The formation of cerebrospinal fluid: nearly a hundred years of interpretations and misinterpretations

Orešković, Darko; Klarica, Marijan

Source / Izvornik: **Brain Research Reviews, 2010, 64, 241 - 262**

Journal article, Accepted version
Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

https://doi.org/10.1016/j.brainresrev.2010.04.006

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:696282

Orešković D., Klarica M. (2010) ***The formation of cerebrospinal fluid: nearly a hundred years of interpretations and misinterpretations.*** **Brain Research Reviews, 64 (2). pp. 241-62. ISSN 0165-0173**

http://www.elsevier.com/locate/issn/01650173

http://www.sciencedirect.com/science/journal/01650173

http://dx.doi.org/10.1016/j.brainresrev.2010.04.006

The formation of cerebrospinal fluid: nearly a hundred years of interpretations and misinterpretations

D. Orešković[1,*], M. Klarica[2]

[1]Ruđer Bošković Institute, Department for Molecular Biology, Zagreb, Croatia

[2]University of Zagreb School of Medicine, Department of Pharmacology and Croatian Institute for Brain Research, Zagreb, Croatia

All authors equally participated in presented work.

[*]Correspondence:

Darko Orešković, PhD

Rudjer Bošković Institute

Department of Molecular Biology

Bijenička 54, 10 000 Zagreb, Croatia

Phone: (+) 385 1 468 0 218

Fax: (+) 385 1 456 1 177

e-mail: doresk@irb.hr

Keywords: Cerebrospinal fluid, Cerebrospinal fluid formation, Cerebrospinal fluid hydrodynamics, Cerebrospinal fluid physiology, Human, Cat,

Abbreviations: BV, brain ventricle; C_i, concentration of a marker in inflow perfusate; C_o, concentration of marker in outflow perfusate; CNS, central nervous system; CSF, cerebrospinal fluid; CM, cisterna magna; ISF, interstitial fluid; ICP, intracranial pressure; LV, lateral ventricle; MR, magnetic resonance; V_f, rate of cerebrospinal fluid formation; V_i, rate of inflow perfusate; V_o, rate of outflow perfusate; Q_{CSF}, cerebrospinal fluid flow;

Abstract

The first scientific and experimental approaches to the study of cerebrospinal fluid (CSF) formation began almost a hundred years ago. Despite researchers being interested for so long, some aspects of CSF formation are still insufficiently understood. Today it is generally believed that CSF formation is an active energy consuming metabolic process which occurs mainly in brain ventricles, in choroid plexuses. CSF formation, together with CSF absorption and circulation, represents the so-called classic hypothesis of CSF hydrodynamics. In spite of the general acceptance of this hypothesis, there is a considerable series of experimental results that do not support the idea of the active nature of CSF formation and the idea that choroid plexuses inside the brain ventricles are the main places of formation. The main goal of this review is to summarize the present understanding of CSF formation and compare this understanding to contradictory experimental results that have been obtained so far. And finally, to try to offer a physiological explanation by which these contradictions could be avoided. We therefore analyzed the main methods that study CSF formation, which enabled such an understanding, and presented their shortcomings, which could also be a reason for the erroneous interpretation of the obtained results. A recent method of direct aqueductal determination of CSF formation is shown in more detail. On the one hand, it provides the possibility of direct insight into CSF formation, and on the other, it clearly indicates that there is no net CSF formation inside the brain ventricles. These results are contradictory to the classic hypothesis and, together with other mentioned contradictory results, strongly support a recently proposed new working hypothesis on the hydrodynamics of CSF. According to this new working hypothesis, CSF is permanently produced and absorbed in the whole CSF system as a consequence of filtration and reabsorption of water volume through the capillary walls into the surrounding brain tissue. The CSF exchange between the entire CSF system and

the surrounding tissue depends on (patho)physiological conditions that predominate within those compartments.

Section 4. Structural Organization of the Brain

Contents

1. Introduction

Cerebrospinal fluid (CSF) is a major part of the extracellular fluid of the central nervous system (CNS) and fine regulation of its composition is vital to the brain's health (Johanson, 2008; Redzic et al., 2005). The CSF has a number of important functions. It is a physiological medium for the brain and provides mechanical support for the brain in the way that the brain floats in the CSF, reducing its effective weight (Segal, 1993). Thus, in the air the brain weighs 1.500 g and its weight in the CSF is 49.5 g, according to Archimedes' law. The CSF appears to serve the brain as a major biological river, transporting humoral messages from one region to another (Johanson et al., 1999; Knigge, 1974) and providing a "sink" serving as an important route for the removal of a variety of waste products produced by cellular metabolism (Segal, 1993; Wolfson, 1974). The CSF in the CNS is divided in two well connected units. The internal unit fills the brain ventricles (the two lateral ones, the third and the fourth) and the central spinal canal, as well as the external unit fills the subarachnoid space with cisterns (Fig. 1). According to experimental scientific interest and the first modern studies of CSF physiology from nearly a century ago (Cushing, 1914; Dandy, 1919; Dandy and Blackfan, 1914; Weed, 1914) the CSF physiology is, after a hundred years of investigation, based on three key premises: 1) the active formation (secretion) of cerebrospinal fluid (Vf); 2) the passive absorption of CSF (Va); and 3) the unidirectional flow of cerebrospinal fluid from the formation to the absorption place. Based on all of the above, the CSF (with blood and lymph) is called the third circulation (Cushing, 1914; Milhorat, 1975; Taketomo and Saito, 1965).

There is an assumption that the CSF is formed mainly by the secretory activity of choroid plexuses in the brain ventricles, and that the majority of the remaining CSF is probably produced by the ependyma (Brown et al., 2004; Davson et al., 1987; Johanson et al., 2008; McComb, 1983; O'Connel, 1970). It is generally accepted that CSF flows/circulates

unidirectional from the brain ventricles to the subarachnoid space with the exchange of various substances (more or less manifested) between the CSF and interstitial compartments (Davson, 1967; Davson et al., 1987; Johanson et al., 2008; Plum and Siesjö, 1975). There is a belief that arachnoid villi of the dural venous sinuses are primarily responsible for the drainage of CSF from the subarachnoid space to the cranial venous blood (Figs. 1 and 2) by means of a hydrostatic gradient (Brodbelt and Stoodley, 2007; Weed, 1935). Welch and coworkers described an open tubular system projecting into the lacuna lateralis or directly into the venous sinus (Welch and Friedman, 1960; Welch and Pollay, 1961). The ultrastructural studies of these structures differed in their support of these pressure-sensitive opening pathway hypotheses (Alksne and Lovings, 1972; Gomez et al., 1974; Jayatilaka, 1965). Since Shabo and Maxwell (1968) showed that the observed tubular system was probably a consequence of tissue histological preparation, and that the endothelium of arachnoid villi was, in fact, intact (Shabo and Maxwell, 1968), Tripathi and Tripathi (1974) proposed that there are temporary transmesothelial channels which allow the passage of CSF in bulk flow from the subarachnoid space to the venous blood (Tripathi, 1974; Tripathi, 1974; Tripathi and Tripathi, 1974). In addition, there is a large amount of literature which suggests that the significant absorption of CSF (Va) occurs from the subarachnoid space to the lymphatic system (Bradbury, 1981; Brierly and Field, 1948; Dandy, 1929; Johnston et al., 2005; Johnston et al., 2004; Koh et al., 2005; Koh et al., 2006; Weed, 1914). Also, in spite of some other proposed places of CSF absorption (choroid plexuses, brain tissue, etc; see later), in physiological conditions the dural sinuses are still the main place of CSF absorption.

According to the above-mentioned data, the CSF physiology conceived this way has been presented as the classic hypothesis of CSF hydrodynamics. Other than humans, the same hydrodynamics is presented in other mammals, and there is no mammalian species in which CSF hydrodynamics is conceived outside the framework of the classic hypothesis. Indeed, the

prevalent experimental data which support the classic hypothesis have been observed in experimental animals. Today this hypothesis, despite the fact that many aspects of CSF hydrodynamics are still insufficiently understood, represents, with minor modifications, a common point of reference in scientific papers, review articles and in numerous textbooks, and it is offered as an unquestionable fact. The hypothesis is also applied to explain some pathological states, such as an increase in intracranial pressure (ICP), the development of hydrocephalus, periventricular edema, etc.

2. Cerebrospinal fluid formation

2.1. The site and the way of cerebrospinal fluid formation

There is, in relation to the classic hypothesis, little doubt that CSF is primarily, if not exclusively, formed inside the cerebral ventricular system. Choroid plexuses are the main site of CSF production (Dandy, 1919; Dandy and Blackfan, 1914; Davson, 1967; Davson et al., 1987; de Rougemont et al., 1960; Johanson et al., 2008; Masserman, 1934; Milhorat, 1987; Netsky and Shuangshoti, 1970; Rubin et al., 1966; Spector and Johanson, 1989; Welch, 1963; Welch, 1975), and other extrachoroidal sources also participate in maintaining the balance (Milhorat, 1987). According to Pollay and Curl (1967), another source of CSF is the ependyma lining the ventricles (Pollay and Curl, 1967). As a working hypothesis, it was also suggested that the blood-brain barrier is probably a fluid generator, but in a very small volume which has no effect on the classic hypothesis (Abbott, 2004; Cserr, 1988). Because it appears that CSF is primarily produced by choroid plexuses, the discussion will continue to focus on the choroidal epithelium as a generator of CSF secretion. The choroid plexuses are branched and highly vascularized structures consisting of numerous villi which project into the ventricles (the lateral, the third and the fourth) of the brain (Fig. 3). The endothelium of the

choroid plexus capillaries is fenestrated, and the first stage in CSF formation is the passage of a plasma ultrafiltrate through the endothelium, which is facilitated by hydrostatic pressure. During the second stage of CSF formation, the ultrafiltrate passes through the choroidal epithelium at the surface of the choroid plexus, and then into the ventricle. The passage through the choroidal epithelium is an active metabolic process which transforms the ultrafiltrate into secretion (cerebrospinal fluid) (Brown et al., 2004; Davson et al., 1987; Johanson et al., 2008; Pollay et al., 1985; Segal and Pollay, 1977). Fluid secretion in epithelia has been found to be dependent on the unidirectional transport of ions, which creates an osmotic gradient inducing the movement of water (Figures 2, 3 and 4).

The basic understanding of this process is incomplete and still speculative. Based on literature (Bergsneider, 2001; Brodbelt and Stoodley, 2007; Brown et al., 2004; Davson et al., 1987; Johanson et al., 2008; Lyons and Meyer, 1990; Pollay 1977; Spector and Johanson, 1989), it could be generally shown that the net osmotic gradient across the choroid plexus is strictly regulated by the transport of Na^+, Cl^-, and HCO_3^- ions. The Na^+/K^+ pump located on the CSF-facing (apical) membrane pumps Na^+ out of the choroid cells, driving down their intracellular Na^+ concentration (Figure 4). The subsequent Na^+ gradient generated on the plasma-facing membrane (basolateral membrane) secondarily activates the transport of Na^+ into the cell via a Na^+/Cl^- cotransport. Cl^- exchange occurs across the apical and basolateral membranes. At the basolateral membrane, an active transport Cl^-/HCO_3^- pump and cotransport $Na^+/K^+/2\ Cl^-$ mechanism allow the influx of Cl^- into the choroid cell. On the apical side, the efflux of intracellular Cl^- occurs through channels and possibly transports proteins. The main function of the basolateral Na^+/H^+ antiport and Cl^-/HCO_3^- pumps is to regulate intracellular pH. Intracellular HCO_3^- generated from the hydration of carbon dioxide, a reaction catalyzed by carbonic anhydrase, helps stimulate Cl^- accumulation on the Cl^-/HCO_3^- exchanger in the basolateral membrane. Water movement across the choroid epithelium seems to be facilitated

and possibly regulated by aquaporin channels. The presence of aquaporins suggests that the movement of water across the choroid epithelia is transcellular and not intercellular (Fig. 4). The secretory character of choroid plexuses that involves pumps, cotransporters and antiporters, ion channels and aquaporins has been confirmed during the past 15 years by the application of molecular biological methods (Brown et al., 2004; Johanson, 2008; Kanaka et al., 2001; Li et al., 2002; Oshio et al., 2005; Praetorius et al., 2004; Speake et al., 2003; Watts et al., 1991; Zlokovic et al., 1993).

It is necessary to emphasize that those experiments were conducted in «ex vivo» and «in vitro» conditions which could significantly differ from those in living organisms. Furthermore, it is known that ionic transport takes place in both directions (in and out of the cell), but with current technology it is impossible to determine which direction is dominant in «in vivo» experiments. Even if there were a dominant direction of active transport from the choroid plexus cell to the CSF, experiments on animals cannot reveal whether such transport has a sufficient capacity to form the volume of CSF that is predicted by the classic concept (Brown et al., 2004). Since even molecular research fails to give clear insight into net ionic transport towards CSF, it is difficult to accept the utmost active creation of CSF as an undoubted fact.

2.2. Formation rate of cerebrospinal fluid as the main generator of flow

The rate of CSF formation has been measured in turtles, rats, sharks, rabbits, cats, monkeys, dogs, goats, and calves at approximately 1.4; 2.2; 3-4; 8-11; 14-21; 19-35; 35-50; 160; 290 µl/min, respectively (Cserr, 1971; Heisey et al., 1962; Milhorat, 1989; Orešković et al., 2000; Rennels et al., 1990; Tripathi, 1974; Wright, 1972). The rate of CSF formation in humans is about 0.3-0.4 ml/min (Cutler et al., 1968; Masserman, 1934; Pollay, 1972; Rubin et al., 1966;

Sjöqvist, 1937), which means that the total production per day is about 430-580 ml. Based on the total CSF volume of 160 ml in the CSF system, it takes about 6 and a half to 9 hours to replace this volume of fluid.

Since CSF formation is an active process, the CSF formation rate should not be significantly altered by moderate changes in intracranial pressure (Cutler et al., 1968; Heisey et al., 1962; Pollay et al., 1983; Rubin et al., 1966; Sklar et al., 1980). If CSF pathways are blocked and active secretion is independent of hydrostatic pressure, the CSF secretion should continue with an accumulation of CSF, an increase in intracranial pressure, the dilatation of the brain ventricles, and eventually result in hydrocephalus. In other words, if Vf in pathological conditions is the generator of hydrocephalus, it is reasonable to expect that Vf is the main generator of CSF circulation in physiological conditions. This assumption has rarely been reported in literature (Di Chiro, 1964; Weed, 1914). It is believed that CSF flow is augmented by the ciliary action of the ventricular ependyma, vascular, choroidal and respiratory pulsations, cardiac systole and diastole and the hydrostatic pressure gradient that exists between the CSF and the venous system (Bering, 1962; Bering and Satto, 1963; Bhadelia et al., 1995; Di Chiro, 1964; Du Boulay, 1966; Du Boulay et al., 1972; Florez et al., 2006; Lee et al., 2004; Mascalchi et al., 1988; Milhorat, 1975; O'Connel, 1970; Ohara et al., 1988; Scollato et al., 2008; Thomsen et al., 1990).

The unquestionable fact is that, in physiological conditions, CSF secretion and absorption inside the CSF space are balanced:

$$V_f = V_a$$

This means that the amount of CSF that is secreted (inside the brain ventricles) must be the same as the amount that is passively absorbed (into the venous sinuses and/or into the lymphatics). Any other relationship will result in an unbalance in the volume of CSF and with

time in a pathological process. The same situation is valid for CSF flow (circulation; Q_{CSF}). Since the secretion and absorption of CSF are positioned in different sites of the CSF system, the flow rate (Q_{CSF}) between secretion and absorption place must be of the same magnitude, so that V_f remains equal to V_a;

$$V_f = Q_{CSF} = V_a$$

Any changes in active CSF secretion should passively follow CSF flow and CSF absorption to maintain physiological relationships in the CSF system. It, therefore, appears logical that active CSF secretion is the main generator of CSF circulation if a constant (physiological) volume of CSF is to be maintained within the CSF system. Due to this assumption, the other above-mentioned reasons for maintaining CSF flow (vascular, choroidal and respiratory pulsations, cardiac systole and diastole) are aggravating the effects of the balanced CSF flow rather than its activators. Also, it is difficult to conceive that CSF flow/circulation could be so accurately regulated by CSF pulsations as these are mostly a consequence of organ functions (heart, lungs) that are located outside the CNS and are not sensitive to hydrodynamic changes within the CSF system. Based on all this, it seems that CSF formation should, due to its active nature, represent a crucial link in the classic hypothesis on CSF hydrodynamics. In light of the classic hypothesis, CSF absorption and circulation could almost be regarded as a consequence of active CSF formation. Considering the significance of the foregoing statement, the manner and methods used to determine CSF formation are of extraordinary importance.

3. Methods of measuring cerebrospinal fluid formation

A number of methods have been applied in the study of CSF formation; we will mention the methods which have been used extensively or for a prolonged period of time, and have been largely responsible for recent advances in our understanding of CSF formation. More

precisely, a description will be provided of only those methods which are still being used in scientific and clinical practice.

3.1. Cerebrospinal fluid drainage

Cerebrospinal fluid drainage is the simplest and the oldest method of determining CSF formation in human subjects and animals. This method involves needle introduction into the CSF space and collection of the CSF that outflows spontaneously through the needle (Flexner and Winters, 1932; Frazier and Peet, 1915; Greeneberg et al., 1943; Sjöqvist, 1937; Weed, 1914). At the beginning a large amount of CSF escapes and it is necessary to wait for a balanced outflow of CSF before the CSF formation rate (V_f) can be determined by measuring the CSF volume in time (ml/min). To obtain reliable V_f values, CSF absorption should be prevented; otherwise CSF formation is reduced by the rate of CSF absorption. CSF absorption is prevented by carrying out CSF collection under negative CSF pressure. According to a modification of this method, a decline in CSF pressure is determined after removing a certain volume of the CSF and thereafter measuring the time needed for the pressure to revert to the initial level. The time measured like this (in minutes) is divided by the amount of the removed CSF (in milliliters) in order to obtain the rate of CSF formation (ml/min). Determination of the CSF formation rate under negative pressure goes completely against this method (see below).

3.2. Radiographic method

The radiographic method (Deck and Potts, 1969; Potts, 1967; Potts and Bergland, 1969; Potts et al., 1971) applied in dogs and in human subjects is based on the introduction of air and

contrast materials into the lateral brain ventricle, which results in a visible difference in the level between the air and the CSF. Before air is injected into the brain ventricle, an equivalent quantity of CSF is removed. CSF formation compresses the air, which results in a shifting of the air-CSF level. By knowing the cross section area of the brain ventricle at the site of the shift, and by measuring the time of shifting of the air-CSF level, it is possible to calculate the CSF formation (ml/min). This method can also be used to measure CSF formation in the way that the CSF is continuously extracted from the brain in an amount that does not cause a significant shift in the air-CSF level. In other words, the CSF that has been removed represents the CSF that has been formed. The validity of the results obtained using both the first and the second method above is doubtful due to air absorption from the CSF system, with an additional problem while implementing the first method related to the determination of the exact cross section area.

3.3. Collection of new CSF on the choroid plexus surface

Collection of new CSF on the choroid plexus surface (de Rougemont et al., 1960) was performed in cats by surgical access to the brain ventricles and by lowering a glass pipette to make contact with the plexus which had been covered with pantopaque oil. Under such circumstances, the new CSF enters the glass capillary and may be collected and measured, which demonstrated the role of choroid plexuses in the secretion of CSF (ml/min). It is appropriate to point out that, in addition to the unphysiological conditions imposed by direct exposure of the choroid plexus, the technique of collecting choroidal fluid from under oil is questionable since it has been shown that pantopaque is a toxic agent which produces acute ventriculitis and inflammatory changes in the choroid plexuses following intraventricular administration (Clark et al., 1971).

3.4. Micropuncture of the principal vein of the choroid plexus

Micropuncture of the principal vein of the choroid plexus (Welch, 1963) was developed in rabbits and is based on the idea that an increase in hemoglobin and hematocrit concentrations in the venous blood of the choroid plexuses occurs after blood flow through the choroid plexus and water loss (CSF secretion). The comparison of hematocrit or hemoglobin concentrations of choroidal venous and mixed arterial blood should give an index of the CSF secretion rate. If blood flow is known, the rates of CSF secretion can be calculated using a derived equation for this case (Welch, 1963; Welch, 1975). However, there are major errors in this technique for estimating choroidal blood flow, and the assumption that the hematocrits of aortic and choroidal blood are equal is probably unjustified (Cserr, 1971).

3.5. Extracorporeal perfusion of the choroid plexus

Extracorporeal perfusion of the choroid plexus (Pollay et al., 1972) was carried out by the rapid removal of the brain of a freshly sacrificed sheep, taking special care to preserve the vessels at the base, which allowed a cold saline infusion into the internal carotid artery within 10 minutes of decapitation. It was possible to perfuse the choroid plexus by ligation of the carotid above the anterior choroidal artery and cannulation of the internal cerebral vein. The CSF secretion was estimated based on the change in hematocrit between the inflowing and outflowing fluid and the rate of outflow, and was calculated the same way as in the method of micropuncture of the principal vein of the choroid plexus. Almost certainly, the unphysiological state of the open evacuated ventricle renders such observations as above invalid. Also, the same objections that were raised regarding the micropuncture of the principal vein of the choroid plexus should be taken into account.

A critical appraisal of the above mentioned methods on humans and different experimental animals as well as an elaboration of their disadvantages are the reason why these methods are practically no longer in use. It should, however, be emphasized that the results obtained by those methods played a pivotal role in the acceptance of the classic hypothesis of CSF formation, and had a critical impact on this way created understanding of the site of CSF formation (brain ventricles/choroid plexuses) and of the active (secretory) character of CSF formation itself.

3.6. Magnetic resonance imaging method

Magnetic resonance (MR) is a noninvasive imaging technique that allows for an evaluation of the direction and velocity of blood throughout the blood vessels (Bradley et al., 1984; Bucciolini et al., 1987; Von Schulthess and Higgins, 1985). Since the aqueduct of Sylvius can be considered a CSF vessel, about 15 mm in length and 1-2 mm in width, that connects the third and the fourth brain ventricles (Brinkmann et al., 2000; Flyger and Hjelmquist, 1957; Longatti et al., 2007; Mascalchi et al., 1988), this technique has also been applied to aqueductal CSF flow (Bradley et al., 1986; Brinkmann et al., 2000; Mark and Feinberg, 1986; Mascalchi et al., 1988). The aqueduct is a suitable location for the characterization of CSF flow since it is a narrow structure, which results in a higher velocity (several cm/s) than, for example, in the third ventricle. Because the aqueduct has a constant diameter over a certain length, a rather constant flow pattern is present as opposed to, for example, the foramina of Monro where irregular flow patterns may be encountered. Cardiac cycle-related cerebral blood volume variations produce bidirectional oscillatory movement of CSF within the aqueduct (Barkhof et al., 1994; Bradley et al., 1986; Florez et al., 2006; Greitz et al., 1993; Lee et al., 2004; Luetmer et al., 2002; Ohara et al., 1988). During systole, the net inflow of

blood increases the intracranial volume and induces craniocaudal (systolic) CSF movement. During diastole, the net outflow of blood decreases the intracranial volume and promotes caudocranial (diastolic) CSF movement (Fig. 5). Since all of the CSF is produced in the cerebral ventricles and absorbed into the arachnoid granulations or lymph, there must be a net outflow (CSF flow/circulation) of CSF through the cerebral aqueduct ($V_f = Q_{csf} = V_a$). With a quantitative MR flow measurement method, an estimate of this net flow can be calculated as the difference between the integrated CSF inflow (diastolic) and outflow (systolic) over one cardiac cycle, representing the production of CSF (V_f; Forner et al., 2007; Gideon et al., 1994; Thomsen et al., 1990).

However, in articles on the subject of hydrodynamics in the aqueduct of Sylvius, the expression for CSF flow is used in many situations without an explanation and/or in the wrong/false context, as for example: average CSF flow, mean CSF flow, maximal systolic and diastolic CSF flow. Such use of terminology for CSF flow leads to confusion and the misunderstanding of CSF hydrodynamics. Due to the classic hypothesis of CSF hydrodynamics, CSF flow is used only in the case when it represents CSF circulation.

By definition of CSF hydrodynamics, CSF flow (unidirectional-craniocaudal) in the aqueduct of Sylvius is CSF volume that passes through the aqueductal cross-sectional surface during a defined period of time (ml/min, or m^3/s in SI units; Figure 5). Because of the bidirectional oscillatory movement of CSF (see above), only the integrated difference between systolic and diastolic CSF movement in ml per unit of time (ml/s; l/day; ml/cardiac cycle) represents the CSF flow. Anything else cannot be called CSF flow. Now that we have defined what CSF flow (circulation) is in the aqueduct of Sylvius, we may state the literature (Table 1) used to get the values of the formed CSF from the measured CSF flow; also, we calculated them from the data available for CSF velocity.

Actually, the determination of CSF formation from the CSF flow rate is based on a relatively simple equation used to calculate the flow (Figure 5):

$$Q_{csf} = A_{aqu}\, v_{csf}$$

where:

Q_{csf} = CSF flow (m^3/s)

A_{aqu} = cross section area of the aqueduct of Sylvius (m^2)

v_{csf} = difference between integrated CSF velocity in cranial and caudal direction (m/s)

When CSF velocity through the aqueduct is measured by MR methods, and information about the aqueduct diameter is provided, there is a possibility to calculate Vf. Thus, since

$$V_f = Q_{csf}$$

then

$$V_f = A_{aqu}\, v_{csf}$$

The area (A) of the aqueduct can be presented as:

$$A = r^2\, \pi$$

where r = d/2 and the diameter of the aqueduct is assumed to be 2.0 mm (Brinkmann et al., 2000; Flyger and Hjelmquist, 1957; Longatti et al., 2007), and thus the calculated results are shown in Table 1.

In Table 1 it is evident that the CSF formation (V_f) obtained in only one study (Tab. 1; No. 3) was within the limits of physiological formation (0.3 – 0.4 ml/min). In two studies (Tab. 1; Nos. 2 and 5), calculated CSF formation was zero, and in the remaining studies V_f values

were higher than the values measured using the other methods (Cutler et al., 1968; Lorenzo et al., 1970; Masserman, 1934; Pollay, 1972; Rubin et al., 1966; Sjöqvist, 1937). It is important to emphasize that in reality these differences are even greater considering that the choroid plexus of the fourth brain ventricle is not included in the study using the MR imaging method to determine V_f as it is outside the field of measurement. This is why it is necessary to correct the obtained values in order to get the total rate of CSF formation. It has been found (Bering and Satto, 1963; Flexner and Winters, 1932; Welch, 1963) that the choroid plexus of the fourth ventricle weighs about 25 per cent of total choroid plexuses mass. If the assumption were made that the amount of fluid formed from a single plexus of any individual animal is approximately a direct function of the plexus weight, then an additional 1/4 of CSF volume should be added to obtain the exact rate of CSF formation (corrected V_f; Table 1). Thus corrected values are (Tab. 1. except for Nos. 2 and 5) approximately 1.5 - 3-fold higher than the values reported in the literature so far (Cutler et al., 1968; Lorenzo et al., 1970; Masserman, 1934; Pollay, 1972; Rubin et al, 1966; Sjöqvist, 1937). Such significant deviation in V_f determination within the MR imaging method itself (from 0 to 1.04 ml/min) and the deviation in relation to other methods are probably the consequence of various techniques being used to measure the CSF flow although the values should in both cases be the same regardless of the different techniques used. Phase-contrast magnetic resonance (MR) imaging is mainly used in cardiovascular imaging for obtaining quantitative information on blood flow. Some error in measurement is inevitable due to the technical limitations of this method, and for blood flow assessment it is estimated to be around 10% (Hoeper et al., 2001; Lotz et al., 2002). It is much more demanding to perform a precise measurement of CSF flow through the mesencephalic aqueduct. Despite the use of the most sophisticated equipment, a significant inaccuracy is found in measurements due to the nonlinearity of the gradients, eddy currents and partial volume effects (Barkhof et al., 1994; Henry-Feugeas et al., 1993; Lee et

al., 2004). This can be clearly seen (see Table 1*)* if the results, which differ in range from 0 to 1.04 ml/min, are compared.

Regrettably, in many other studies where CSF movement in the aqueduct of Sylvius has been examined in physiological conditions by different MR imaging techniques, the obtained results did not allow presentation of the rate of CSF formation (V_f) neither from reported flow values nor from the CSF velocity (Baledent et al., 2006; Barkhof et al., 1994; Florez et al., 2006; Kim et al., 1999; Lee et al., 2004; Luetmer et al., 2002; Mase et al., 1998; Schroeder et al., 2000; Scollato et al., 2008; Stahlberg et al., 1989). The reason for this lies in the fact that attempts have been made to diagnose pathologic changes by means of other, more easily measurable hydrodynamic parameters in the aqueduct of Sylvius (see above).

MR imaging is a noninvasive, highly sophisticated method and, when used in animals, is a rather expensive and limited method based on the magnitude of the animals' aqueduct (Brinkmann et al., 2000). On the other hand, as CSF formation, circulation and absorption are mainly investigated on experimental animals, the perfusion method of CSF spaces remains the method of choice for this purpose.

3.7. Perfusion method of cerebrospinal fluid spaces

Much of our recent understanding of CSF hydrodynamics comes from the quantitative utilization of the technique of ventriculo-cisternal perfusion. This method is widely accepted and represents a basic tool by which CSF physiology has been studied. Mostly thanks to the results of experiments obtained by this method, a general hypothesis of CSF hydrodynamics has been confirmed. Although this method is used extensively in animals, its clinical usage has been very limited as the procedure is invasive. This method was developed by Pappenheimer et al. (1962) and Heisey et al. (1962) on a goat and has been considered a

precise and physiological method for studying cerebrospinal fluid secretion (Lorenzo et al., 1970). Perfusion is performed from the lateral brain ventricle to the cisterna magna (ventriculo-cisternal perfusion; Figure 6) by a mock CSF that contains a marker (inulin, albumin or dextran). Determination of CSF formation is based on the assumption that marker dilution in perfusate occurs due to CSF secretion within the brain ventricles, implying that higher CSF secretion increases marker dilution. Apart from ventriculo-cisternal perfusion, a series of modifications to this method has been presented in the literature; they differ only in terms of the entry and exit sites of the perfusate in the CSF space (e.g., ventriculo-ventricular perfusion; ventriculo-aqueductal perfusion, ventriculo-lumbar, etc.). Since all these methods are founded on the same hypotheses as ventriculo-cisternal perfusion, a detailed presentation of only the ventriculo-cisternal perfusion method is sufficient.

Perfusion of the brain ventricles from the lateral ventricle to the cisterna magna begins with introducing one metal cannula into the lateral brain ventricle (inflow cannula), and the second into the cisterna magna (outflow cannula). The inflow cannula is attached to a T-shaped connector, and for the purpose of measuring CSF pressure, on one side it is connected via polyethylene tubing to a manometer (polygraph), and on the other side to the syringe containing artificial fluid with the diluted marker. The syringe is fixed on the perfusion pump that allows the artificial fluid with the marker (inflowing perfusate) to flow into the lateral ventricle at a constant rate of perfusion (ml/min). The outflowing cannula is connected to a plastic tube with the outflowing perfusate from the brain ventricles and the cisterna magna that is collected in test tubes (Figure 6). Perfusion is performed under a certain hydrostatic pressure that can be adjusted by setting the end of the outflowing polyethylene tubing above (positive pressure) or below (negative pressure) the external auditory channel whose level is assumed to be the zero value of the hydrostatic pressure.

The method itself is indirect because the rate of CSF formation is calculated by marker dilution in the outflowing perfusate. Equations for the calculation of CSF formation founded on test substance dilution were elaborated by Heisey et al. (1962) on the basis of the known principle for calculation of the glomerular filtration quantity by means of inulin clearance in renal physiology.

However, certain preconditions must be fulfilled to use this method and calculate CSF formation. Above all, in order to apply the analogy from renal physiology, it is necessary that CSF behaves in accordance with the classic hypothesis of secretion, circulation and absorption of CSF (like the third circulation), which at the same time represents the pivotal hypothesis that is the origin of all the other derived hypotheses. Almost all of the lost marker from the CSF can be accounted for by the bulk absorption distal to the fourth ventricle. Any dilution of the marker during passage through the ventricles should result from newly formed CSF. All CSF should be produced within the brain ventricles and absorbed outside in subarachnoid space. As a marker, a substance can only be used that is otherwise absent from the CSF. If CSF is absorbed within the brain ventricles, the measurement of the formed CSF is reduced by the amount of the CSF absorbed within the ventricles. Provided that these conditions are fulfilled, the following equation for calculating CSF formation can be applied:

$$V_f = V_i \times (C_i - C_o)/C_o$$

where:

V_f = rate of CSF formation (ml/min)

V_i = rate of inflow perfusate (ml/min)

C_i = concentration of the marker in inflow perfusate (mg/ml)

C_o = concentration of the marker in outflow perfusate (mg/ml)

Although the classic hypothesis of net CSF formation is mostly due to the development of the widely used ventriculo-cisternal perfusion method for the measurement of V_f, the mentioned assumptions have not been critically evaluated. However, it is known that a marker can pass from the CSF into the brain parenchyma (Bergsneider, 2001; Curran et al., 1970; Davson et al., 1987; Fenstermacher et al., 1974; Orešković and Bulat, 1993), and that inulin enters rapidly into perivascular CNS spaces, reaches a very large surface area of capillaries and, by slow diffusion across microvascular walls, reaches the bloodstream and is rapidly eliminated into the urine (Vladić et al., 2009). Due to the fact that the marker passes from the perfusate to the adjacent tissue, correction factors were used to calculate V_f (Cserr, 1971; Curran et al., 1970; Heisey et al., 1962; Pappenheimer et al., 1962). During ventriculo-cisternal perfusion with the perfusate containing ^{3}H-water and blue dextran (m.w. $2x10^6$), it was observed that the increase of hydrostatic pressure in the perfusate from negative (-10 cm H_2O) to positive (20 cm H_2O) values had opposite effects on these two substances: the augmentation of blue dextran and a decrease in ^{3}H-water concentration occurred in the perfusate (Orešković and Bulat, 1993). If we take into account that it was demonstrated that a significant bulk absorption of ^{3}H-water (Bulat et al., 2008) occurs within the brain ventricles, all these losses of the marker from the perfusate and the changes in marker concentration caused by loss of water should give a false calculation of net CSF formation. Furthermore, the higher rates of CSF formation are calculated at lower rates of ventriculo-cisternal perfusion than at higher perfusion rates (Orešković et al., 2003), indicating that an inherent defect in the method is present, probably caused by a different mixing of the marker and the native CSF at a different perfusion rate. Also, the CSF formation rate measured by prolonged ventriculo-cisternal perfusion declines spontaneously with time for a reason that remains obscure, and this reduction may be an artifact of the method (Martins et al., 1977). The results registered in sacrificed cats showed persisting CSF formation (between 3 and 5 µl/min) 80 minutes after

the animals were sacrificed (Orešković et al., 2008). As CSF could not possibly form in dead animals, it is possible that an error in the method is in question. A potential artifact of the method was also observed in experiments performed in a plastic cylinder. In this method (Orešković et al., 2008) the absorption of the marker into the surrounding tissue was prevented, all "CSF was secreted" above the collection site, "CSF absorption" did not occur before this site and "CSF was formed" by simulating CSF formation by means of an infusion pump. The calculated results of "CSF formation" by Heisey et al. (1962) obtained under the same circumstances as the result obtained through simulation by means of the pump, were significantly higher although they should be the same in both cases. If this occurred in dead animals and in the plastic cylinder, a reasonable question arises as to what perfusion itself does in live animals.

To summarize, of utmost importance in this indirect method is that the concentration of the marker substance can be changed only by newly formed CSF if we are to obtain the correct result of the CSF formation rate (V_f) by using the equation of Heisey et al. (1962). Since a change of the marker substance is not caused only by newly formed CSF, but also by absorption of the marker substance into the surrounding ventricular tissue, and inflow (Bering, 1952) or outflow (Bulat et al., 2008) of water inside the brain ventricles, the method itself will always (e.g. when Vf is studied in dead and alive animals, "in vitro" experiments, during perfusion of other parts of the CSF system, such as the spinal cord, etc.) give some kind of result by which the CSF formation rate could be calculated. For these reasons, all the results concerning CSF secretion obtained by the perfusion method should be reevaluated, both the results showing an increased secretion (in papillomas and choroid plexus carcinomas) or a decreased secretion (for example, after the application of carboanhydrase inhibitors).

3.8. Direct aqueductal method for measuring CSF formation

Due to the above mentioned problems with the indirect method, a direct method for V_f calculation has been developed on cats by draining the CSF out of the aqueduct of Sylvius. This method is in fact a modification to the drainage method of the aqueduct of Sylvius by Flexner and Winters (1932). The main problem with the Flexner and Winters (1932) method was the high rate of oscillation of active aqueduct flow. The period of active flow from the aqueduct (10 µl/min) was followed by a 30 minute cessation period. It seems that the main reason for this oscillation was the manner of blocking the aqueduct of Sylvius. Actually, the blockade was made using a distended balloon positioned in the fourth ventricle. The pressure within the balloon was two times higher than the physiological one (about 160 mm H_2O) and this unphysiological pressure, by influencing the sensitive adjacent tissue, probably caused a significant disturbance in the CSF hydrodynamics and brain blood flow surrounding the balloon.

To avoid the mentioned problems, a method was developed on a cat, in which the aqueduct of Sylvius was blocked by a plastic cannula without increased pressure on the adjacent tissue (Orešković et al., 2002). Surgical reconstruction was done so that the CSF system was completely protected against any uncontrolled influences of atmospheric pressure and CSF leakage from subarachnoid and ventricular spaces. Thus, the relationship between the ventricular and subarachnoid CSF pressure was established in a physiological range without a transmantle pressure gradient which is a crucial advantage of the model (Klarica et al., 2009). Actually, in case of pathway obstruction, the absence of a transmantle gradient would not cause the possible CSF flow from the ventricles through the brain tissue into the subarachnoid space, as was suggested by Milhorat (1989). Therefore, if CSF is mainly formed inside the brain ventricles and absorbed in the subarachnoid space, it has to circulate at a physiological CSF pressure through the aqueduct of Sylvius or, as is the case in this model, through the

plastic cannula positioned in the aqueduct. The direct (visual) observation of the CSF outflow throughout the external end of the cannula (adjusted to the physiological CSF pressure) should represent CSF formation (Figure 7). The collected volume of CSF divided by the time of collection therefore represents the rate of CSF formation (Orešković et al., 2002). But during the examined period, CSF permanently pulsated near the external end of the plastic cannula, the flow of the CSF volume (circulation) did not exist and not a single drop of CSF was observed in the collection tube in any of the investigated animal. These results closely corresponded to some results observed by the MR imaging technique on humans (Bradley et al., 1986; Enzmann and Pelc, 1991) and the results obtained by a similar method on cats (Orešković et al., 2001). To test the sensitivity of the method, i.e. check if the physiological CSF formation rate can be measured by this method, the "CSF secretion" was under the same conditions experimentally imitated by infusing the artificial CSF into the lateral brain ventricle by means of an infusion pump (Figure 7). The infusion rate ("CSF secretion") was adjusted according to the limits of the expected rate of CSF secretion (13 µl/min) suggested by the classic hypothesis (Flexner and Winters, 1932; Orešković et al., 2000; Plum and Siesjö, 1975; Pollay, 1975; Pollay, 1977; Welch, 1963). Under these experimental conditions, CSF formation could be represented as:

$$V_f = V_o - V_i$$

Since no difference between the rate of experimentally "secreted CSF" (infused volume) and the rate of the outflowing perfusate (V_o) was observed, i.e. as much "CSF was secreted" into the brain ventricles, that much of the "net CSF formed" was measured, obviously CSF formation did not exist.

$$V_f = 13\ \mu l/min - 13\ \mu l/min = 0$$

In this way it was also demonstrated that anything that is produced within the brain ventricles (13 µl/min by pump) is accurately measured by this method (13 µl/min outflow rate).

When the CSF formation was measured simultaneously by this direct and indirect perfusion method under the above mentioned conditions (Orešković et al., 2005), the rate of CSF formation calculated by Heisey et al. was about 5 µl/min (the same result was also obtained in cats by applying ventriculo-ventricular perfusion in isolated ventricles; Orešković et al., 1991) although V_f does not exist ($V_f = 0$). The calculated rate of CSF formation was obviously not the consequence of CSF formation within the brain ventricles, but probably the consequence of the absorption of the marker into the surrounding tissue (see *Perfusion method of cerebrospinal fluid spaces*).

Could this paradoxical observation be explained and incorporated into the classic hypothesis of the CSF hydrodynamics?

4. Criticism of the classic hypothesis of cerebrospinal fluid formation

Despite the generally accepted CSF formation hypothesis where CSF is actively produced (secreted) mainly within the brain ventricles by choroid plexuses, there are experimental results which are not consistent with this hypothesis. Development of this classic hypothesis was crucially affected by early experimental studies done by the pioneers of CSF physiology research, Weed (1914) and especially Dandy (1919). Weed (1914) concluded that the usage of the method of ventricular catheterization provides evidence of a more direct nature that the choroid plexuses of the cerebral ventricles are the generators of cerebrospinal fluid.

Yet, out of the various observations that have come about as proof that the choroid plexuses actively form (secret) CSF, none has influenced modern thinking more than Dandy's crucial

experiment (1919) concerning the consequences of choroid plexecotomy. If the choroid plexus of one lateral ventricle was removed, and if foramina of Monro of both lateral ventricles were obstructed, it was reported that the ventricle containing a choroid plexus would dilate and the ventricle lacking a choroid plexus would collapse. This observation led Dandy to conclude that the choroid plexuses were the sole source of CSF secretion. This experiment, besides being the source of CSF formation and its active nature (secretion), still points to a few more facts that are crucial in terms of forming a general hypothesis of CSF hydrodynamics. If the obstructed lateral ventricle containing a choroid plexus dilated, it is obvious that CSF would not be absorbed inside the brain ventricles. If absorbed outside the brain ventricles, the CSF should flow to the place of CSF absorption. When there is an obstruction of the CSF system between the place of CSF secretion and the place of CSF absorption, the brain ventricles should, because of the continuity of CSF secretion by the choroid plexuses, dilate and produce hydrocephalus. In fact, the whole classic hypothesis of CSF hydrodynamics could be postulated and confirmed by this experiment. The problem with Dandy's crucial experiment (1919) is that the presented observation was obtained in a single dog experiment. If the fact that this experiment has not been able to be reproduced is neglected and the obtained results are accepted as fact, the logical consequence of that experiment was Dandy's approach to the treatment of hydrocephalus by choroid plexectomy. For many years this surgical procedure was the most popular form of treatment for infantile hydrocephalus in the United States. However, over decades it became clear that bilateral extirpation and/or cauterization of the choroid plexuses invariably failed to benefit the patients. Because of universally poor results, choroid plexectomy was abandoned by neurosurgeons as a treatment for hydrocephalus, and today it is an operation of historic interest only and has no place in the treatment of hydrocephalus. But the failure of choroid

plexectomy to cure, or at least ameliorate progressive hydrocephalus was incompatible with the thesis that the choroid plexuses are the main source of active CSF formation.

Beside unsuccessful medical treatment, there are experimental data that have provided evidence of extrachoroidal CSF secretion. Thus, for example, attempts to repeat the classic experiment by Dandy (1919) were unable to confirm Dandy's finding that the plexectomized ventricle collapsed (Hassin, 1924; Milhorat, 1969). Moreover, the lateral ventricle became markedly dilated, which indicates that hydrocephalus can occur rapidly and progressively in the plexectomized ventricular system and that the choroid plexus is not essential as a source of CSF secretion (Milhorat, 1969). Furthermore, when the rate of CSF formation within the lateral ventricles, the third ventricle, and the aqueduct of Sylvius were compared in control and bilaterally plexectomized animals, the rates in the plexectomized group were found to be on average about 70% of the norm (Milhorat, 1975; Milhorat et al., 1971), or CSF formation in plexectomized patient remained similar to that in hydrocephalus-free individuals (Milhorat et al., 1976). Also, choroid plexectomy was not found to alter the chemical composition of CSF, thus indicating, at least, that the sites other than the choroid plexuses can produce a fluid whose composition of water, electrolytes, and protein is the same as that of normal CSF (Hammock and Milhorat, 1973; Milhorat, 1969; Milhorat, 1975; Milhorat et al., 1976).

With the development of the endoscopic methods in the mid 90s, new attempts were made to cure hydrocephalus using different surgical procedures on the choroid plexuses (Enchev and Oi, 2008; Pople and Ettles 1995; Wellons et al., 2002). Although the results of the treatment were somewhat better than those using a classic surgical approach, the same problems still persisted. The ventricular size was not significantly reduced by choroid plexus coagulation and only 35% of patients achieved long-term control without cerebrospinal fluid shunts (Pople and Ettles 1995). In another study (Griffith and Jamjoom, 1990), in 48% of the cases, shunting was required, which was done from one week to thirteen months after the choroid

plexus coagulation. And so, even when the plexus is removed, the development of hydrocephalus can still occur. These results show that the role of the choroid plexus in the pathophysiology of hydrocephalus is still unclear, that our knowledge of the hydrocephalus pathophysiology is still insufficient, and the results clearly confirm the above mentioned claims about CSF formation related to the choroid plexuses.

We must wonder how anybody could, based on those results, consider the choroid plexuses as the main site of CSF formation, and whether such contradictory results initiated another interpretation of the nature of CSF formation. The answer to the latter question is that they did not, and there are, in our opinion, several reasons for that.

First, the authors of those results did not introduce a significant change in the classic concept of CSF hydrodynamics. Rather, they concluded that, although a significant fraction of CSF is formed extrachoroidally (across the cerebral endothelium), a considerable volume of CSF is formed continuously within the cerebral ventricles and the choroid plexuses contribute to this formation. The nature of CSF formation should involve combined processes of ultrafiltration and active transport, and the CSF should circulate along CSF pathways until the major sites of absorption (arachnoid villi) are reached (Milhorat, 1976).

Another reason is the understanding that hydrocephalus occurs due to interrupted communication (obstruction) between the CSF formation site and the CSF absorption site, which was shown in different animal models of CSF pathway obstruction. Thus, for the development of hydrocephalus to happen, it is necessary that the CSF is actively formed (by secretion) and that the formation occurs before obstruction, which significantly supports the classic hypothesis. Although hydrocephalus is not a matter of interest here, in this article, it is impossible to avoid discussion on this pathologic state. The etiopathogenesis of hydrocephalus is still not well known. For example, it is not clear if obstruction of CSF

pathways is so important, and if hydrocephalus may develop only as a result of the obstruction of the circulating pathways and in reduction in ability to absorb cerebrospinal fluid.

The most cogent reason for questioning the traditionally accepted relationship of stenosis as cause and hydrocephalus as result is that it is relatively common to find a mild degree of stenosis in conjunction with hydrocephalus when it is clear that the function of the patency of the aqueduct as a conduit for CSF was unimpaired. It is possible that aqueductal narrowing or even closure may occur as a result of hydrocephalus and that it may, in the past, have been wrongly blamed for being the cause of hydrocephalus, when in fact it only contributed to it in the final stages (Williams, 1973). Moreover, Masters et al. (1977) have shown that an infection by reovirus type 1 in mice causes hydrocephalus developments in proportion to the degree of inflammatory/fibrotic changes within the cerebrospinal fluid pathways. As the hydrocephalic state progresses, axial herniation and compression of the midbrain results in the appearance of aqueduct stenosis. It was demonstrated that the stenosis of the aqueduct was a secondary phenomenon, not causally related to the pathogenesis of hydrocephalus. Furthermore, Adeloye and Warkany (1976) have shown that on rats with a zinc-deficient diet throughout pregnancy stenosis of the aqueduct can cause hydrocephalus only late in fetal life when the aqueductal lumen normally becomes small. Earlier in development, when the dysgenesis of the anterior neurospore occurs, the aqueduct is quite capacious and cannot obstruct CSF flow. In some animals the aqueduct was deformed but not occluded, and yet hydrocephalus was present throughout the ventricular system. Other animal experiments using a hypovitaminosis A diet in rabbits (Millen and Woolam, 1958) or a pteroylglutamic acid-deficient diet in rats (Monie et al., 1961) also suggested that stenosis of the aqueduct of Sylvius was the result of hydrocephalus. Borit and Sidman (1972) reported that in mutant mice there was a genetically determined postnatal communicating hydrocephalus which

secondarily produced aqueductal stenosis by compression of the mesencephalon. The best clinical evidence of secondary aqueductal stenosis was given by Foltz and Shurtleff (1966) who found that among 27 patients with communicating hydrocephalus, 12 developed secondary aqueductal stenosis or aqueductal occlusion during chronic venticulo-atrial shunting.

It is also not clear if the pressure gradient is often associated with the occurrence of hydrocephalus, particularly the acute one, and some authors view it as the fundamental mechanism of hydrocephalus development regardless of whether a low gradient (Conner et al., 1984; Hakim and Hakim, 1984; Levine, 2008; Penn et al., 2005) or a high gradient is in question (Kaczmarek et al., 1997; Nagashima et al., 1987; Smillic et al., 2005). There are, nevertheless, some other authors who believe that CSF pressure gradient is not possible within the cranium firmly enclosed by bones, mostly because they did not observe such a gradient neither in experiments involving animals (Shapiro et al., 1987) nor in patients with communicating or non-communicating hydrocephalus (Stephensen et al., 2002).

Since the data regarding the gradient-related results in literature are so contradictory, the question arises as to whether the transmantle pressure gradient is necessary for the development of hydrocephalus or some other factors may play an important role in such a process with occlusion or the stenosis of CSF pathways. It was shown in cats that 3 weeks after the application of kaolin in the cisterna magna with the obstruction of cervical subarachnoid space, or the stenosis of the aqueduct with a plastic screw, dilatation of ventricles is developed without a rise in the ventricular CSF pressure (Miše et al. 1996). The acute experiments show that an occlusion of the aqueduct itself does not cause the rise of CSF pressure in isolated ventricles and their dilatation (Klarica et al., 2009). However, during a prolonged occlusion or stenosis of the aqueduct, the development of a ventricular dilatation probably without an increase in the ventricular pressure should be expected. This idea is

supported by the observation that in patients with communicative and non-communicative hydrocephalus the transmantle pressure is absent (Stephensen et al., 2002). Furthermore, Holtzer and de Lange (1973) observed in some children with communicative and non-communicative hydrocephalus that after the shunt obstruction the hydrocephalus did not progress suggesting that this pathological process was compensated. All of this evidence supports the idea that the transmantle pressure gradient may not be necessary or instrumental for the development of hydrocephalus, and that some other factors may play an important role in the development of that pathological process (see below).

All of these mechanisms indicate that hydrocephalus develops over a prolonged period. We assume that hydrocephalus is essentially a chronic process which may change into its acute form under certain conditions (ventricular dilatation with a high CSF pressure) due to the appearance of the transmantle pressure gradient. If pathological changes take place along with an interruption of communication before the obstruction and they result in a CSF pressure increase in the ventricles (e.g. bleeding, infection, a tumour, a cysticercous cyst), this should lead to the pressure gradient appearance, an accelerated ventricular dilatation and the occurrence of the acute hydrocephalus phase. Previously, Zulch (1958) described many cases of arrested hydrocephalus that remained dormant for years, with the aggravation occurring only when some other pathological process took place within the cranium.

The cause of hydrocephalus in the case of diffuse villous hyperplasia of the choroid plexus, choroid plexus papilloma or choroid plexus carcinoma remains controversial. It is commonly stated that the cause of hydrocephalus is CSF oversecretion. However, other causes are also mentioned, such as arachnoiditis, inflammatory ependymitis, and CSF pathway obstruction caused by the tumorous mass itself, or by its metastases (metastases are usually observed in a cerebello-pontine angle, the third ventricle, the suprasellar region, and the spine: foramen magnum, cervical and lumbo-sacral areas) (Akil et al., 2008; Davson et al., 1987; Nagib and

O'Fallon, 2000). Vascularity is increased and the enhancement of the choroid plexus following an injection of gadolinium is very evident in cases of papillomas (Fujimura et al., 2004). Inside a carcinoma, areas of necrosis and cystic formations have been observed, and the permeability of such a structure is much higher than that of a healthy choroid plexus. Thus, the CSF is abnormal in 60% of cases (increased protein levels, xanthochromia or both) (Hawkins, 1980). It was also observed in animals that the amount of proteins in the CSF, as well as the number of white blood cells, is increased in the case of a choroid plexus tumor (Bailey, 1986). Since blood can be found in the CSF, hydrocephalus could develop because of subarachnoid scarring (recurrent bleeding from the tumor) (Barber et al., 2008; Davson et al., 1987; Nagib and O'Fallon, 2000). The ventricle on the side of the lateral ventricular papilloma is usually the larger of the two (Davson et al., 1987) – the development of unilateral hydrocephalus with the entire CSF pathways patent. Cases of a partial enlargement of only one part of the ventricle have also been detected. Pencalet et al. (1998) have shown 25 cases of papilloma and 13 cases of choroid plexus carcinoma in children. Hydrocephalus was present in 33 patients and poorly correlated with the size, site, and pathological characteristics of the tumor.

Unfortunately, the treatment of such pathological conditions is not satisfactory. In a series of 24 choroid plexus tumors, a total surgical excision was performed in 20 cases. The postoperative mortality was 8% (mortality within one month of surgery), but the overall mortality was 25% (Lena et al., 1990).

Oversecretion of choroid plexus papilloma is shown using the technique of CSF drainage (Fairburn, 1960; Nagib and O'Fallon, 2000; Tamburrini et al., 2006), and a “scientifically acceptable method” of ventriculo-spinal perfusion (Eisenberg et al., 1974; Milhorat et al., 1976). In some cases, 400 to 960 ml/day have been collected by external drainage, under the conditions of pressure of 5 cm H_2O (Fairburn, 1960). By using CSF drainage, it was

determined that more than 2 l of CSF a day could be drained preoperatively (Nagib and O'Fallon, 2000; Tamburrini et al., 2006). However, after a complete tumor resection, in most cases it was observed (cca 2/3 of the cases, according to the literature data) that the CSF extensively drains even more than 72 hours after the operation, and because of this it was necessary to insert a shunt (Lena et al., 1990; Nagib and O'Fallon, 2000). Tamburrini et al. (2006) have shown that, even after a choroid plexus resection, the CSF drains at the rate of 1000 ml/day. The progression of hydrocephalus after a complete resection of the tumorous tissue has also been observed, so a shunt had to be placed inside both ventricles, even 3 years after the surgery (Husag et al., 1984). In 9 out of 33 children (Pencalet et al., 1998), a ventriculoperitoneal shunt was required after tumor excision, which questions the notion that cerebrospinal fluid oversecretion is the only cause of hydrocephalus.

Hirano et al. (1994) and Britz et al. (1996) measured the daily CSF production rate by way of external drainage (collection of ventricular CSF). This procedure can cause fluid and electrolyte imbalance (due to the large amount of fluid loss). It is believed that the creation of CSF determined by a method of drainage in these cases has flaws, like the fact that a decrease in pressure during drainage creates new hydrostatic conditions inside the cranium, and therefore an inability to determine the volume of CSF that is absorbed. By using the ventriculo-spinal perfusion in children (^{125}I albumine), it has been detected that the creation is 4-5 times larger than normal (1.43 ml/min), while the calculated absorption was within normal range, 0.59 ml/min (Eisenberg et al., 1974). The normal value is the result which Cutler et al. (1968) have observed in children without CSF pathway obstruction, and in those that had communicating hydrocephalus, obtained by ventriculo-lumbar perfusion (creation 0.30 ml/min; absorption 0.61 ml/min). In this method, the quantity of CSF secretion is determined based on the dilution of the marker substance (see section **3 - Methods of measuring cerebrospinal fluid formation**). Taking into account that the pathologically

changed choroid plexus is a structure more permeable for large molecules (more proteins in the CSF), it is possible that a part of the marker substance is lost from the CSF, in a larger amount than in patients with a healthy choroid plexus, thus providing the illusion of hypersecretion. On the other hand, an increased amount of the proteins inside the CSF increases the colloid-osmotic CSF pressure, which has to lead to increased water withdrawal from the interstitium and the cerebral capillaries. This could explain why more CSF is being formed during external drainage under the lower hydrostatic pressure, in the case of a choroid plexus papilloma.

If we add to the above mentioned, and also relevant to papilloma tumors, a discussion in section **4 - Criticism of the classic hypothesis of cerebrospinal fluid formation** related to choroid plexectomy, it becomes clear that in accordance with the explanation of the hydrocephalus, pathophysiology in these conditions does not exist, and that most studies presented here do not fit into the classic CSF secretion, circulation and absorption hypothesis.

Moreover, numerous hypotheses of hydrocephalus development are mentioned in today's literature, which are not in accordance with the classic hypothesis. For example, some other factors, such as an increase in the ventricular CSF pulse pressure (Di Rocco et al., 1978), an impairment of systolic-diastolic displacement of the CSF with the development of periventricular ischemia (Miše et al., 1996), cardiac disease (Luciano and Dombrowski, 2007), changes in arterial pulsations (Greitz, 2004 and 2007) and venous compliance (Bateman, 2000 and 2003) may play an important role in the development of that pathological process.

The third reason why the choroid plexuses are considered to the main site of formation should be the fact that the observations mentioned so far were accompanied by a significant number of concurrent studies that pointed to CSF formation by the choroid plexuses (Dandy, 1919; de Rougemont et al., 1960; Pollay et al., 1972; Weed, 1914; Welch, 1963), as was extensively

stated in the section *Methods of measuring cerebrospinal fluid formation*. Despite the significant unphysiological conditions under which those experiments were mostly performed, the results that indicated CSF formation by the choroid plexus and the reasons stated above helped keep the choroid plexus as the main place of CSF formation. Even more, until today the whole mechanism of CSF secretion by the choroid plexuses was proposed and elaborated (Figures 2, 3 and 4; see section *The site and the way of cerebrospinal fluid formation*), and the secretory character of the choroid plexuses was confirmed by the application of the molecular biological methods during the past two decades (Brown et al., 2004; Johanson, 2008; Kanaka et al., 2001; Li et al., 2002; Oshio et al., 2005; Praetorius et al., 2004; Speake et al., 2003; Watts et al., 1991; Zlokovic et al., 1993).

Furthermore, a significant characteristic of CSF formation is, according to the classic hypothesis, its active nature (CSF secretion). Since CSF formation is an active process, the rate of CSF formation should not be altered following changes in intracranial pressure (ICP). Several authors have tried to establish a relationship between CSF formation and ICP. From the data obtained during the CSF perfusion technique, it was concluded that the alteration of ICP had little or no significant effect on CSF formation (Artru, 1988; Bering and Satto, 1963; Cutler et al., 1968; Heisey et al., 1962; Rubin et al., 1966; Sklar et al., 1980). However, other studies have demonstrated that a change in ICP interferes with the rate of CSF formation (Calhoun et al., 1967; Flexner, 1933; Frier et al., 1972; Hochwald and Sahar, 1971; Martins et al., 1977; Orešković et al., 2000; Weiss and Wertman, 1978) and that the rate of CSF formation declines as pressure in the CSF system is elevated. These results, obtained from different experiments on animals, cannot be explained by the classic hypothesis of CSF formation since Vf is considered to be active and energy consuming, and should bear on ICP, especially because a force under + 30 cm H_2O is not sufficient to affect an active process (Pollay et al., 1983). In other words, it seems that CSF formation may occur as a result of a

passive process rather than an active one, and that the volume of CSF is regulated by hydrostatic pressure (Bulat, 1993; Orešković et al., 2000; Orešković et al., 1991).

It should also be pointed out that experimental results, which suggest a significant portion of CSF formation outside the brain ventricles were obtained (Di Chiro, 1964; Orešković et al., 1991; Sato et al., 1972; Sato et al., 1971; Sato and Bering, 1967). On the basis of these studies, it may be concluded that CSF is formed within the entire fluid system and that it is not mainly related to the brain ventricles and consequently to the choroid plexuses. Included with the indirect evidence consistent with this view is also the observation that CSF is formed within the neural tube of fetal pigs (animal) (Weed, 1917) and humans (Kappers, 1958) before the choroid plexuses anlage appears, and the observation that CSF is formed within the ventricular cavities of some lower vertebrates that lack choroid plexuses (Cserr, 1971; Kappers, 1958; Oppelt et al., 1964). Furthermore, in some sharks, where the choroid plexuses are present in the brain ventricles, no open communication has been found between internal and external CSF. In spite of the existence of the choroid plexuses in isolated brain ventricles there is no tendency for the dilatation of the brain ventricles in physiological conditions (Kappers, 1958; Oppelt et al., 1964). If we add to this the results demonstrating that CSF formation within isolated brain ventricles in cats is, at physiological pressure, in balance with CSF absorption (Orešković et al., 1991;) and that, under physiological conditions in free moving cats, CSF absorption within the ventricles takes place, to a significant extent, also in an open ventricular system (Bulat et al., 2008), it may be expected that a significant and constant exchange occurs between CSF and the surrounding ventricular tissue.

At the end we should ask, if choroid plexuses are not the place of CSF secretion, what is the role of the brain plexuses? This is not an easy question to answer. However, we can say with certainty that the function of the choroid plexuses is not of vital significance to a living organism. Namely, if the choroid plexuses are removed, the patient will continue to live

without disturbance (Enchev and Oi, 2008; Milhorat et al., 1976; Pople and Ettles 1995; Wellons et al., 2002). We also know that it is a highly vascularized structure immerged into the brain ventricles' CSF, with an expressed active metabolic nature (Brown et al., 2004; Johanson, 2008; Kanaka et al., 2001; Li et al., 2002; Oshio et al., 2005). Based on all of this, it can be presumed that (in terms of evolution) by bringing the blood vessels in close contact (immersing into) with CSF this could accomplish a significant and fast exchange of matter between the blood and the CSF, and vice versa, with the purpose of supporting the maintenance of the biochemical balance of the CSF as an important physiological medium for normal CNS functioning. This theory is also supported by a rather old idea that the choroid plexuses (undoubtedly a secretory active tissue) participate in CSF absorption in a manner of two way traffic (Brightman, 1968; Cserr, 1971; Dodge and Fishman, 1970; Foley, 1921; Hassin, 1924; Wright, 1972). After all, it could be said that everything that has been discussed calls for a new approach and hypothesis of CSF hydrodynamics.

5. The new working hypothesis of cerebrospinal fluid hydrodynamics

On the basis of the mentioned foregoing evident discrepancies between experimental results and the classic hypothesis of CSF hydrodynamics, a recent working hypothesis (Figure 8) has been proposed that is more appropriate according to experimental facts. According to this hypothesis (Bulat and Klarica, 2005; Bulat et al., 2008), the mentioned results can be easily explained: during the filtration of water from the arterial capillaries under high hydrostatic pressure (Figure 8B), plasma osmolytes are retained since their permeability across the cerebral capillary wall is very poor (reflection coefficient of main electrolytes Na^+ and Cl^- is 0.98 and it is very similar to that of proteins - 0.999), therefore an osmotic counter-pressure is generated which opposes the water filtration. When such hyperosmolal plasma reaches the

venous capillaries and postcapillary venules where the hydrostatic pressure is low, it becomes instrumental in water reabsorption from interstial fluid (ISF) and CSF (Figure 8B) (Bulat and Klarica, 2005; Bulat et al., 2008). In the brain parenchyma, arterial capillaries with high hydrostatic pressure can be situated near the vessels with low hydrostatic pressure, as shown in Figure 8B. Thus, a rapid turnover of water, which constitutes 99% of ISF-CSF volume, continuously takes place between plasma and ISF-CSF (Bulat et al., 2008). Since the surface of the choroid plexus is about 5000 times smaller than the surface of cerebral capillaries (Crone, 1963; Raichle, 1983), this and all of the above suggests that "formation" and "absorption" of ISF-CSF mainly take place at the cerebral capillaries (Figure 8B). This hypothesis is supported by the observation that, when ^{3}H-water in physiological saline was slowly infused into the lateral ventricle of cats, it was not delivered to the cisterna magna but rather locally absorbed into the periventricular capillaries and drained via the great cerebral vein of Galeni into the confluence of the sinuses (Bulat, 1993; Bulat et al., 2008). In other words, there is no net CSF formation under normal conditions, especially not only inside the brain ventricles, and it seems that CSF appeared and disappeared everywhere in the CSF system. Experimental results (Di Chiro, 1964; Orešković et al., 1991; Sato et al., 1972; Sato et al., 1971; Sato and Bering, 1967) also show that CSF is formed outside the brain ventricles (in subarachnoid space). The results also show that CSF absorption occurs not only through villi arachnoidales, but also inside the brain ventricles (Brightman, 1968; Bulat et al., 2008; Cserr, 1971; Dodge and Fishman, 1970; Foley, 1921; Hassin, 1924; Orešković et al., 1991; Wright, 1972), along the nerve roots (Kido et al., 1976), from the subarachnoid space into the lymphatic system (Bradbury, 1981; Brierly and Field, 1948; Dandy, 1929; Johnston et al., 2005; Johnston et al., 2004; Koh et al., 2005; Koh et al., 2006; Weed, 1914), and high intraspinal absorption (Edsbagge et al., 2004).

The control of CSF volume is under the influence of hydrostatic and osmotic forces (Bulat, 1993; Orešković et al., 2000; Orešković et al., 2002; Orešković et al., 1991) between the CSF system and the surrounding tissue (Figure 8D), and CSF volume will change depending on the prevalence of those forces, caused by (patho)physiological reasons inside or outside of the CSF system, in fact, in a manner similar to the way they regulate the volume of extracellular fluid in other organs (Figure 8).

Of course, that one of the most important mechanisms of maintaining the physiological homeostasis in the CNS is the active transport of substances. Such active and energy consuming process is omnipresent in the CNS; inside the cells, on the membrane of CNS cells (including cells of choroid plexuses) as well as the blood-brain-barrier. Active transport of substances proceeds in both directions, in and out of the cell, directly impacting homeostasis of the CNS (Strikić et al., 1994; Vladić et al., 2000; Zmajević et al., 2002), and is thereby also included in the regulation of osmotic balance and subsequently into maintenance of the CSF volume (see above; Fig. 8D).

Such approach to CSF physiology was strongly supported by our recent investigation using a new experimental model of acute aqueductal blockade by plastic cannula in cats (Klarica et al., 2009; Orešković et al., 2001; Orešković et al., 2002), as was shown in detail in the section *Direct aqueductal method for measuring CSF formation* (Figure 7). It is only necessary to mention that if CSF is mainly formed inside the brain ventricles and absorbed outside, in our model it has to circulate through the cannula positioned in the aqueduct. While under physiological CSF pressure the CSF was collected from isolated brain ventricles for 120 min (Orešković et al., 2001; Orešković et al., 2002), and it permanently pulsated near the external end of the cannula and not a single drop of CSF was observed in the collection tube, which clearly showed that there was no net formation of CSF. Furthermore, according to a new working hypothesis, it is expected that osmotic force also plays an important role in the

regulation of CSF volume, and it seems that the imbalance of CSF osmolality would result in a change in CSF volume. To test this thesis, infusion was done on the same mentioned model (Figure 7) but with hyperosmolal fluid (Orešković et al., 2002). A significant increase in outflow was observed in comparison with isoosmolal infusion, i.e. it is unquestionable that osmolality significantly influences the control of CSF volume and that the increase in CSF osmolality enhanced the CSF volume inside the brain ventricles. In other words, the net CSF formation does not exist in brain ventricles in physiological conditions, but in pathophysiological conditions (hyperosmolal CSF) there is a false impression of CSF formation that could consequently result in an increase in CSF volume, or in our model in the CSF outflow throughout the external end of the plastic cannula.

In addition, the effect on cerebrospinal fluid pressure and the size of the brain ventricles has been studied in the same type of experiment (Klarica et al., 2009) after an acute aqueductal blockade in cats. We showed that the CSF pressure in the isolated ventricles and cisterna magna were practically equal over 120 min, and in some cases up to 190 min, after the aqueductal blockade, and similar to the pressure recorded in an open CSF system (control). These data contradict the classic hypothesis according to which the CSF secreted into the ventricles cannot be absorbed (due to aqueductal occlusion) at the hypothetic CSF absorption site outside the ventricles, so that CSF accumulation in the ventricles should lead to a significant rise in CSF pressure. There is a hypothetical possibility that the elevation of CSF pressure could be avoided by simultaneous dilatation of the isolated ventricles. For that reason, the X-ray measurements of the cross-sectioned area of the lateral ventricle were done in control conditions (aqueduct open) and two hours after the aqueductal blockade (Klarica et al., 2009). Since they did not disclose any significant dilatation of the isolated ventricle, it is clear that CSF does not secrete within the brain ventricles.

In the end, it is necessary to say that after decades of unsuccessful medical treatment of hydrocephalic patients by way of plexectomy, after experimental results which have shown dependency of CSF formation on hydrostatic pressure, after a method for determining CSF formation that has not been sufficiently critically evaluated, and finally after recently obtained results which have clearly shown that there is no net CSF formation in the brain ventricles, the time has definitively come to abandon the classic hypothesis as a framework of thinking in CSF physiology. The time has also come to abandon the classic hypothesis as a common point of reference in scientific papers and in review articles, as an unquestionable fact in text-books for students, and as a schematic illustration in books and anatomic atlases. The time has come for a new hypothesis which can offer a new explanation in CSF physiology that will correspond more closely with experimental facts.

6. Concluding remarks

We may conclude that after almost a hundred years of scientific experimental research in the field of CSF hydrodynamics there is no scientifically founded knowledge of CSF formation that is supported by relevant facts, but CSF formation is still interpreted by means of the classic hypothesis of CSF hydrodynamics. The hypothesis, ever since being founded, has been accompanied by a series of results that cannot be explained or integrated into this hypothesis. The methods that have been used to determine CSF formation have been insufficiently scientifically evaluated, and have very frequently been performed under unphysiological conditions. The obtained results are therefore of questionable reliability and do not ensure relevant data that could provide an answer regarding the nature of CSF establishment. As it has recently been shown that there is no net CSF formation within the brain ventricles under physiological conditions, a new working hypothesis has been proposed

instead of the classic one. According to the new working hypothesis, osmotic and hydrostatic forces are crucial to the regulation of ISF-CSF volume. Considering the capacity of fluid exchange, the cerebral capillaries are the dominant place and the choroid plexuses are a less relevant place for this process. There is a permanent fluid and substances exchange between the CSF system and the surrounding tissue which depends on (patho)physiological conditions that predominate within those compartments. Regardless, our results and those mentioned above indicate that a new approach to the physiology and pathology of CSF is necessary.

References

Abbott, N.J., 2004. Evidence for bulk flow of brain interstitial fluid: significance for physiology and pathology. Neurochem Int. 45, 545-552.

Adeloye, A., Warkany, J., 1976. Experimental congenital hydrocephalus. A review with special consideration of hydrocephalus produced by zinc deficiency. Child's Brain. 2, 325-360.

Alksne, J.F., Lovings, E.T., 1972. The role of the arachnoid villus in the removal of red blood cells from subarachnoid spaces: an electron microscope study in the dog. J Neurosurg.. 36, 192-200.

Akil, H., Coupe, N. J., Singh, J., 2008. Spinal deposits of a benign choroid plexus papilloma. J Clin Neurosci. 15, 708-712.

Artru, A.A., 1988. Dose related changes in the rate of cerebrospinal fluid following administration of thiopental, midazolam and etomidate in dogs. Anesthesiol. 69, 541-546.

Bailey, C. S., Higgins, R. J., 1986. Characteristics of cisternal cerebrospinal fluid associated with primary brain tumors in the dogs: a retrospective study. J Am Vet Med Assoc. 188, 414-417.

Baledent, O., Gondry-Jouet, C., Stoquart-Elsankari, S., Bouzerar, R., LeGars, D., Meyer, M.E., 2006. Value of phase contrast magnetic resonance imaging for investigation of cerebral hydrodynamics. J Neuroradiol. 33, 292-303.

Barber, M. A., Eguiluz, I., Plasencia, W., Medina, M., Valle, L., 2008. Intracranial fetal hemorrhage due to choroid plexus papilloma. Int J Gynecol Obst. 105, 172-173.

Bateman, G.A., 2000. Vascular compliance in normal pressure hydrocephalus. Am J Neuroradiol. 21, 1574-1585.

Bateman, G. A., 2003. The reversibility of reduced cortical vein compliance in normal-pressure hydrocephalus following shunt insertion. Neuroradiol. 45, 65-70.

Barkhof, K., Kouwenhoven, M., Scheltens, P., Sprenger, M., Algra, P., Valk, J., 1994. Phase-contrast cine MR imaging of normal aqueductal CSF flow. Effect of aging and relation to CSF void on modules MR. Acta Radiol. 35, 123-130.

Bergsneider, M., 2001. Evolving concepts of cerebrospinal fluid physiology. Neurosurg Clin N Am. 12, 631-638.

Bering, E.A.Jr., 1952. Water exchange of central nervous system and cerebrospinal fluid. J Neurosurg. 9, 275-287.

Bering, E.A.Jr., 1955. Choroid plexus and arterial pulsation of cerebrospinal fluid. Arch Neurol Psychiat. 73, 165-172.

Bering, E.A.Jr., 1962. Circulation of the cerebrospinal fluid: Demonstration of the choroid plexuses as the generator of the force for flow of fluid and ventricular enlargement. J Neurosurg. 19, 405-413.

Bering, E.A.Jr., Satto, O., 1963. Hydrocephalus: changes in formation and absorption of cerebrospinal fluid within the cerebral ventricles. J Neurosurg. 20, 1050-1063.

Bhadelia, R.A., Bogdan, A.R., Wolpaert, S.M., 1995. Analysis of cerebrospinal fluid flow waveforms with gated phase-contrast MR velocity measurements. Am J Neuroradiol. 16, 389-400.

Borit, A., Sidman, R. L., 1972. New mutant mouse with communicating hydrocephalus and secondary aqueductal stenosis. Acta Neuropath. 21, 316-331.

Bradbury, M.W.B., 1981. Lymphatics and central nervous system. Trends In Neurosc. 4, 100-101.

Bradley, W.G., Feinberg, D.A., Openshaw, K.L., Klein, B., Otto, R., 1986. Comparison of MR cardiac-gated aqueductal flow velocity measurements in healthy individuals and in patients with hydrocephalus. Radiology. 161(P), 194.

Bradley, W.G., Kortman, K.E., Burgoyne, B., 1986. Flowing cerebrospinal fluid in normal and hydrocephalic states: Appearance on MR images. Radiology. 159, 611-616.

Bradley, W.G., Waluch, V., Lai, K.S., Fernandes, E.J., Spatler, C., 1984. The appearance of rapidly flowing blood on magnetic resonance images. Am J Rradiol. 143, 1167-1174.

Brierly, J.F., Field, E.J., 1948. The connections of the cerebrospinal fluid space with the lymphatic system. J Anat. 82, 153-166.

Brightman, M.W., 1968. The intracerebral movement of proteins injected into blood and cerebrospinal fluid of mice. In: Progress in brain research. Brain barrier system, Vol. 29, A. Lajth., D.H. Ford, eds. Elsevier Pub. Comp., Amsterdam pp.19-40.

Brinkmann, G., Harlandt, O., Muhle, C., Brossman, J., Heller, M., 2000. Quantification of fluid flow in magnetic resonance tomography: an experimental study of flow model and liquid flow measurements in the cerebral aqueduct in volunteers. Fortschr Röntgenstr. 172, 1043-1051.

Britz, G. W., Kim, D. K., Loeser, J. D., 1996. Hydrocephalus secondary to diffuse villous hyperplasia of the choroid plexus. Case report. J Neurosurg. 85, 689-691.

Brodbelt, A., Stoodley, M., 2007. CSF pathways: a review. Br J Neurosurg. 21, 510-520.

Brown, P.D,, Davies, S.L., Speake, T., Millar, I.D., 2004. Molecular mechanisms of cerebrospinal fluid production. Neurosci. 129, 957-970.

Bucciolini, M., Casolo, G.C., Giani, M., Ciraolo, L., Galanti, G., 1987. Preliminary approach to quantitative evaluation of blood floe velocity by MR imaging. Phys Med. 2, 115-127.

Bulat, M., 1993. Dynamics and statics of the cerebrospinal fluid: the classic and new hypothesis. In: Intracranial Pressure VIII. C.J.J.Avezaat, J.H.N. van Eijndhoven, A.I.R. Maas, J.Th.J. Tans, eds. Springer-Verlag, Berlin, pp. 731-734.

Bulat, M., Klarica, M., 2005. Fluid filtration and reabsorption across microvascular walls: control by oncotic or osmotic pressure? Period Biol. 107, 147-152.

Bulat, M., Lupret, V., Orešković, D., Klarica, M., 2008. Transventricular and transpial absorption of cerebrospinal fluid into cerebral microvessels. Coll Antropol. (Suppl 3) 31, 43-50.

Calhoun, M.C., Hurt, H.D., Eaton, H.D., Rousseau, H.D. Jr., Hall, R.C. Jr., 1967. Rates of formation and absorption of cerebrospinal fluid in Holstein male calves. Storrs Agric Expl Station, Univ Conn Bull. 401, 22-26.

Clark, R.G., Milhorat, T.H., Stanley, W.C., DiChiro, G., 1971. Experimental Pantopaque ventriculography. J Neurosurg. 34, 387-395.

Conner, E. S., Foley, L., Black, P. M., 1984. Experimental normal-pressure hydrocephalus is accompanied by increased transmantle pressure. J Neurosurg. 61, 322-327.

Crone, C., 1963. The permeability of capillaries in various organs as determined by use of the indicator diffusion method. Acta Physiol Scand. 58, 292-305.

Cserr, H.F., 1971. Physiology of the choroid plexus. Physiol Rev. 51, 273-311.

Cserr, H.F., 1988. Role of secretion and bulk flow of brain interstitial fluid in brain volume regulation. Ann N Y Acad Sci. 529, 9-20.

Curran, R.E., Mosher, M.B., Owens, E.S., Fenstermacher, J.D., 1970. Cerebrospinal fluid production rates determined by simultaneous albumin and inulin perfusion. Exp Neurol. 29, 546-553.

Cushing, H., 1914. Studies on the cerebrospinal fluid. J Med Res. 31, 1-19.

Cutler, R.W.P., Page, L., Galichich, J., Waters, G.V., 1968. Formation and absorption of cerebrospinal fluid in man. Brain. 91, 707-720.

Dandy, W.E., 1919. Experimental hydrocephalus. Ann Surg. 70, 129-142.

Dandy, W.E., 1929. Where is cerebrospinal fluid absorbed? JAMA. 92, 2012-2014.

Dandy, W.E., Blackfan, K.D., 1914. Internal hydrocephalus: experimental, clinical and pathological study. Am J Dis Child. 8, 406-482.

Davson, H., 1967. Physiology of the cerebrospinal fluid. Churchill, London

Davson, H., Welch, K., Segal, M.B., 1987. Physiology and pathophysiology of the cerebrospinal fluid. Churchill-Livingstone, Edinburgh

Deck, M.D.F., Potts, G., 1969. Movements of ventricular fluid levels due to cerebrospinal fluid formation. Am J Roentgenol Radium Ther Nucl Med. 106, 354-368.

de Rougemont, J., Ames, A. 3rd., Nesbett, F.B., Hofmann, H.F., 1960. Fluid formed by choroid plexus; a technique for its collection and comparison of its electrolyte composition with serum and cisternal fluids. J Neurophysiol. 23, 485-495.

Di Chiro, G., 1964. Movement of the cerebrospinal fluid in human beings. Nature. 204, 290-291.

Di Rocco, C., Pettorossi, V. E., Caldarelli. M., Mancinelli, R., Velardi, F., 1978. Communicating hydrocephalus induced by mechanically increased amplitude of the intraventricular cerebrospinal fluid pressure: experimental studies. Exp Neurol. 59, 40-52.

Dodge, P.R., Fishman, M.A., 1970. The choroid plexus – two way traffic? New Engl J Med. 283, 316-317.

Du Boulay, G., 1966. Pulsatile movements in the CSF pathways. J. Radiol. 39, 255-262.

Du Boulay, G., O'Connel, J., Bostic, T.; Verity, P., 1972. Further investigation on pulsatile movements in the cerebrospinal fluid pathways. Acta Radiol Diagn. 13, 496-523.

Edsbagge, M., Tisell, M., Jacobsson, L., Wikkelso, C., 2004. Spinal CSF absorption in healthy individuals. Am J Physiol Regul Integr Comp Physiol. 287, R1450-R1455.

Eisenberg, H. M., McComb, J. G., Lorenzo, A.V., 1974. Cerebrospinal fluid overproduction and hydrocephalus associated with choroid plexus papilloma. J Neurosurg. 40, 381-385.

Enchev, Y, Oi S, 2008. Historical trends of neuroendoscopic surgical techniques in the treatment of hydrocephalus. Neurosurg Rev. 31, DOI 10.1007/s10143-008-0131-y

Enzmann, D.R., Pelc, N.J., 1991. Normal flow patterns of intracranial and spinal cerebrospinal fluid defined with phase-contrast cine MR imaging. Radiology. 178, 467-474.

Fairburn, B., 1960. Choroid plexus papilloma and its relation to hydrocephalus. J Neurosurg. 17, 166-171.

Fenstermacher, J.D., Patlak, C.S., Blasberg, R.G., 1974. Transport of material between brain extracellular fluid, brain cells and blood. Fed Proc. 33, 2070-2074.

Flexner, L.B., 1933. The water of the cerebrospinal fluid. Variations of its rate of flow with variation of ventricular pressure. Am J Physiol. 106, 170-174.

Flexner, I.B., Winters, H., 1932. The rate of formation of cerebrospinal fluid in etherized cats. Am J Physiol. 101, 697-710.

Florez, Y.N., Moratal, D., Forner, J., Mari-Bonmati, L., Arana, E., Guajardo-Hernandez, U., Millet-Roig, J., 2006. Semiautomatic analysis of phase contrast magnetic imaging of cerbrospinal fluid flow through the aqueduct of Sylvius. Magn Resson Mater Phy. 19, 78-87.

Flyger, G., Hjelmquist, U., 1957. Normal variation in the caliber of the human cerebral aqueduct. Anat Rec. 127, 151-162.

Foley, F., 1921. Resorption of the cerebrospinal fluid by the choroid plexuses under the influence of intravenous injection of hypertonic salt solution. Arch Neurol Psychiat. 5, 744-745.

Foltz, E. L., Shurtleff, D. B., 1966. Conversion of communicating hydrocephalus to stenosis or occlusion of the aqueduct during ventricular shunt. J Neurosurg. 24, 520-529.

Forner, J., Florez, N., Valero Merino, C., Marti-Bonmati, L., Moratal, D., Piquer, J., Elso, L., Arana, E., 2007. Assessment of reliable quantification of the dynamics of cerebrospinal fluid by magnetic resonance imaging in idiopathic normal pressure hydrocephalus. Neurologia. 22, 213-220.

Frazier, C.H., Peet, M.M., 1915. The action of glandular extracts on the secretion of cerebrospinal fluid. Am J Physiol. 36, 464-487.

Frier, H.I., Gallina, A.M., Rousseau, J.E.Jr., Eaton, H.D., 1972. Rates of formation and absorption of cerebrospinal fluid in the very young calf. J Dairy Sci. 55, 339-344.

Fujimura, M., Onuma, T., Kameyama, M., Motohashi, O., Kon, H., Yamamoto, K., Ishii, K., Tominaga, T., 2004. Hydrocephalus due to cerebrospinal fluid overproduction of bilateral choroid plexus papillomas. Child Nerv Syst. 20, 485-488.

Gideon, P., Stahlberg, F., Thomsen, C., Gjerris, F., Sørensen, P.S., Henriksen, O., 1994. Cerebrospinal fluid flow production in patient with normal pressure hydrocephalus studied by MRI. Neuroradiology. 36, 210-215.

Gomez, D.G., Potts, D.G., Deonarine, V., 1974. Arachnoid granulations of the sheep. Structural and ultrastructural changes with varying pressure differences. Arch Neurol. 30, 169-174.

Greeneberg, D.M., Aird, R.B., Boetler, M.D.D., Campbell, W.W., Cohn, W.E., Murayama, M.M.A., 1943. A study with radioactive isotope of the permeability of the blood-cerebrospinal fluid barrier to ions. Am J Physiol. 140, 47-64.

Greitz, D., 2004. Radiological assessment of hydrocephalus: new theories and implications for therapy. Neurosurg Rev. 27, 145-165.

Greitz, D., 2007. Paradigm shift in hydrocephalus research in legacy of Dandy's pioneering work: rationale for third ventriculostomy in communicating hydrocephalus. Childs Nerv Syst. 23, 487-489.

Greitz, D., Franck, A., Nordell, B., 1993. On the pulsatile nature of intracranial and spinal CSF-circulation demonstrated by MR imaging. Acta Radiol. 34, 321-328.

Griffith, H. J., Jamjoom, A. B., 1990. The treatment of childhood hydrocephalus by choroid plexus coagulation and artificial cerebrospinal fluid perfusion. Br J Neurosurg. 4, 95-100.

Hakim, S., Hakim, C., 1984. A biomechanical model of hydrocephalus and its relationship to treatment. In: Hydrocephalus, K. Shapiro, A. Marmarou, H. Portnoy, eds, pp. 143-160. New York: Raven Press.

Hammock, M.K., Milhorat, T.H., 1973. Recent studies on the formation of cerebrospinal fluid. Dev Med Child Neurol Suppl. 15, 27-34.

Hassin, G.B., 1924. Notes of the nature and origin of the cerebrospinal fluid. J Nerv Ment Dis. 59, 113-121.

Hawkins, J. C., 1980. Treatment of choroid plexus papillomas in children: a brief analysis of twenty years' experience. Neurosurgery. 6, 380-384.

Heisey, S.R., Held, D., Pappenheimer, J.R., 1962. Bulk flow and diffusion in the cerebrospinal fluid system of the goat. Am J Physiol. 203, 775-781.

Henry-Feugeas, M.C., Idy-Peretti, I., Blanchet, B., Hassine, D., Zannoli, G., Schouman-Claeys, E., 1993. Temporal and spatial assessment of normal cerebrospinal fluid dynamics with MR imaging. Magn Reson Imaging.11, 1107-18.

Hirano, H., Hirahara, K., Asakura, T., 1994. Hydrocephalus due to villous hypertrophy of the choroid plexus in the lateral ventricle. J Neurosurg. 80, 321-323.

Hochwald, G.M., Sahar, A., 1971. Effect of spinal fluid pressure on cerebrospinal fluid formation. Expl Neurol. 32, 30-40.

Hoeper, M.M., Tongers, J., Leppert, A., Baus, S., Maier, R., Lotz, J., 2001. Evaluation of right ventricular performance with a right ventricular ejection fraction thermodilution catheter and magnetic resonance imaging in patients with pulmonary hypertension. Chest. 120, 502–507.

Holtzer, G. J., de Lange, S. A., 1973. Shunt-independent arrest of hydrocephalus. J Neurosurg. 39, 698-701.

Husag, L., Costabile, G., Probst, C.. 1984. Persistent hydrocephalus following removal of choroid plexus papilloma of the lateral ventricle. Neurochirurgia (Stuttg). 27, 82-85.

Jayatilaka, A.D.P., 1965. An electron microscope study of sheep arachnoid granulations. J Anat. 99, 635-649.

Johanson, C.E., 2008. Choroid plexus-CSF circulatory dynamics: Impact on brain growth, metabolism and repair. In Neuroscience in Medicine. C. P. Totowa, ed. The Humana Press, New Jersey, pp. 173-200.

Johanson, C.E., Duncan, J.A. III., Klinge, P.M., Brinker, T., Stopa, E.G., Silveberg, G.D., 2008. Multiplicity of cerebrospinal fluid functions: new challenges in health and disease. Cerebrospinal Fluid Res. 5, 10 doi:10.1186/1743-8454-**5**-10

Johanson, C.E., Preston, J.E., Chodobski, A., Stopa, E.G., Szmydynger-Chodobska, J., McMillan, P.N., 1999. AVP V_1 receptor-mediated decrease in Cl^- efflux and increase in dark cell number in choroid plexus epithelium. Am J Physiol. 276, C82-C90.

Johnston. M., Zakharov, A., Koh, L., Armstrong, D., 2005. Subarachnoid injection of Microfil reveals connections between cerebrospinal fluid and nasal lymphatic in the non-human primate. Neuropathol Appl Neurobiol. 31, 632-640.

Johnston, M., Zakharov, A., Papaiconomou, C., Salmasi, G., Armstrong, D., 2004. Evidence of connections between cerebrospinal fluid and nasal lymphatic vessels in humans, non-human primates and other mammalian species. Cerebrospinal Fluid Res. 1, 10 doi: 10.1186/1743-8454-1-2.

Kaczmarek, M., Subramaniam, R. P., Neff, S. R., 1997. The hydromechanics of hydrocephalus: steady-state solutions for cylindrical geometry. Bull Math Biol. 59, 295-323.

Kanaka, C., Ohno, K., Okabe, A., Kuryama, K., Itoh, T., Fukuda, A., Sato, K., 2001. The differential expression patterns of messenger RNAs encoding K-Cl cotransporters (KCC1,2) and Na-K-2Cl cotransporter (NKCC1) in the rat nervous system. Neurosci. 104, 933-946.

Kappers, J.A., 1958. Structural and functional changes in the telencephalic choroid plexus during human ontogenesis. In: Ciba Found Symp Cerebrospinal Fluid. G.E.W. Wolstenholme, G.M. O'Connor, eds. Little Brown, Boston, pp. 3-31.

Kido, D. K., Gomez, D.G., Pavese, A. M. Jr., Pots, D. G., 1976. Human spinal arachnoid villi granulations. Neuroradiology. 11, 221-228.

Kim, D-S., Choi, J-U., Huh, R., Yun, P-H., Kim, D-I., 1999. Quantitative assessment of cerebrospinal fluid hydrodynamics using a phase-contrast cine MR image in hydrocephalus. Child's Nerv Syst. 15, 461-467.

Klarica, M., Orešković, D., Božić, B., Vukić, M., Butković, V., Bulat, M., 2009. New experimental model of acute aqueductal blockade in cats: Effects on cerebrospinal fluid pressure and the size of brain ventricles. Neurosci. 158, 1397-1405.

Knigge, K.M., 1974. Role of the ventricular system in neuroendocrine processes. Initial studies on the role of catecholamines in transport of thyrotropin-releasing factor. Frontiers in Neurology and Neuroscience Research, P. Seeman, G.M. Brown, eds. University of Toronto Press, Toronto, pp. 40-47.

Koh, L., Zakharov, A., Johnston, M., 2005. Integration of the subarachnoid space and lymphatics: Is it time to embrace a new concept of cerebrospinal fluid absorption? Cerebrospinal Fluid Res. 2, 1-11.

Koh, L., Zakharov, A., Nagra, G., Armstrong, D., Friendship, R., Johnston, M., 2006. Development of cerebrospinal fluid absorption sites in the pig and rat: connections between the subarachnoid space and lymphatic vessels in the olfactory turbinates. Anat Embryol. 211, 335-344.

Lee, J.H., Lee, H.K., Kim, J.K., Kim, H.J., Park, J.K., Choi, C.G. 2004. CSF flow quantification of the cerebral aqueduct in normal volunteers using phase contrast cine MR imaging. Korean J Radiol. 5, 81-86.

Lena, G., Genitori, L., Molina, J., Legatte, J. R. S., Choux, M., 1990. Choroid plexus tumors in children. Review of 24 cases. Acta Neurochir (Wien). 106, 68-72.

Levine, D. N., 2008. Intracranial pressure and ventricular expansion in hydrocephalus: Have been asking wrong question? J Neurolog Sci. 269, 1-11.

Li, H., Tornberg, J., Kaila, K., Airaksinen, M.S., Rivera, C., 2002. Patterns of cation-chloride cotransporter expression during embryonic rodent CNS development. Eur J Neurosci. 16, 2358-2370.

Longatti, P., Fiorindi, A., Perin, A., Martinuzzi, A., 2007. Endoscopic anatomy of the cerebral aqueduct. Neurosurg. 61, ONS1-ONS5.

Lorenzo, A.V., Page, L.K., Waters, G.V., 1970. Relationship between cerebrospinal fluid formation, absorption and pressure in human hydrocephalus. Brain. 93, 679-692.

Lotz, J., Meier, C., Leppert, A., Galanski, M., 2002. Cardiovascular flow measurement with phase-contrast MR imaging: basic facts and implementation. Radiographics. 22, 651-671.

Luciano, M., Dombrovski, S., 2007. Hydrocephalus and the heart: Interactions of the first and third circulations. Cleveland Cinic J Med. 74, S128-S131.

Luetmer, P.H., Huston, J., Friedman, J.A., Dixon, G.R., Petersen, R.C., Jack, C.R., McClelland, R.L., Ebersold, M.J., 2002. Measurement of cerebrospinal fluid flow at the cerebral aqueduct by use of phase-contrast Magnetic Resonance imaging: Technique validation and utility in diagnosing idiopathic normal pressure hydrocephalus. Neurosurg. 50, 534-542.

Lyons, M.K., Meyer, F.B., 1990. Cerebrospinal fluid physiology and the management of increased intracranial pressure. Mayo Clin Proc. 65, 684-707.

Mark, A., Feinberg, D.A., 1986. CSF flow: correlation between signal void and CSF velocity measured by gated velocity phase-encoded MR imaging. Radiology. 161(P), 195.

Martins, A.N., Newby, N., Doyle, T.F., 1977. Sources of error in measuring cerebrospinal fluid formation by ventriculocisternal perfusion. J Neurol Neurosurg Psych. 40, 645-650.

Mascalchi, M., Ciraolo, L., Tanfani, G., Taverni, N., Inzitari, D., Siracusa, G.F., Dal Pozzo, G.C., 1988. Cardiac-gated phase MR imaging of aqueductal CSF flow. J Computer Assisted Tomography. 12, 923-926.

Mase, M., Yamada, K., Banno, T., Miyachi, T., Ohara, S., Matsumoto, T., 1998. Quantitative analysis of CSF flow dynamics using MRI in normal pressure hydrocephalus. Acta Neurochir. 71, 350-353.

Masserman, J.H., 1934. Cerebrospinal hydrodynamics. 4 Clinical experimental studies. Arch Neurol Pshyatry (Lond). 32, 523-553.

Masters, C., Alpers, M., Kakulas, B., 1977. Pathogenesis of reovirus type 1 hydrocephalus in mice. Significance of aqueductal changes. Arch Neurol. 34, 18-28.

McComb, J.G., 1983. Recent research into nature of cerebrospinal fluid formation and absorption. J Neurosurg. 59, 369-383.

Milhorat, T.H., 1969. Choroid plexus and cerebrospinal fluid production. Science. 166, 1514-1516.

Milhorat, T.H., 1975. The third circulation revisited. J Neurosurg. 42, 628-645.

Milhorat, T.H., 1976. Structure and function of the choroid plexus and other sites of cerebrospinal fluid formation. Int Rev Cytol. 47, 225-288.

Milhorat, T.H., 1987. Physiology of the cerebrospinal fluid. In Cerebrospinal fluid and the brain edemas. New York: Neuroscience Society of New York. 39-73.

Milhorat, T.H., 1989. Circulation of the cerebrospinal fluid. In: Pediatric Neurosurgery, 2nd edition. R.L. McLaurin, L. Schult, J.L. Venes, F. Epstein, eds. WB Saunders Co, Philadelphia, pp. 170-179.

Milhorat, T. H., Hammock, M. K., Davis, D. A., Fenstermacher, J. D., 1976. Choroid plexus papilloma. I. Proof of cerebrospinal fluid overproduction. Child's Brain. 2, 273-289.

Milhorat, T.H., Hammock, M.K., Chien, T., Davis, D.A., 1976. Normal rate of cerebrospinal fluid formation five years after bilateral choroid plexectomy. Case report. J. Neurosurg. 44, 735-739.

Milhorat, T.H., Hammock, M.K., Fenstermacher, J.D., Rall, D.P., Levin, V.A., 1971. Science. 173, 330

Millen, J., Woolam, D. H. W., 1958. Vitamins and cerebrospinal fluid. In: CIBA Foundation Symposium on the Cerebrospinal fluid, G. E. W. Wolstenholme, C. M. O'Connor, eds. Churchill, London, pp.168-188.

Miše, B., Klarica, M., Seiwerth, S., Bulat, M., 1996. Experimental hydrocephalus and hydromyelia: a new insight in mechanism of their development. Acta Neurochir. 138, 862-869.

Monie, I. W., Armstrong, R. M., Nelson, M. M., 1961. Hydrocephalus and other abnormalities in rat young resulting from maternal pteroylglutamic acid deficiency from the eight to the tenth days of pregnancy. Abstr Terat Soc. 1, 8-14.

Nagashima, T., Tamaki, N., Matsumoto, S., Horwitz, B., Seguchi, Y., 1987. Biomechanics of hydrocephalus: a new theoretical model. Neurosurg. 21, 898-904.

Nagib, M. G., O'Fallon, T. M., 2000. Lateral ventricle choroid plexus papilloma in childhood: management and complications. Surg Neurol. 54. 366-372.

Netsky, M.G., Shuangshoti, S., 1970. Studies on the choroid plexus. Neurosci Res (NY). 3, 131-173.

Nitz, W.R., Bradley, W.G.Jr., Watanabe, A.S., Lee, R.R., Burgoyne, B., O'Sullivan, R.M., Herbst, M.D., 1992. Flow dynamics of cerebrospinal fluid: Assessment with phase-contrast velocity MR imaging performed with retrospective cardiac gating. Radiology. 183: 395-405.

O'Connel, J.E.A., 1970. Cerebrospinal fluid mechanics. Proc Roy Soc Med. 63, 507-518.

Ohara, S., Negai, H., Matsumoto, T., Banno, T. 1988. MR imaging of CSF pulsatory flow and its relation to intracranial pressure. J Neurosurg. 69, 675-682.

Oppelt, W.W., Patlak, C.S,, Zubrod, C.G., Rall, D.P., 1964. Ventricular fluid production rates and turnover in Elasmobranchii. Comp Biochem Physiol. 12, 171-177.

Orešković, D., Bulat, M., 1993. Hydrostatic force in regulation of CSF volume. In: Intracranial Pressure VIII. C.J.J. Avezaat, J.H.N. van Eijndhoven, A.I.R. Maas, J.Th.J. Tans eds. Springer-Verlag, Berlin, pp. 731-734.

Orešković, D., Klarica, M., Lupret, V., Vukić, M., 2000. The character of the cerebrospinal fluid production. Neurosci Research Communications. 26, 69-76.

Orešković, D., Klarica, M., Vukić, M., 2001. Does the secretion and circulation of the cerebrospinal fluid really exist?. Medical Hypotheses. 56, 622-624.

Orešković, D., Klarica, M., Vukić, M., 2002. The formation and circulation of cerebrospinal fluid inside the cat brain ventricles: a fact or an illusion?. Neurosc Lett. 327, 103-106.

Orešković, D., Klarica, M., Vukić, M., Maraković, J., 2003. Evaluation of ventriculo-cisternal perfusion model as a method to study cerebrospinal fluid formation. Croatian Med J. 44, 161-164.

Orešković, D., Maraković, J., Vukić, M., Radoš, M., Klarica, M., 2008. Fluid perfusion as a method of cerebrospinal fluid formation rate – Critical appraisal. Coll Antropol. 32, 133-137.

Orešković, D., Vukić, M., Klarica, M., Bulat, M., 2005. The investigation of CSF formation by ventriculo-aqueductal perfusion method in cats. Acta Neurochir. (Suppl) 95, 433-436.

Orešković, D., Whitton, P.S., Lupret, V., 1991. Effect of intracranial pressure on cerebrospinal fluid formation in isolated brain ventricles. Neurosci. 41, 773-777.

Oshio, K., Watanabe, H., Song, Y., Verkman, A.S., Manley, G.T., 2005. Reduced cerebrospinal fluid production and intracranial pressure in mice lacking choroid plexus water channel Aquaporin-I. Faseb J. 19, 76-78.

Pappenheimer, J.R., Heisey, S.R., Jordan, E.F., Downer, J.deC., 1962. Perfusion of the cerebral ventricular system in anaesthetized goats. Am J Physiol. 203, 763-774.

Pencalet, P., Sainte-Rose, C., Lellouch-Tubiana, A., Kalifa, C., Brunelle, F., Sgouros, S., Meyer, P., Cinalli, G., Zerah, M., Pierre-Kahn, A., Renier, D.. 1998. Papillomas and carcinomas of the choroid plexus in children. J Neurosurg. 88, 521-8.

Penn, R. D., Lee, M. C., Linninger, A. A., Miesel, K., Ning Lu, S., Stylos, L., 2005, Pressure gradient in the brain in an experimental model of hydrocephalus. J Neurosurg. 102, 1069-1075.

Plum, F., Siesjö, B.K., 1975. Recent advances in CSF physiology. Anesthesiology. 42, 708-730.

Pollay, M., 1972. CSF formation and mechanism of drainage. In: Cisternography and hydrocephalus. J.C. Harbert, D.C. McCullogh, A.S. Lussenhop, G. DiChiro eds. Charles C Thomas, Springfield, pp. 13-24

Pollay, M., 1975. Formation of cerebrospinal fluid. J Neurosurg. 42, 665-673.

Pollay, M., 1977. Review of spinal fluid physiology: Production and absorption in relation to pressure. Clin Neurosurg. 24, 254-269.

Pollay, M., Curl, F., 1967. Secretion of cerebrospinal fluid by the ventricular ependyma of the rabbit. Am J Physiol. 213, 1031-1038.

Pollay, M., Hisey, B., Reynolds, E., 1985. Choroid plexus Na^+/K^+ activated Adenosine Triphosphatase and cerebrospinal fluid formation. Neurosurg. 17, 768-772.

Pollay, M., Stevensen, A., Estrada, E., Kaplan, R., 1972. Extracorporeal perfusion of choroid plexus. J Appl Physiol. 32, 612-617.

Pollay, M., Stevens, A., Roberts, P.A., 1983. Alteration in choroid plexus blood flow and cerebrospinal fluid formation by increased ventricular pressure. In: Neurobiology of cerebrospinal fluid 2. J.H. Wood, ed. Plenum Press, New York, pp. 687-695.

Potts, D.G., 1967. Measurement of the net rate of cerebrospinal fluid formation in a portion of the human lateral ventricle. Radiology. 89, 1093-1095.

Pople, I. K., Ettles D., 1995 The role of endoscopic choroid plexus coagulation in the management of hydrocephalus. Neurosurg. 36, 698-702.

Potts, D.G., Bergland, R.M., 1969. Roentgenologic studies of cerebrospinal fluid formation in the dog. Am J Roentgenol Radium Ther Nucl Med. 105, 756-762.

Potts, D.G., Deck, M.D., Deonarine, V., 1971. Measurement of the rate of cerebrospinal fluid formation in the lateral ventricles of the dog. Radiology. 98, 605-610.

Praetorius, J., Nejsum, L.N., Nielsen, S., 2004. A SCL4A10 gene product maps selectively to the basolateral membrane of choroid plexus epithelial cells. Am J Physiol. 286, C601-C610.

Raichle, M.E., 1983. Neurogenic control of blood-brain barrier permeability. Acta Neuropathol. (Suppl) 8, 75-79.

Redzic, Z.B., Preston, J.E., Duncan, J.A., Chodobski, A., Szmydynger-Chodobska, J., 2005. The choroid plexus-cerebrospinal fluid system: from development to aging. Cur Top Dev Biol. 71, 1-52.

Rennels, M.L., Blaumanis, O.R., Grady, P.A., 1990. Rapid solute transport throughout the brain via paravascular fluid path ways. Adv Neurol. 52, 431-439.

Rubin, R.C., Henderson, E.S., Ommaya, A.K., Walker, M.D., Rall, D.P., 1966. The production of cerebrospinal fluid in man and its modification by acetazolamide. J Neurosurg. 25, 430-436.

Sato, O., Asai, T., Amano, Y., Hara, M., Tsugane, R., Yagi, M., 1972. Extraventricular origin of cerebrospinal fluid: formation rate qualitatively measured in the subarachnoid space of dogs. J Neurosurg. 36, 276-282.

Sato, O., Asai, T., Amano, Y., Hara, M., Tsugane, R., Yagi, M., 1971. Formation of cerebrospinal fluid in spinal subarachnoidal space. Nature. 233, 129-130.

Sato, O., Bering, E.A., 1967. Extra-ventricular formation of cerebrospinal fluid. Brain Nerve. 19, 883-885.

Schroeder, H.W.S., Schweim, C., Schweim, K.H., Gaab, M.R., 2000. Analysis of aqueductal flow after endoscopic aqueductoplasty by using cine phase-contrast magnetic resonance imaging. J Neurosurg. 93, 237-244.

Scollato, A., Tenenbaum, R., Bahl, G., Celerini, M., Salani, B., Di Lorenzo, N., 2008. Changes in aqueductal CSF stroke volume and progression of symptoms in patient with unshunted idiopathic normal pressure hydrocephalus. Am J Neuroradiol. 29, 192-197.

Segal, M.B., 1993. Extracellular and cerebrospinal fluid. J Inherit Metabol Dis. 16, 617-638.

Segal, M.B., Pollay, M., 1977. The secretion of cerebrospinal fluid. Exp Eye Res. (Suppl) 25, 128-147.

Shabo, A.L., Maxwell, D.S., 1968. The morphology of the arachnoid villi: a light and electron microscopic study in the monkey. J. Neurosurg. 29, 451-463.

Shapiro, K., Kohn, I. J., Takei, F., Zee, C., 1987. Progressive ventricular enlargement in cats in the absence of transmantle pressure gradients. J Neurosurg. 67, 88-92.

Sjöqvist, O., 1937. Beobachtungen über die Liqursekretion beim Menschen. Zentralbl Neurochir. 2, 8-18.

Sklar, F.H., Reisch, J., Elashvili, I., Smith, T., Long, D.M., 1980. Effects on pressure on cerebrospinal fluid formation: nonsteady-state measurement in dogs. Am J Physiol. 239, R277-R284.

Smillic, A., Sobey, I., Molnar, Z., 2005. A hydroelastic model of hydrocephalus. J Fluid Mech. 539, 417-433.

Speake, T., Freeman, L.J., Brown, P.D., 2003. Expression of aquaporin-4 water channels in rat choroid plexus. Biochem Biophys Acta. 1609, 80-86.

Spector, R., Johanson, C.E., 1989. The mammalian choroid plexus. Sci Am. 261, 68-74.

Stahlberg, F., Mogelvang, J., Thomsen, C., Nordell, B., Stubgaard, M., Ericsson, A., Sperberg, G., Gretz, D., Larsson, H., Henriksen, O., Persson, B., 1989. A method for MR quantification of flow velocities in blood and csf using interleaved gradient-echo pulse sequences. Magnetic Resonance Imaging. 7, 655-667.

Stephensen, H., Tisell, M., Wikkelsö, C., 2002. There is no pressure gradient in communicating or noncommunicating hydrocephalus. Neurosurg. 50, 763-773.

Strikić, N., Klarica, M., Vladić, A., Bulat, M., 1994. Effect of active transport on distribution and concentration gradients of [^{3}H] benzylpenicillin in the cerebrospinal fluid. Neurosci. Lett. 169, 159-162.

Taketomo, T., Saito, A., 1965. Experimental studies on cerebrospinal fluid flow. Neurology. 15, 578-586.

Tamburrini, G., Caldarelli, M., DiRocco, F., Massimi, L., D'Angelo, L., Fasano, T., DiRocco, C., 2006. The role of endoscopic choroid plexus coagulation in the surgical management of bilateral choroid plexuses hyperplasia. Childs Nerv Syst. 22, 605-608.

Thomsen, C., Stahlberg, F., Stubgard, M., Nordell, B., 1990. Fourier analysis of cerebrospinal fluid flow velocities: MR imaging study. Radiology. 177, 659-665.

Tripathi, R., 1974. Light and electron microscopical studies of the exit pathways of cerebrospinal fluid. J Anat. 118, 379-380.

Tripathi, R., 1974. Tracing the bulk outflow route of cerebrospinal fluid by transmission and scanning electron microscopy. Brain Res. 80, 503-506.

Tripathi, B.S., Tripathi, R., 1974. Vacuolar transcellular channels as a drainage pathways for cerebrospinal fluid J. Physiol (Lond). 239, 195-206.

Vladić, A., Klarica, M., Bulat, M., 2009. Dynamics of distribution of ^{3}H-inulin between the cerebrospinal fluid compartments. Brain Res. 1248, 127-135.

Vladić, A., Strikić, N., Jurčić, D., Zmajević, M., Klarica, M., Bulat, M., 2000. Homeostatic role of the active transport in elimination of [^{3}H] benzylpenicillin out of the cerebrospinal fluid system, Life Sci. 67, 2375–2385.

Von Schulthess, G.K., Higgins, C.B., 1985. Blood flow imaging with MR: spin-phase phenomena. Radiology. 157, 687-695.

Watts, A.G., Sanchez-Watts, G., Emanuel, J.R., Levenson, R., 1991. Cell specific expression of mRNAs encoding Na^+, K^+-ATPase alpha- and beta-subunit isoforms within the rat central nervous system. Proc Nati Acad Sci USA. 88, 7425-7429.

Weed, L.H., 1914. The dual source of CSF. J Med Res. 26, 93-113.

Weed, L.H., 1917. The development of the cerebrospinal spaces in pig and in man. Contrib Embryol. 5, 1-116.

Weed, L.H., 1935. Forces concerned in the absorption of the cerebrospinal fluid. Am J Physiol. 114, 40-45.

Weiss, M.H., Wertman, N., 1978. Modulation of CSF production by alterations in cerebral perfusion pressure. Arch Neurol. 35, 527-529.

Welch, K., 1963. Secretion of cerebrospinal fluid by choroid plexus of the rabbit. Am J Physiol. 205, 617-624.

Welch, K., 1975. The principles of physiology of the cerebrospinal fluid in relation to hydrocephalus including normal pressure hydrocephalus. In: Advances in Neurology. W.J. Friedlander, ed. Raven Press, New York, pp. 247-332.

Welch, K., Friedman, V., 1960. The cerebrospinal fluid valves. Brain. 83, 454-469.

Welch, K., Pollay, M., 1961. Perfusion of particles through arachnoid villi of the monkey. Am J Physiol. 201, 651-654.

Wellons, J. C., Tubbs, R. S., Leveque, J. A., Blount, J. P., Oakes, W. J., 2002. Choroid plexectomy reduced neurosurgical intervention in patients with hydranencephaly. Pediatr Neurosurg. 36, 148-152.

Williams, B., 1973. Is aqueduct stenosis a result of hydrocephalus? Brain. 96, 399-412.

Wolfson, L.I., Katzman, R., Escriva, A., 1974. Clearance of amine metabolite from the cerebrospinal fluid: The brain as a «sink». Neurology. 24, 772-779.

Wright, E. M., 1972. Mechanisms of ion transport across the choroid plexus. J Physiol (London). 226, 545-571.

Zlokovic, B.V., Makic, J.B., Wang, L., McComb, J.G., McDonough, A., 1993. Differential expression of Na, K-ATPase alfa and beta subunit isoforms at the blood brain barrier and the choroid plexus. J Biol Chem. 268, 8019-8025.

Zmajević, M., Klarica, M., Varda, R., Kudelić, N., Bulat, M., 2002. Elimination of phenolsulfonphthalein from the cerebrospinal fluid via capillaries in central nervous system in cats by active transport. Neurosci. Lett. 321, 123-125.

Zülch, K. J., 1958. Neuropathological observation on the cerebrospinal fluid pathway. In: Wolstenholme GEW, C. H. O'Connor, ed, pp 230-242. Boston: Little Brown and Co.

Figures legend

Figure 1. Location scheme of the choroid plexuses and the distribution of CSF in the human central nervous system. CSF is shown as the gray area and the arrows point the direction of CSF circulation and the sites of CSF absorption.

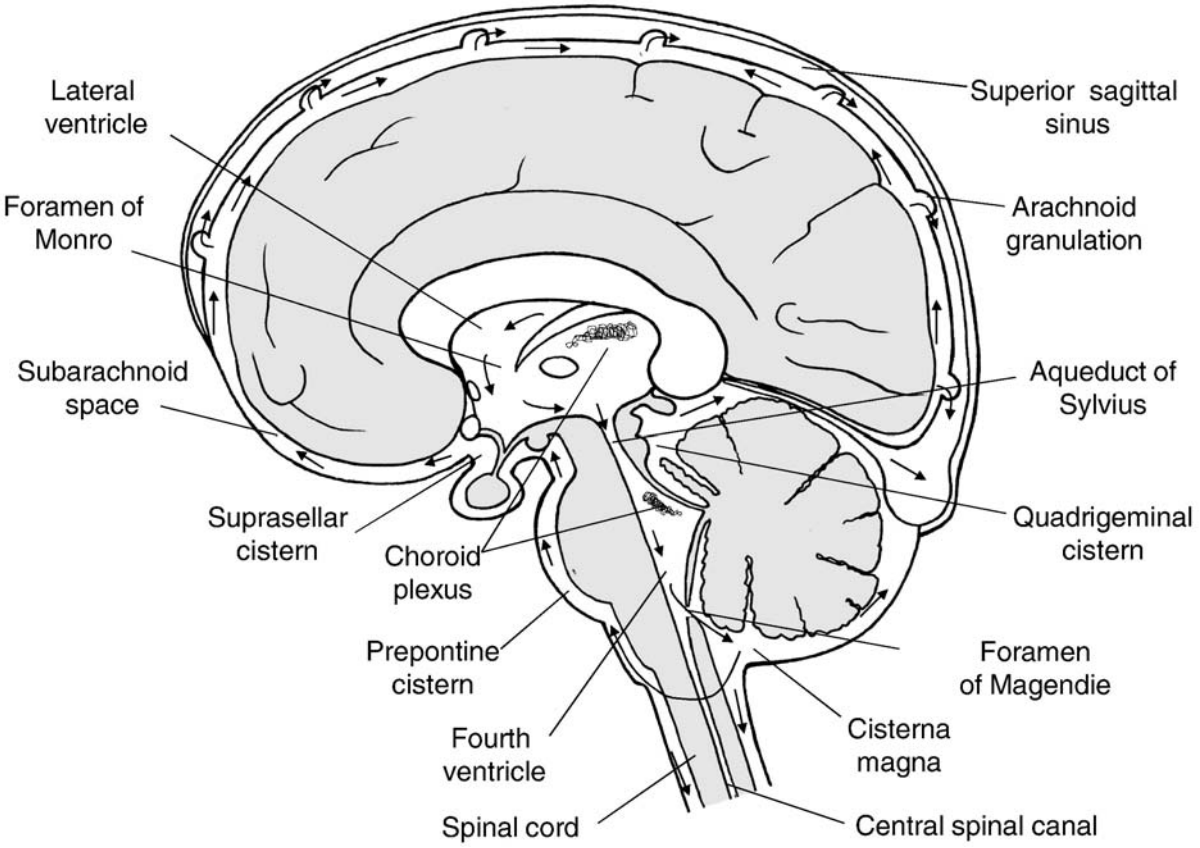

Figure 2. Scheme of the main site of CSF absorption in relation to the arachnoid granulations and dural sinuses, shown in the coronal plane.

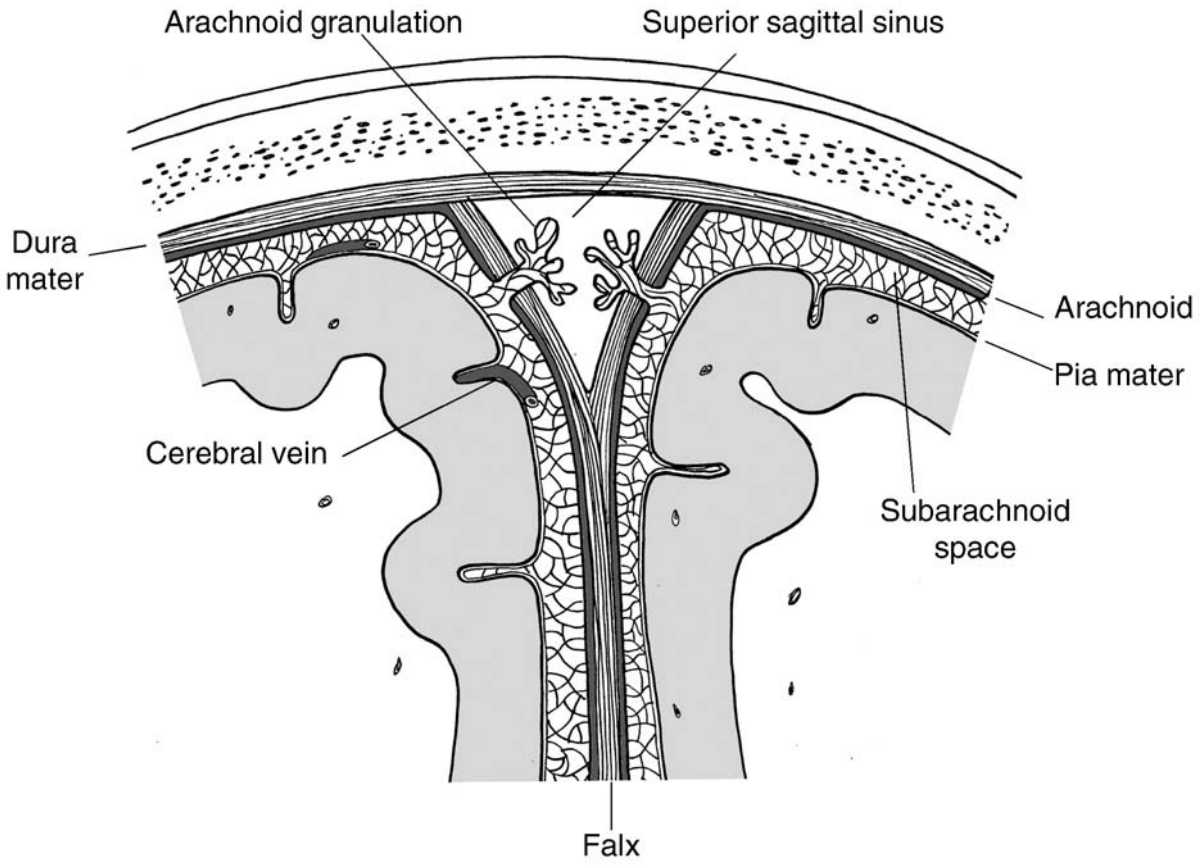

Figure 3. Structure scheme of the choroid plexus. Branched structure of the choroid plexus with villi projecting into the ventricle of the brain. Each plexus consists of a network of capillaries covered by a single layer of cuboidal epithelial cells.

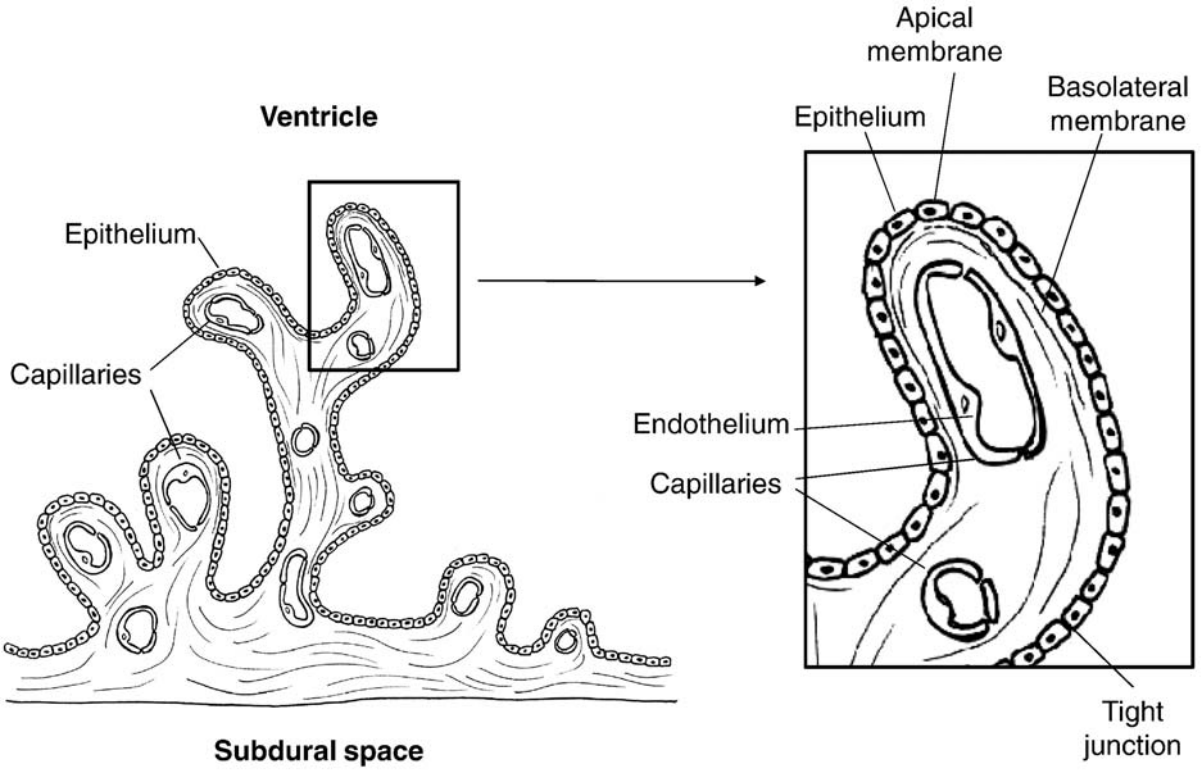

Figure 4. Schematic representation of CSF secretion in the choroid plexus. Inset shows the proposed Na^+ - K^+ pump placed on the apical (CSF-facing) membrane. Aquaporin channels exist on the apical and basolateral membrane.

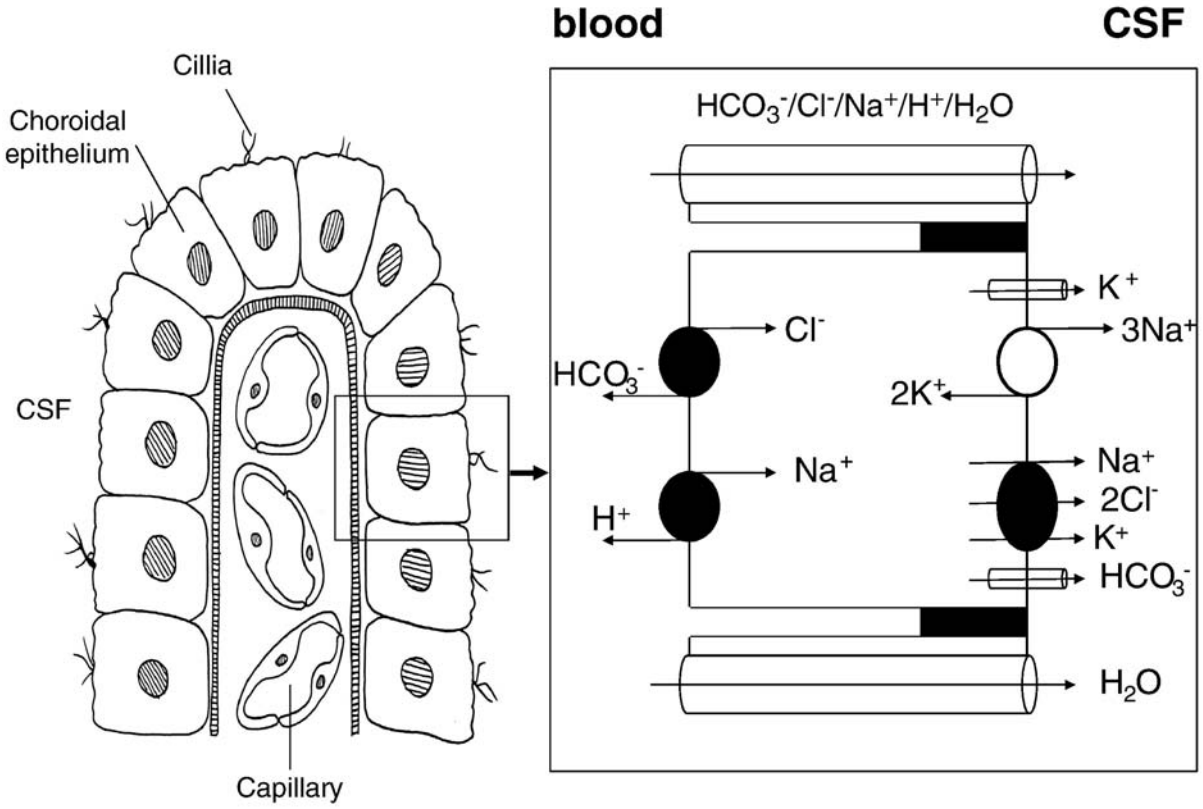

Figure 5. Scheme of the human brain ventricles with emphasized choroid plexuses. Inset shows the aqueduct of Sylvius, a part of the CSF system and the equations which enable the calculation of CSF formation based on CSF velocity using the MRI method.

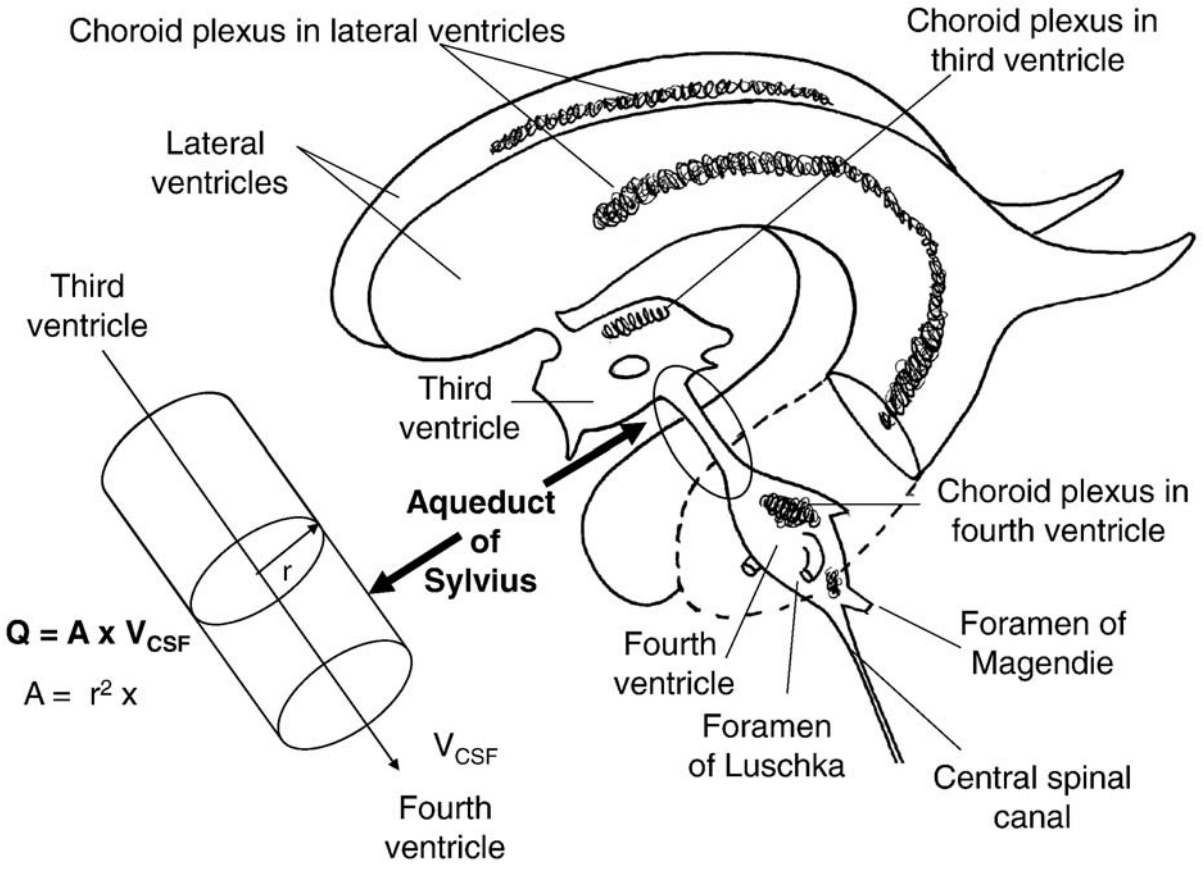

Figure 6. Scheme of the ventriculo-cisternal perfusion in cats.

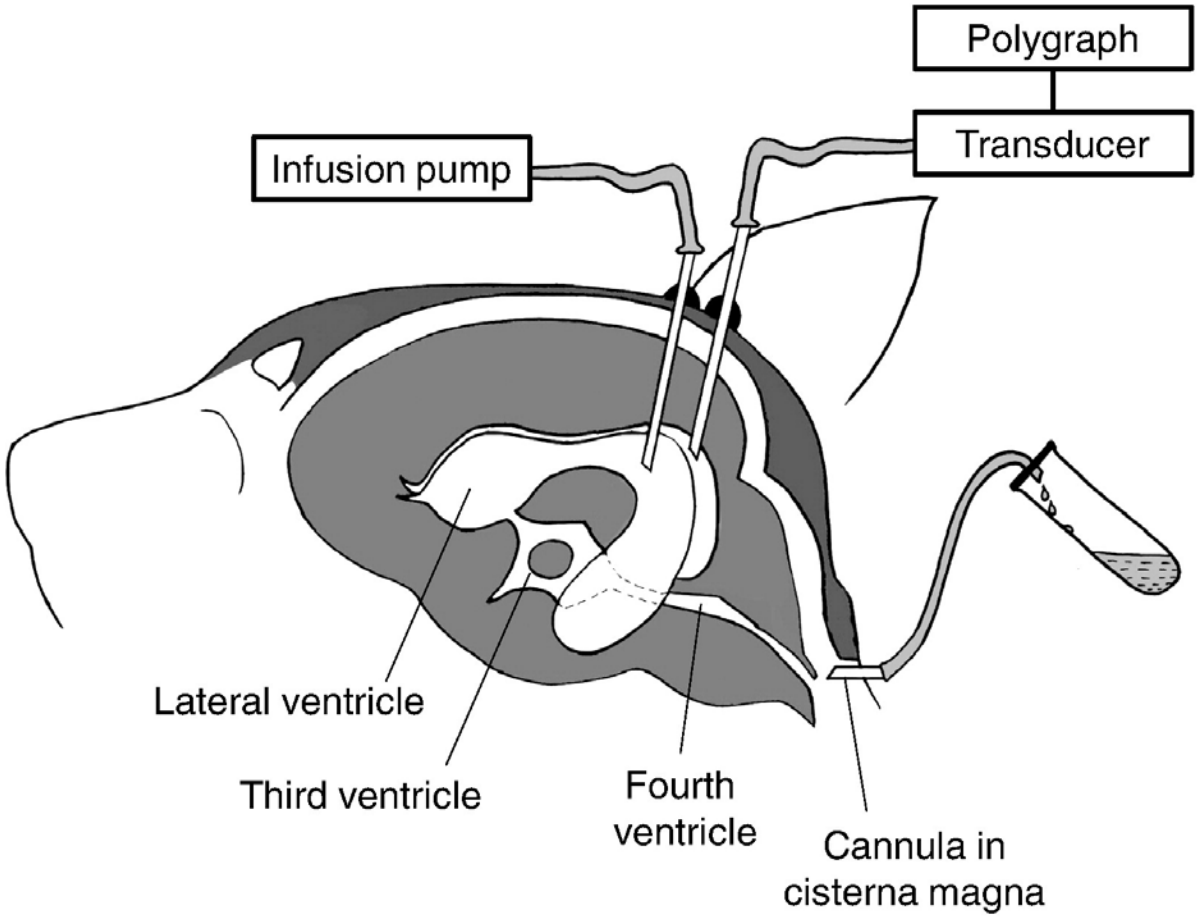

Figure 7. Scheme of the experimental model (direct aqueductal method) showing the position of the cannula for the infusion of artificial CSF in the left lateral ventricle, cannula in the right lateral ventricle for CSF pressure recording, and cannula in the aqueduct of Sylvius for CSF collection or outflow of perfusate.

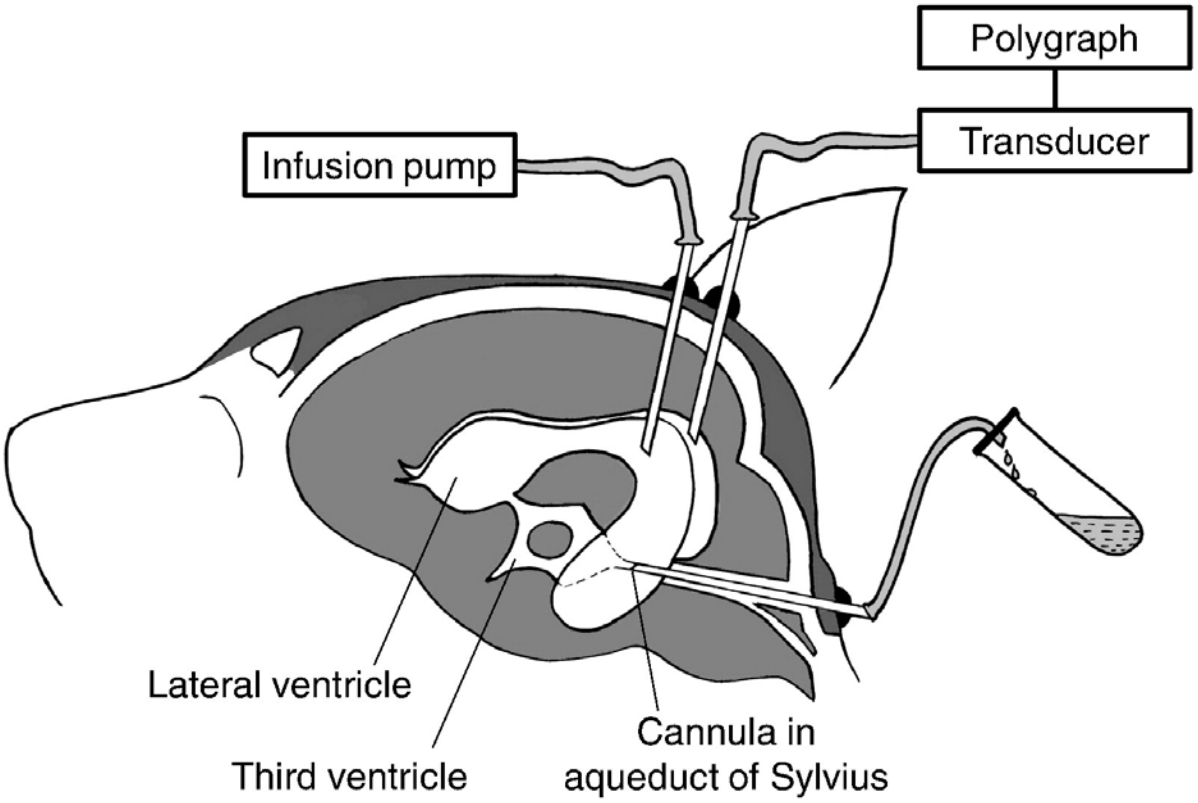

Figure 8. A scheme of the interrelation between cerebrospinal fluid (CSF), interstitial fluid (ISF) and cerebral blood vessels, and the exchange of water and substances between the blood and ISF-CSF through the blood-brain barrier.

A. Larger blood vessels enter deep into the brain tissue. The substances with large m.w. can, after being applied into the CSF system, rapidly enter deep into the tissue via perivascular spaces and reach the vast capillary net. Due to the slow elimination from ISF-CSF into the blood, those substances should be widely distributed inside the brain parenchyma and along CSF system. On the other hand, smaller molecules like water can rapidly reach the capillary net situated under the pia mater after application into the CSF, and then they can be removed from the ISF. Similar to that, the molecules of water from the ventricles can rapidly reach the choroid plexus and the capillaries under the ependyma surrounding the ventricles.

B. The contact surface of the capillaries inside the brain is vast (250cm^2/g of the tissue), and it is about 5000 times larger than the surface of the capillaries inside the choroid plexus. Apart from this, the surface of the arachnoid villi and perineural sheaths of the cranial and spinal nerves are not assumed to be higher than 10 cm^2. Filtration of water from the blood to the ISF takes place at the arterial capillaries (high capillary pressure), and absorption is observed at the vessels under low hydrostatic pressure (venous capillaries, postcapillary venules). The rapid turnover of water volume between the cerebral capillaries and ISF-CSF takes place. Due to great differences between the contact surface of capillaries in brain tissue and in the choroid plexus, it should be expected that the volume of CSF-ISF is predominantly regulated inside the brain parenchyma. The differences in hydrostatic pressure inside the capillaries are shown by the intensity of the color gray.

C. A scheme of the relationship between a cerebral capillary endothelial cell and the surrounding structures (pericytes, neurons, astrocyte end-feets, basement membrane) which contribute to the blood-brain barrier function.

D. The ways substances pass through the membranes of the cerebral capillaries' endothelial cells. A passive diffusion is highly expressed regarding liposoluble substances, and it is conducted under gradient of concentration. The net transport of water depends on the gradients of hydrostatic (hydrostatic capillary pressure-HPc, and hydrostatic interstitial pressure-HPi) and osmotic (osmotic capillary pressure-Opc, and osmotic interstitial pressure-Opi) pressures. The transport systems enable the entrance of more hydrophilic and larger molecules from the blood to the ISF (influx; the straight arrow at the top of the figure). There are also the transport systems which enable the return of molecules from ISF-CSF into the bloodstream (efflux; the straight arrow at the bottom of the figure). The transport of molecules with large m.w. often occurs via endosomes (the formation of the endocytic vesicles; it can also be receptor-dependent)

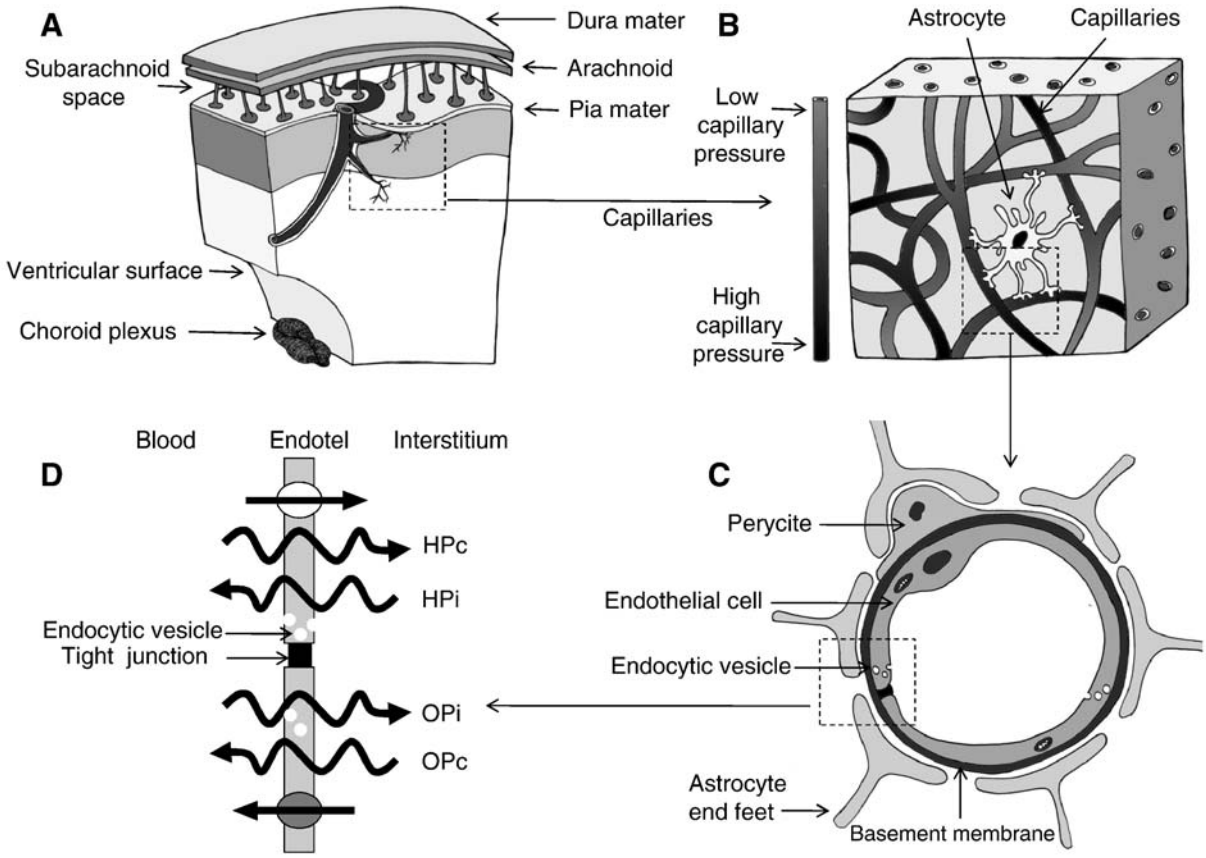
A
Dura mater
Subarachnoid space
Arachnoid
Pia mater
Capillaries
Ventricular surface
Choroid plexus
B
Astrocyte
Capillaries
Low capillary pressure
High capillary pressure
C
Perycite
Endothelial cell
Endocytic vesicle
Astrocyte end feet
Basement membrane
D
Blood
Endotel
Interstitium
HPc
HPi
Endocytic vesicle
Tight junction
OPi
OPc

Table 1. Rates of cerebrospinal fluid formation in humans estimated by Magnetic Resonance Imaging methods

Acknowledgements

This work has been supported by the Ministry of Science, Education and Sport, Republic of Croatia (Projects: 1. Hydrodynamics of the cerebrospinal fluid No. 098-1080231-2328 and 2. Pathophysiology of the cerebrospinal fluid and intracranial pressure No. 108-1080231-0023).

Table 1. Rates of cerebrospinal fluid formation in human estimated by Magnetic Resonance Imaging methods

	Authors	CSF flow ml/min			CSF velocity mm/s			V_f ml/min		
		systolic	diastolic	difference	systolic	diastolic	difference	flow..... based *	velocity. based **	corrected ..... ***
1	Thomsen et al., (1990)	--	--	--	--	--	--	**0.42-0.83**	**--**	**0.53-1.04**
2	Mascalchi et al., (1988)	--	--	--	3-5	3-5	0	**--**	**0**	**0**
3	Forner et al., (2007)	--	--	--	--	--	--	**0.4**	**--**	**0.5**
4	Nitz et al. (1992)	3.42	2.67	0.75	21.5	17.5	4.0	**0.75**	**0.75**	**0.94**
5	Enzman et al., (1991)	--	--	--	11.6	11.8	0	**--**	**0**	**0**
6	Gideon et al., (1994)	--	--	--	--	--	--	**0.68**	**--**	**0.85**

*V_f was obtained by measuring the CSF flow; **V_f was obtained by calculation based on CSF velocity; ***Corrected V_f was obtained by adding V_f from IV brain ventricle