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# Fluid Perfusion as a Method of Cerebrospinal Fluid Formation Rate – Critical Appraisal

Dariko Orešković<sup>1</sup>, Jurica Maraković<sup>2</sup>, Miroslav Vukić<sup>3</sup>, Milan Radoš<sup>4</sup> and Marijan Klarica<sup>4</sup>

<sup>1</sup> Laboratory of Neurochemistry and Molecular Neurobiology, Department of Molecular Biology, »Ruđer Bošković« Institute, Zagreb, Croatia

<sup>2</sup> Department of Neurosurgery, University Hospital »Dubrava«, Zagreb, Croatia

<sup>3</sup> Department of Neurosurgery, University Hospital Center »Zagreb« and School of Medicine, University of Zagreb, Zagreb, Croatia

<sup>4</sup> Department of Pharmacology and Croatian, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia

## ABSTRACT

*The aim of the study was to evaluate whether or not cerebrospinal fluid formation rate (Vf) calculated according to the equation of Heisey et al.<sup>4</sup>, truly show the produced cerebrospinal fluid. For this reason Vf was simulated (40.6 µL/min) by an infusion pump in a plastic cylinder and the evaluation was done by comparing the results obtained between the calculated Vf and the simulated one. In both cases the result should be the same (40.6 µL/min). Other types of experiments were carried out by ventriculocisternal perfusion (92.4 µL/min) on anaesthetized and sacrificed cats. If the equation is correct, the calculated Vf for sacrificed animals should be zero, because there is no Vf in dead animals. The fact that the calculated Vf (46.5 µL/min) in the plastic cylinder was different ( $p < 0.0001$ ) from the simulated one (40.6 µL/min) and that Vf was calculated even for dead animals (3–5 µL/min) clearly shows that the perfusion method may not be an accurate method for determination of Vf.*

**Key words:** cerebrospinal fluid, cerebrospinal fluid formation rate, ventriculocisternal perfusion

## Introduction

According to the generally accepted hypothesis of cerebrospinal fluid (CSF) dynamics, CSF is produced within the cerebral ventricular system, and circulates slowly from the brain ventricles towards the cortical subarachnoid space, to be absorbed into the venous sinuses across the arachnoid villi. It is believed that CSF is formed mainly by the secretory activity of the choroid plexuses in the brain ventricles and that the passage through the choroidal epithelium is an active metabolic process which transforms the ultrafiltrate into a secretion, i.e. CSF. The CSF formation rate (Vf) in animals has been extensively studied by the ventriculocisternal perfusion technique, which is still regarded as the most precise method<sup>1–4</sup>. The method and the equation for the calculation of the CSF formation rate have been established by Heisey et al.<sup>4</sup> and Pappenheimer et al.<sup>5</sup>, who assumed that the dilution of the indicator substance is a conse-

quence of the newly formed CSF, i.e. that a higher CSF formation rate will result in a higher degree of dilution of the indicator substance. Therefore, any mistake in the interpretation of the degree of dilution of the indicator substance in the perfusate caused by other reasons (escape of indicator substances or water into the brain tissue, or irregular mixing) will result in questionable and often contradictory conclusions regarding CSF formation rates<sup>6–11</sup>.

For this reason we have developed a perfusion method in a plastic cylinder (see Materials and Methods) where the »formation rate« was simulated experimentally (40.6 µL/min) and the »rate of formation« (»Vf«) was calculated using the equation developed by Heisey et al.<sup>4</sup>. If the method was correct and precise, no significant differences between the calculated Vf and simulated Vf should be expected. In addition, we have tested the perfusion

method on sacrificed animals. Two things are important on this model: a) the anatomical CSF relationship was strictly preserved (which is important for the real perfusion flow and regular mixing of indicator substance) and b) the CSF formation certainly does not exist. On the assumption that the perfusion method is correct, the CSF formation rate should be zero on dead animals.

## Material and Methods

### *Perfusion in a plastic chamber*

Perfusion in a plastic cylinder (Figure 1) was performed in a 2 mL plastic syringe. Two plastic cannulas (O.D. 1.2 mm) connected with perfusion pumps were introduced from the back of the syringe. »CSF was formed« (»simulated Vf«: 40.6  $\mu\text{L}/\text{min}$ , rate of infusion of artificial CSF) through the upper cannula by the first pump (Palmer, London, England) and the plastic cylinder was perfused (252.0  $\mu\text{L}/\text{min}$ ) through the lower cannula by the second pump (Harvard, Boston, USA) using the method of Heisey et al.<sup>4</sup>. The specimens were collected through the cannula connected to the front syringe orifice and the end of cannula was positioned at 10 cm below. All connections to the syringe were hermetically closed by cyano acrylic glue.

When the plastic cylinder was filled with artificial CSF, the infusion pump for the simulation of Vf was turned on, and after one hour of steady-state period, the specimens were collected in plastic tubes every 15 minutes during the following two hours and weighed (Mettler, Toledo, Switzerland) for volume determination. Under the same experimental conditions, perfusion in the plastic cylinder was then performed with an indicator substance (dextran blue, Pharmacia, Uppsala; Sweden) dissolved in artificial CSF (1 mg/mL) using the method of Heisey et al.<sup>4</sup>.

### *Animals*

Experiments were performed on domestic cats of both sexes ( $n=4$ ) weighing between 1.9 and 3.3 kg in compliance with the Law on Animal Rights and Animal Protection of the Republic of Croatia. Animal quarters were

kept at a temperature of 23 °C, with natural light–dark cycles, and entered between noon and two p.m. for cleaning and supplying fresh water and food. The animals were housed in individual cages and fed commercial cat food (SP215 Feline, Hill's, Topeka, Kansas, USA). Before any experimental procedure was undertaken, the cats were quarantined for 30 days.

The animals were anesthetized with chloralose (Chloralose, Fluka Chemika, Buchs, Switzerland, 100 mg/kg, i.p.) and the anesthesia was maintained by the administration of the anaesthetic via a polyethylene cannula in the femoral vein. The cats were positioned in a stereotaxic frame (Cat model, D. Kopf, Tujunga, California, USA) with their heads elevated, the external auditory meatus being at 15 cm above the stereotaxic table (sphinx position). Ventriculocisternal perfusion was performed with an indicator substance (dextran blue) dissolved in artificial CSF (1 mg/mL) using the method of Heisey et al.<sup>4</sup>, modified for use in cats<sup>6</sup>. A 22-gauge needle was placed by a micromanipulator into the left lateral ventricle at coordinates 4.5 mm anterior and 9 mm lateral from the zero point of the stereotaxic atlas, and 8–10 mm vertically from the dural surface, until free communication with the CSF was obtained. The needle was connected via a polyethylene tubing to a perfusion pump and the perfusion solution infused at a desired rate. From a second needle, which punctured the cisterna magna, 20-min samples of the perfusate were collected in glass tubes (Figure 2). The perfusion was allowed to proceed for 60 min to stabilize prior to collecting the first sample, as well as after changing the perfusion rate. Intracranial pressure (ICP) was measured at the inflow needle, using a Statham strain gauge feeding into a polygraph (7D, Grass, Quincy, Massachusetts, USA). ICP was adjusted at  $-10\text{ cm H}_2\text{O}$  by positioning the outflow tubing 10 cm below the external auditory meatus. The level of the external auditory meatus was taken as pressure zero.

Body temperature was maintained at 37 °C using an infrared lamp connected to an electronic thermometer placed in the rectum. The femoral artery was cannulated for blood pressure recording and blood sampling for acid-base balance determination. During the experiments, no significant changes in the monitored physiological

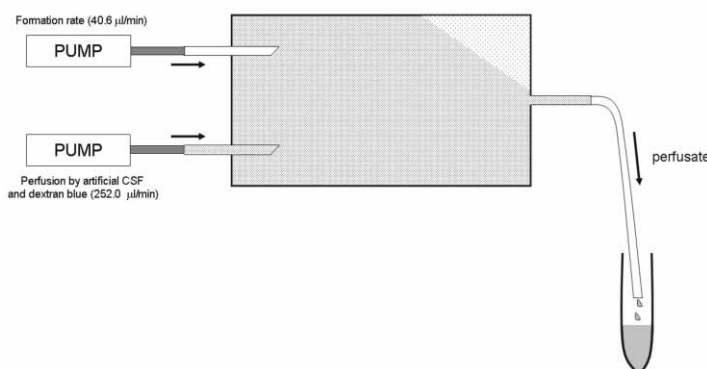


Fig. 1. Scheme of the perfusion in plastic chamber.

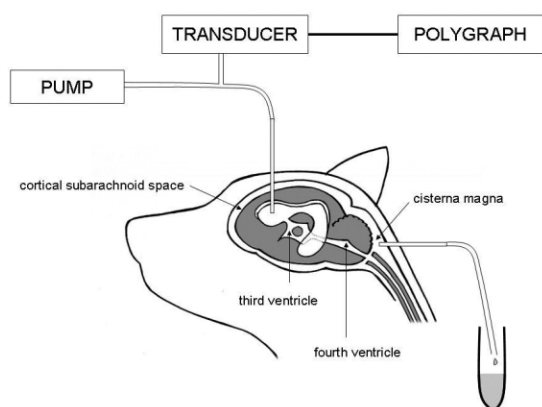


Fig. 2. Scheme of the ventriculocisternal perfusion in cats.

parameters were observed while the cats were breathing spontaneously.

The animals were sacrificed by an anesthetic overdose and, after 30 min of perfusion under the same conditions as on anesthetized cats (see above), samples were collected every 20 minutes. After the collection, the samples were centrifuged at 3000 rpm for 5 min to remove particular matter, and the optical density of the perfusate was measured using a spectrophotometer (55B, Perkin-Elmer, Norwalk, Connecticut, USA) at a wavelength of 635 nm.

#### Calculation of CSF formation rate

The CSF formation rate (Vf) was calculated according to the equation derived from Heisey et al.<sup>4</sup>:

$$Vf = Vi (Ci - Co) / Co$$

where Vi is the inflow perfusate rate, Ci is the concentration of the indicator substance in the inflow perfusate, and Co is the concentration of the indicator substance in the outflow perfusate (sample, mg/mL). The calculation of Vf is based on the dilution of the indicator in the outflow perfusate. For this reason, it is of utmost importance that the indicator does not diffuse from the perfusate into the surrounding nervous tissue. Therefore, dextran blue, as a large molecule ( $2 \times 10^6$  m.w.), was used as the indicator substance in our experiments. The CSF formation rate (Vf) was expressed as  $\mu\text{L}$  of CSF per min. For statistical evaluation Student's t-test was used and  $p < 0.05$  was taken as statistically significant.

#### Results

Table 1 shows the results of four experiments conducted in the plastic cylinder to calculate CSF formation (Vf) according to the equation of Heisey et al.<sup>4</sup> at a perfusion rate of  $252.0 \mu\text{L}/\text{min}$ , and the results for the CSF obtained by simulated formation using a pump at infusion rate of  $40.6 \mu\text{L}/\text{min}$  during two hours. The calculated Vf value is evidently statistically significantly different ( $p < 0.0001$ ) from the Vf produced by simulation using a pump, although results should be identical in both cases ( $40.6 \mu\text{L}/\text{min}$ ).

**TABLE 1**  
RATE OF INFUSION OF ARTIFICIAL CSF (SIMULATED »Vf«) IN PLASTIC CYLINDER AND CALCULATED »Vf« ACCORDING HEISEY METHOD IN FOUR EXPERIMENTS (MEAN AND S.E.M.) DURING 120 MIN.

Time (min)	Simulated »Vf« ( $\mu\text{L}/\text{min}$ )	Calculated »Vf« ( $\mu\text{L}/\text{min}$ )
0–15	$40.2 \pm 3.5^*$	$45.9 \pm 7.0$
15–30	$40.1 \pm 3.7^*$	$46.4 \pm 6.5$
30–45	$40.5 \pm 3.6^*$	$46.4 \pm 11.1$
45–60	$40.5 \pm 3.4^*$	$46.7 \pm 11.5$
60–75	$40.7 \pm 3.0^*$	$47.2 \pm 12.9$
75–90	$39.9 \pm 3.1^*$	$46.1 \pm 9.4$
90–105	$40.1 \pm 3.5^*$	$46.4 \pm 10.0$
105–120	$40.0 \pm 3.6^*$	$46.4 \pm 9.4$
0–120	$40.6 \pm 1.1^{**}$	$46.5 \pm 3.2$

\* $p < 0.001$ , \*\* $p < 0.0001$ , Vf – formation rate

The volume of the calculated CSF obtained by ventriculocisternal perfusion on anaesthetized and sacrificed cats is shown in Figure 3. The perfusion was performed using dextran blue as the indicator substance ( $1 \text{ mg}/\text{mL}$ ) diluted in artificial CSF at the rate of  $92.4 \mu\text{L}/\text{min}$ , in both cases during 80 min. The calculated CSF formation was evidently present in observation periods both prior to (about  $16.0 \mu\text{L}/\text{min}$ ) and after (about  $3.0 \mu\text{L}/\text{min}$ ) animal sacrificing. As no CSF formation is possible in dead animals, what occurred was evidently an error of the method rather than detection of the values that would stand for Vf.

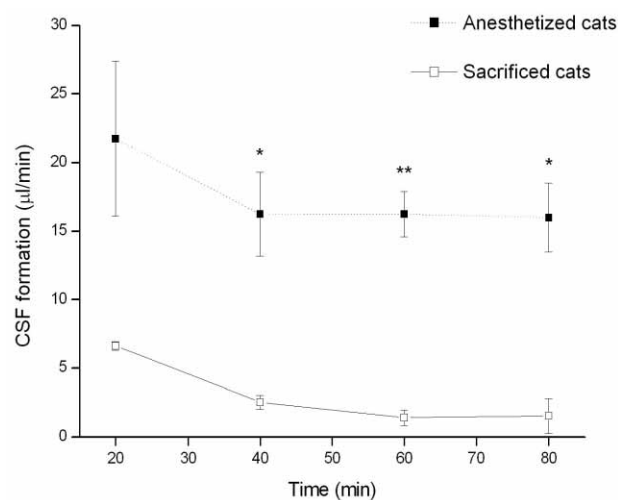


Fig. 3. The cerebrospinal fluid (CSF) formation rate ( $\mu\text{L}/\text{min}$ ) calculated by equation of Heisey et al. obtained by ventriculocisternal perfusion ( $92.4 \mu\text{L}/\text{min}$ ) with dextran blue ( $1 \text{ mg}/\text{mL}$ ) dissolved in the artificial CSF in a four cats before (closed square) and after sacrifice (open square) at  $-10 \text{ cm H}_2\text{O}$ . The vertical lines show the standard error of mean value. Differences between the anaesthetized and sacrificed animals are statistically significant in each observed period except after 20 min (\* $p < 0.05$ , \*\* $p < 0.01$ ).

## Discussion

Current hypothesis of CSF physiology (see Introduction) is based on CSF formation and absorption, in addition to CSF circulation, as its crucial assumptions. The results obtained by Heisey method for Vf determination have been most frequently used as the key evidence to confirm such a hypothesis. On the other hand, the authors of this method (Heisey et al.<sup>4</sup>) have determined the conditions to be met for the successful application of the method, although these conditions have actually not been the subject of a serious scientific analysis. This primarily refers to the indicator substance that should not be absorbed by the surrounding tissue during perfusion, and to presumptions that all CSF should be formed before the connection point with the outflow cannula (see Material and Methods), and absorbed exclusively thereafter. If a substance is absorbed by the surrounding tissue, this will change the outflow concentration of the substance and produce false result. If CSF is formed after the outflow cannula, its volume will not be measured, and each CSF absorption episode occurring before the outflow cannula will result in an error that will be manifested by a reduced CSF formation level in both cases.

As in the meantime some warranted doubts have been put forward regarding both the hypothesis itself<sup>12–15</sup> and the method of perfusion<sup>6,8–10</sup>, it is essential that both should be subject to a serious scientific evaluation. Therefore, in this study we have evaluated the perfusion method by applying two novel approaches: one was perfusion in a plastic cylinder and the other on sacrificed animals.

In the experiments performed in the plastic cylinder, we met all the aforementioned conditions (we prevented absorption of the indicator substance into the surrounding tissue, all CSF was produced above the collection site and there was no CSF absorption before this site), and we were also aware of how much CSF is formed by simulating CSF formation by means of the infusion pump (40.6  $\mu\text{L}/\text{min}$ ). Providing that the method of Heisey et al.

was correct, the results obtained by this method in the controlled conditions as described above, should match the ones obtained through simulation by means of a pump, which did not happen. The calculated results (Table 1) were statistically significantly higher than the actual results ( $p < 0.0001$ ), which clearly points to an error that occurs during the Vf calculation. Which error is it precisely? The answer to that question cannot be obtained based on this study, but it will be a subject of our further investigations.

Also, the results registered in another group of experiments involving sacrificed cats (Figure 3) were quite opposite to those expected. In fact, although 80 minutes passed after the animals were sacrificed, the calculated values continued to show persisting CSF formation (between 3 and 5  $\mu\text{L}/\text{min}$ ). As CSF could not possibly be formed in dead animals, it is evident that an error of the method is in question. It is quite likely that at negative pressure ( $-10 \text{ cm H}_2\text{O}$ ) perfusion itself, conducted on a dead animal, caused flushing of the CSF system and thus demonstrated certain »formation«. However, if this occurred in dead animals, a reasonable question arises as to what perfusion itself does in live animals.

In conclusion, the obtained results have clearly demonstrated that the perfusion method by Heisey et al.<sup>4</sup> cannot not be used with precision as a method to calculate CSF formation (Vf) since the calculated values significantly deviate from the actual experimental condition.

## Acknowledgements

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D. Orešković

Department of Molecular Biology, Ruđer Bošković Institute, Bijenička c. 54, 10 000 Zagreb, Croatia  
e-mail: doresk@rudjer.irb.hr

## KRITIČKO VREDNOVANJE PERFUZIONIJE METODE KAO METODE ZA ODREĐIVANJE STVARANJA CEREBROSPINALNOG LIKVORA

### SAŽETAK

Svrha prikazanih istraživanja bila je provjeriti da li perfuziona metoda izračunavanja stvaranja cerebrospinalnog likvora (Vf) po Heisey i sur.<sup>4</sup> zaista prikazuje stvaranje likvora. U tu svrhu »Vf« je u plastičnoj komorici simuliran pomoću infuzione pumpe (40,6  $\mu\text{L}/\text{min}$ ), a provjera je vršena uspoređivanjem rezultata izračunanog Vf-a s pomoću Heiseyve perfuzione metode i onog simuliranog s pomoću pumpe. U slučaju da je perfuziona metoda točna, rezultat bi trebao biti u oba slučaja isti (40,6  $\mu\text{L}/\text{min}$ ). U drugoj skupini pokusa vršena je ventrikulocisternalna perfuzija (brzina 92,4  $\mu\text{L}/\text{min}$ ) na anesteziranim i žrtvovanim mačkama. Ukoliko je ispitivana perfuziona metoda točna izračunane vrijednosti na žrtvovanim mačkama Vf-a bi morale iznositi 0  $\mu\text{L}/\text{min}$ , jer ne postoji stvaranje likvora na mrtvim životinjama. Budući da se izračunani »Vf« (46,5  $\mu\text{L}/\text{min}$ ) u plastičnoj komorici značajno razlikuje ( $p < 0.0001$ ) od simuliranog s pomoću pumpe (40,6  $\mu\text{L}/\text{min}$ ) i budući da je izračunani Vf kod mrtvih životinja varirao od 3–5  $\mu\text{L}/\text{min}$ , onda to jasno ukazuje da se perfuziona metoda ne može koristiti kao pouzdana metoda za izračunavanje stvaranja cerebrospinalnog likvora (Vf).