnoid spaces of 12 to 15 kg dogs were found to be 120.1 cm² and 119.8 cm² respectively. Since the CSF production rate in the spinal subarachnoid space was 0.018 mL/min, this similarity could be worthy of note.

Although no new information has been provided from these experiments concerning the mechanism of CSF formation, it is important that this extraventricular production of fluid be recognized.

**Summary**

The spinal subarachnoid space of the dog was perfused with an artificial CSF containing inulin as a tracer, and extraventricular CSF formation in this space was demonstrated at the rate of 0.018 mL/min in 12 to 15 kg dogs. The fact that perfusions were conducted in a cephalad direction provided adequate physiological isolation of the spinal subarachnoid space without ligating or dissecting the spinal cord. The rate of CSF formation in the space was independent of hydrostatic pressure. The surface area of the intracranial and spinal subarachnoid spaces have been briefly discussed in relation to possible corresponding CSF formation rates in these compartments.

**References**


18. Katzenelbogen S: *The Cerebrospinal Fluid and its Relation to the Blood. A Physiological and*


33. Wallace GB, Brodie BB: On the source of the cerebrospinal fluid. The distribution of bromide and iodine throughout the central nervous system. J Pharmacol Exp Ther 70:418–427, 1940


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