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Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:670973

Download date / Datum preuzimanja: **2025-03-29**

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Vladić, A., Klarica, M., Bulat, M. (2008). *Dynamics of distribution of (3)H-inulin between the cerebrospinal fluid compartments.* Brain Research [Epub ahead of print, Corrected Proof].

Dynamics of distribution of ^{3}H-inulin between the cerebrospinal fluid compartments

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Number of pages of the whole manuscript: 26

Number of figures: 6

Number of tables: 2

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Abstract

Since the distribution of substances between various cerebrospinal fluid (CSF) compartments is poorly understood, we studied ^{3}H-inulin distribution, over time, after its injection into cisterna magna (CM) or lateral ventricle (LV) or cisterna corporis callosi (CCC) in dogs. After the injection into CM ^{3}H-inulin was well distributed to cisterna basalis (CB), lumbar (LSS) and cortical (CSS) subarachnoid spaces and less distributed to LV. When injected in LV ^{3}H-inulin was well distributed to all CSF compartments. However, after injection into CCC ^{3}H-inulin was mostly localized in CCC and adjacent CSS, while its concentrations were much lower in CM and CB and very low in LSS and LV. Concentrations of ^{3}H-inulin in venous plasma of superior sagittal sinus and arterial plasma were very low and did not differ significantly, while its concentration in urine was very high. In ^{3}H-inulin distribution it seems that two simultaneous processes are relevant: a) the pulsation of CSF with to-and-fro displacement of CSF and its mixing, carrying ^{3}H-inulin in all directions, and b) the passage of ^{3}H-inulin from CSF into nervous parenchyma and its rapid distribution to a huge surface area of capillaries by vessels pulsations. ^{3}H-inulin then slowly diffuses across capillary walls into the bloodstream to be eliminated in the urine.

Section 6: Regulatory systems

1. Introduction

The knowledge of the distribution of drugs injected into the cerebrospinal fluid (CSF) is of clinical interest for prediction of their passage and potential therapeutic effect in adjacent nervous parenchyma (Shapiro et al., 1975; Kimelberg et al., 1978). When penicillin was injected into cisterna magna (CM) of dogs, it was well distributed to adjacent cisterna basalis (CB) where pulsations of large brain vessels are prominent, while only its traces were present in lumbar subarachnoid space (LSS), cortical subarachnoid space (CSS) and lateral brain ventricle (LV) under control conditions (Vladić et al., 2000). However, when active transport of penicillin into cerebral capillaries was blocked by probenecid, its distribution from CM to other CSF compartments greatly increased (Vladić et al., 2000). This indicates that the rate of the removal of substances into capillaries of the central nervous system (CNS) is instrumental in the substance distribution between various CSF compartments (Vladić et al., 2000; Strikić et al., 1994; Zmajević et al., 2002).

Since the distribution of substances in CSF is poorly understood, we studied the inulin distribution between various CSF compartments after its injection in CM or LV, or cisterna corporis callosi (CCC) in dogs. Inulin is a relatively large molecule (m.w. 5,500) which poorly diffuses across the blood-brain barrier (Crone, 1963). However, inulin passes easily from CSF into CNS parenchyma (Kimelberg et al., 1978), and it is used as a marker of the CNS extracellular space, i.e. „inulin space" (Levin et al., 1970). Due to the poor passage of inulin across the capillary walls in CNS, it is expected that its distribution between CSF compartments should be efficient.

• *Abbreviations*: CB, cisterna basalis; CCC, cisterna corporis callosi; CM, cisterna magna; CNS, central nervous system; CSF, cerebrospinal fluid; CSS, cortical subarachnoid space;

LSS, lumbar subarachnoid space; LV, lateral ventricle; SEM, standard error of the mean; SSS, superior sagittal sinus.

2. Results

Table 1 shows that the total volume of all brain ventricles measured in 3 dogs is 5.1 ml, while volumes of lateral ventricles, the third ventricle and the fourth ventricle are 3.8 ml, 0.5 ml and 0.8 ml, respectively. Thus, it appears that lateral ventricles contain about 75% of the total CSF volume in ventricles, while CSF volumes of the third and the fourth ventricle are relatively small.

Table 1. Volume of brain ventricles in dogs

Ventricles	Volume (ml)
Lateral ventricles	3.8 ± 0.17 *
Third ventricle	0.5 ± 0.06
Fourth ventricle	0.8 ± 0.08
All ventricles	5.1 ± 0.31

* Results are shown as mean ± SEM of three dogs weighting 13, 19 and 25 kg, respectively.

The length and the diameter of lateral ventricle (anterior horn, middle part and inferior horn) are shown in Table 2. It is seen that the length of lateral ventricle from the tip of anterior horn to the tip of inferior horn is 6.4 cm, while diameters at indicated points in anterior horn, middle part and inferior horn are 0.6 cm, 0.8 cm and 0.8 cm, respectively. The diameter of interventricular foramen (Monro) is 0.4 cm, while lengths of the third ventricle and cerebral aqueduct (Sylvius) are 1.8 and 1.2 cm, respectively. The length of the fourth ventricle is 2.6 cm, while its width and height at the level of colliculus facialis were 1.2 cm and 0.8 cm, respectively.

Table 2. Some dimensions of CSF compartments in dogs

	Dimension (cm)
LATERAL VENTRICLE	
Anterior horn	
diameter (0.5 cm anterior to foramen Monro)	0.6 ± 0.03 *
length (from tip of anterior horn to foramen Monro)	1.4 ± 0.17
Middle part	
diameter at foramen Monro	0.7 ± 0.09
diameter at concavity of trigone	0.8 ± 0.13
length (from foramen Monro to concavity of trigone)	2.0 ± 0.30
Inferior horn	
diameter (0.5 cm anterior to concavity of trigone)	0.8 ± 1.0
length (from concavity of trigone to tip of horn)	3.0 ± 0.15
FORAMEN MONRO	
diameter	0.4 ± 0.03
THIRD VENTRICLE	
length (from foramen Monro to aqueduct)	1.8 ± 0.12
CEREBRAL AQUEDUCT	
diameter (at exit in forth ventricle)	0.2 ± 0.01
length	1.2 ± 0.06
FOURTH VENTRICLE	
length (from aqueduct to central cannal of spinal cord)	2.6 ± 0.12
width (at level of colliculus facialis)	1.2 ± 0.05
height (at level of colliculus facialis)	0.8 ± 0.06
SUBARACHNOID SPACES	
Distances	
from cisterna magna to cisterna ambiens:	
- via brain convexity	4.0 ± 0.24
- via brain base	5.1 ± 0.36
from cisterna ambiens to cisterna corporis callosi	
(0.5 cm posterior to bregma)	3.9 ± 0.21
from cisterna magna to cisterna basalis	6.4 ± 0.23
from cisterna magna to spinous process of fifth lumbar vertebra	69.3 ± 4.30

Table 2 (continued)

Distances between some subarachnoid spaces are shown in Table 2. The distance between CM and CB is 6.4 cm, while the distance from CM to CCC (via cisterna ambiens either by brain convexity or brain base) is about 8-9 cm. The distance between CM and lumbar CSF at the level of the fifth lumbar vertebra is very long, i.e. 69,3 cm.

One and an half hour after the injection into CM a relatively high concentration of ^{3}H-inulin is reached in CB in comparison to LSS, CSS and LV (Fig. 1). Between 3 and 7 hours after the injection the concentrations of ^{3}H-inulin in CM and CB show a drastic fall, while its concentrations in LSS and CSS significantly increase and overshoot the concentrations in CM and CB. Initial low concentration of ^{3}H-inulin in LV rises between 3 and 7 hours. From 7 to 12 hours ^{3}H-inulin concentrations show fall in all CSF compartments so that at 24 hours they were very low except in LSS.

Three hours after the injection into right LV (R-LV), the concentration of ^{3}H-inulin in this ventricle was about twice higher than in CM and CB and much higher than in other CSF compartments, i.e. left LV (L-LV), CSS and LSS (Fig. 2). From 3 to 7 hours the concentration of ^{3}H-inulin in R-LV shows drastic fall while its concentrations in CM, CB and L-LV show slight decrease, and those in CSS and LSS increase. From 7 to 12 hours ^{3}H-inulin concentrations in LSS shows great increase, while in all other the CSF compartments a decrease of ^{3}H-inulin is observed.

Three hours after ^{3}H-inulin injection in CCC, its concentrations in CCC and CSS were high in comparison to CM and CB, while ^{3}H-inulin concentrations in LV and LSS were low (Fig. 3). From 3 to 7 hours concentrations of ^{3}H-inulin in CCC and CSS decreased while those in CM and CB increased, and concentrations in LV and LSS remained low.

Fig. 4 shows concentrations of ^{3}H-inulin in arterial plasma, venous plasma of superior sagittal sinus and urine at 3, 7 and 12 hours after its injection in CM (see Fig. 1). At 3 and 7 hours concentrations of ^{3}H-inulin in arterial and venous plasma were very low in comparison to those in the CSF (see Fig. 1), while concentrations of ^{3}H-inulin in urine were very high. At 12 hours plasma concentrations of ^{3}H-inulin were equal or close to blank value, while concentrations in urine significantly decreased in comparison to those at 7 and 12 hours. No significant differences were detected between ^{3}H-inulin concentrations in arterial plasma and venous plasma of superior sagittal sinuses ($p > 0.05$).

Fig. 5 shows concentrations of ^{3}H-inulin in arterial plasma, venous plasma of superior sagittal sinus and urine at 3 and 7 hours after its injection into CCC (see Fig. 3). Concentrations of ^{3}H-inulin in arterial and sinus plasma at 3 and 7 hours did not differ significantly ($p > 0.05$) and were somewhat lower than after cisternal injection of ^{3}H-inulin (compare Fig. 4 and Fig. 5), while ^{3}H-inulin concentration in urine increased drastically between 3 and 7 hours. In 3 animals the blood samples from superior cerebral vein were obtained at 7 hours after its injection into CCC, and plasma concentration of ^{3}H-inulin did not differ significantly from those in arterial and sinus plasma ($p > 0.05$).

3. Discussion

3.1. Mechanism of substances distribution in CSF and CNS

Three processes can contribute to the distribution of substances throughout various compartments of body fluids: diffusion, unidirectional fluid volume flow (circulation) and mixing by currents within body fluids (Riggs, 1972). Since diffusion is a very slow process, it cannot contribute significantly to substance distribution, if diffusion distances between fluid compartments are long. It was calculated by Riggs (1972) that for the diffusion of nitrogen in water at 20 °C along distances 0.001, 0.01, 0.1 and 1.0 cm, the time required for reaching 95% equilibrium is 6.3 sec, 630 sec (11 min), 63,000 sec (18 hr) and 6,300,000 sec (73 days), respectively. Namely, the time required for the diffusion process to attain any specified fraction of its final equilibrium concentration increases with the square of the distance over which the diffusion occurs (Riggs, 1972). Thus, a tenfold increase in the diffusion distance means that the diffusion process will require 100 times longer to reach a given degree of completion.

In our dogs the distance between CM and LV (tip of inferior horn) is about 10 cm, between CM and CCC (via cisterna ambiens) is about 8-9 cm and between CM and lumbar (fifth lumbar vertebra) CSF is about 70 cm (Table 2). Such long distances between various CSF compartments in dogs indicate that the diffusion of substances between them is not a relevant process. On the other hand, classical concept of the distribution of substances by unidirectional CSF flow (circulation) along CSF spaces should be critically analyzed (see below).

Several factors could influence concentration and distribution of inulin along CSF spaces during time such as systolic-diastolic displacement of CSF volume and its mixing, the passage of inulin from CSF into CNS and its elimination into bloodstream. The distribution of substances by stirring and mixing of CSF is due to respiratory and systolic-diastolic

changes of CSF pressure with to-and-fro displacement of CSF volume. This dislocation and mixing of CSF seems to be brought about in the first place by distensibility of the spinal dura (Martins et al., 1972) and compressibility and emptying of veins in spinal and cranial cavities (Foltz, 1984). NM imaging suggests that during systolic brain expansion an almost simultaneous craniocaudal CSF displacement occurs, while during diastole a caudocranial dislocation of CSF appears due to the recoil of CSF displaced in spinal region (Feinberg and Mark, 1987; Enzmann and Pelc, 1991). These to-and-fro displacements of CSF should cause mixing of CSF and bidirectional distribution of substances between the CSF compartments (Vladić et al., 2000).

An important factor in the distribution of substances between the CSF compartments is physical activity. It was shown in dogs that the distribution of substances in CSF is much better in free moving than in anaesthetized animals (Vladić and Bulat, 1988). For this reason in the present experiments ^{3}H-inulin was injected into CSF in short ether anesthesia and thereafter the animals woke up continuing normal physical activity.

Dynamics of the distribution of ^{3}H-inulin along CSF spaces over time should be also affected by its rate of passage from CSF into adjacent nervous parenchyma and elimination into capillaries. It seems that the distribution of substances from CSF into nervous extracellular space is controlled by systolic-diastolic pulse pressure and mixing of perivascular fluid. It was shown by Rennels et al. (1985) that after horseradish peroxidase (HRP; m.w. 40,000) was infused into lateral ventricle or cisterna magna during 4-10 min in cats and dogs under normal CSF pressure, it was distributed in perivascular (Virchow-Robin) spaces of whole brain parenchyma so that all blood vessels including capillaries were outlined. This rapid perivascular distribution of HRP in the brain can be prevented by stopping or diminishing the pulsations of cerebral arteries by aortic occlusion or by partial ligation of the brachiocephalic artery (Rennels et al., 1985). Thus, the pulse pressure in CSF

and parenchyma (DiRocco, 1984) should be instrumental in rapid perivascular distribution of substances from CSF but not a slow diffusional process (Rennels et al., 1985). Furthermore, when HRP and some other substances are infused into brain parenchyma, they are distributed along the perivascular spaces (Cserr, 1984; Hadaczek et al., 2006) and pass into CSF (Cserr, 1984). Nonrestricted passage of organic acids between nervous parenchyma and CSF (Bulat and Živković, 1971; Bulat and Živković, 1978) and in the opposite direction (Zmajević et al., 2002) was also demonstrated.

Since inulin passes easily from CSF into extracellular space of brain, it is often used as a marker of extracellular space, the so-called „inulin space" (Levin et al., 1970). After application in CSF, ^{14}C-inulin and methotrexate are distributed in the whole brain parenchyma and over time their concentrations tend to equilibrate between these two compartments (Kimelberg et al., 1978). Thus, in our experiments ^{3}H-inulin applied in CSF should rapidly enter perivascular spaces of nervous parenchyma and reach large surface of capillaries. Since inulin diffuses slowly across the blood-brain barrier (Crone, 1963), its passage in the opposite direction, i.e. across the brain-blood barrier, should be a similar diffusional process. However, since capillary surface area is very large, the elimination of inulin into the bloodstream over time could be considerable. Crone (1963) estimated that the surface area of cerebral capillaries is 240 cm^2/g. Taking the brain weight 88 g for dogs (Levin et al., 1970), the calculated capillary surface area would be 21,120 cm^2. To this value, an unknown capillary surface area of spinal cord should be added. On the other hand, the surface area of arachnoid villi and perineural sheets of cranial and spinal nerves is not known but we assume that is not higher than 10 cm^2 so that the elimination of inulin from CSF via those surface areas is probably very limited under normal CSF pressure (see below).

3.2. Distribution of ^{3}H-inulin between CSF compartments

During first 3 hours after the injection of ^{3}H-inulin in CM, its concentration in CB was higher than in other CSF compartments indicating a good stirring and mixing of CSF between these adjacent CSF compartments due to the pulsation of large arteries (internal carotid arteries, basilar artery) at the base of brain (Fig. 1). From 3 to 7 hours concentrations of ^{3}H-inulin in CM and CB drastically falls while its concentrations in LSS and CSS significantly increases indicating that the fluid mixing over that time effectively distributes ^{3}H-inulin along CSF system. For the understanding of the intensity of the distribution of substances among various CSF compartments several factors should be considered such as their proximity, the cross-sectional area of communication, the vehemence of CSF pulsation and mixing, the passage in adjacent nervous parenchyma and the rate of elimination into capillaries (Vladić et al., 2000). A relatively limited distribution of ^{3}H-inulin to LV (Fig. 1) can be explained by a small cross-sectional area of communication and high tortuosity of CSF spaces which include foramen Magendie and foramina Luschka, the fourth ventricle, the aqueduct of Sylvius, the third ventricle and foramina Monro. All these factors should postpone and limit the distribution of ^{3}H-inulin from CM to LV. However, the fact that ^{3}H-inulin is distributed to LV after intracisternal injection speaks in favor of bidirectional distribution of substances between CSF compartments due to fluid mixing and not to unidirectional flow of CSF from ventricles to subarachnoid space (Bulat et al., 2008). It should be mentioned that after lumbar injection of methotrexate in patients, it was distributed to lateral ventricle and in most cases reached assumed therapeutic concentration (Shapiro et al., 1975). From 7 to 24 hours after intracisternal injection ^{3}H-inulin falls in all CSF compartments, its concentration being the highest in LSS, despite the long distance between CM and LSS of about 70 cm (see Table 2). Since spinal subarachnoid space is relatively straight, where systolic-diastolic pulsations and the mixing of CSF are prominent (Martins et al., 1972; Feinberg and Mark, 1987; Enzmann

and Pelc, 1991; Alperin et al., 2005), this explains good distribution of ^{3}H-inulin from CM to LSS. Continuous fall of ^{3}H-inulin in all CSF compartments from 7 to 24 hours (Fig. 1) is explained by its passage from CSF into nervous parenchyma and elimination into microvessels (see above).

Three hours after the injection into right LV (R-LV) ^{3}H-inulin was well distributed to CM and CB, somewhat less to left LV (L-LV) and CSS, while its distribution to LSS was very low (Fig. 2). Between 7 and 12 hours ^{3}H-inulin concentrations in all CSF compartments showed continuous fall except in LSS where a large increase was observed indicating good CSF mixing along spinal cord (see above). If CSF was continuously formed in lateral ventricles and flowed unidirectionally to subarachnoid space, the distribution of ^{3}H-inulin from R-LV to L-LV should not be expected. However, the observed distribution of ^{3}H-inulin to L-LV from R-LV (Fig.2) indicates that systolic-diastolic displacement and mixing of CSF occurs so that the bidirectional distribution of ^{3}H-inulin between the CSF compartments takes place. This is supported by the observation that ^{3}H-inulin is distributed not only from LV to CM (Fig. 2) but also from CM to LV (Fig. 1). Furthermore, ^{3}H-inulin is distributed from CM to CSS (Fig. 1) but also in the opposite direction, i.e. from CSS to CM (Bulat et al., 2008). All this evidence indicate that the bidirectional distribution of substances such as macromolecule inulin, between the CSF compartments is due to the pulsation of CSF and to-and-fro displacements and mixing of CSF volume.

Efficient distribution of ^{3}H-inulin from LV to subarachnoid spaces (Fig. 2) could be explained by large CSF pulsations in brain ventricles (Bering, 1955). These intraventricular CSF pulsations are probably due to the pulsation of choroidal arteries and the transmission of pulse pressure from numerous meningeal arteries which penetrate perpendicularly across a large spherical surface of brain cortex toward centrally located ventricular CSF. This idea is supported by our unpublished observation that, when aqueduct of Sylvius is occluded,

increased CSF pressure in subarachnoid space, obtained by the infusion of artificial CSF into CM, is immediately and faithfully transmitted across brain parenchyma into isolated ventricles. Dynamics of ^{3}H-inulin distribution from R-LV and CM seems to be similar. When concentrations of ^{3}H-inulin in R-LV (Fig. 2) and CM (Fig. 1) are presented on semi-log scale from 3 to 12 hours after their injection (Fig 6.), they show practically the same kinetics with the half-times of 3 hours. This indicates that CSF pulsations in these CSF compartments are prominent and efficacious in the distribution of ^{3}H-inulin along CSF compartments.

After the injection of ^{3}H-inulin in CCC, its concentrations in CCC and CSS were very high between 3 and 7 hours, and showed a continuous decrease, while concentrations in CM and CB were much lower and increased during that time interval (Fig. 3). It would appear that the pulsations of large arteries at the base of the brain produce CSF currents toward the top of the brain and postpone the distribution of ^{3}H-inulin in the opposite direction, i.e. from CCC toward to CM and CB. Between 3 and 7 hr after the injection of ^{3}H-inulin in CCC, its concentrations in LV and LSS were very low (Fig. 3).

3.3. Absorption of ^{3}H-inulin from CSF into blood

Since it is usually assumed that CSF is drained into superior sagittal sinus (SSS) (Davson et al., 1987; Brodbelt and Stoodley, 2007), we measured ^{3}H-inulin concentration in the SSS plasma, arterial plasma and urine at different time intervals after its injection in CM (Fig. 4) and CCC (Fig. 5) to follow its fate in body. Fig. 4 shows concentrations of ^{3}H-inulin in those fluids at 3, 7 and 12 hours after its application in CM. At 3 and 7 hr no significant concentration differences of ^{3}H-inulin could be detected between SSS plasma and arterial plasma, while at 12 hr these concentrations were close to blank value. On the other hand, in comparison to plasma the ^{3}H-inulin concentrations in urine were very high at 3 and 7 hr and showed significant fall at 12 hr. After the injection into CCC the ^{3}H-inulin concentrations in

sinus and arterial plasma at 3 and 7 hr were low and similar while its excretion into urine was high (Fig. 5). At 7 hr the concentration of ^{3}H-inulin in the plasma of superior cerebral vein, which drains into SSS, was similar to those in sinus and arterial plasma (Fig. 5).

It should be mentioned that during the infusion of radioiodinated serum albumin (RISA) into CSF no difference of its concentration could be detected between sinus and arterial blood in rabbits and cats, suggesting that no preferential absorption of RISA from CSF into SSS takes place (McComb et al., 1982; McComb et al., 1984). Our experiments support such a conclusion for the absorption of ^{3}H-inulin from CSF into bloodstream (Fig. 4 and 5). As discussed above, inulin should enter rapidly into perivascular spaces of brain parenchyma from CSF and reach capillaries which have great surface area and diffuse slowly into the bloodstream across capillary walls. The concentration of ^{3}H-inulin in bloodstream is maintained low due to this slow diffusion across capillary walls, its distribution from the blood to various organs and rapid excretion in the urine (Fig. 4 and 5).

For the study of substances absorption into the bloodstream in physiological conditions the normal CSF pressure should be maintained during their application into CSF. Butler (1984) showed that when HRP solution was infused into CSF under normal CSF pressure it remained within CSF, but its application under high CSF pressure damages layers of arachnoid cells connected by tight junctions adjacent to the dura, so that HRP entered the highly vascularized dura. Furthermore, when HRP solution was infused into CSF under high CSF pressure, the endothelial cells of arachnoid villi developed vesicules and transcellular channels while resistance to fluid infusion greatly decreased indicating unphysiological increase of fluid absorption (Butler, 1984). In addition, McComb et al. (1982; 1984) showed that when RISA was infused into CSF of cats and rabbits under raised CSF pressure, its concentration greatly increased in the optic nerves, olfactory bulbs, episcleral tissue and deep

cervical lymph nodes, while in animals with RISA infusion at normal CSF pressure only its traces were detected in mentioned structures.

In our experiments before the injection of small volumes of ^{3}H-inulin solution into CSF, the same volume of CSF was removed so that significant changes of CSF pressure were not expected. Since in anaesthetized animals distribution of substances along CSF spaces is greatly retarded (Vladić and Bulat, 1988), in our experiments ^{3}H-inulin injection into CSF was performed in short ether anesthesia and thereafter the animals woke up and continued normal physical activity. Taking mentioned precautions in our experiments, we assume that the distribution of ^{3}H-inulin in CSF and its absorption into the bloodstream was performed under reasonable physiological conditions.

It seems that the extent of the distribution of various substances along CSF spaces is determined by the rate of their elimination into CNS capillaries. As already mentioned, organic acids (e.g. penicillin, 5-hydroxyindoleacetic acid, phenolsulphonphtalein) show limited distribution along CSF space because they enter adjacent CNS parenchyma and are rapidly eliminated into capillaries by powerful active transport. However, when this active transport is blocked competitively by probenecid, residence time of organic acids in CNS and CSF is greatly increased so that their distribution over time to all CSF compartments is greatly enhanced (Vladić et al., 2000; Strikić et al., 1994; Zmajević et al., 2002). This clearly shows the important role of capillaries in the elimination of substances from interstial fluid and CSF and in the distribution of substances between CNS and CSF compartments.

Of special interest is the fate of water in CSF and interstial fluid since water constitutes 99% of their volume. When ^{3}H-water is slowly infused into LV in cats, it is rapidly absorbed transventricularly into adjacent capillaries which drain via vena cerebri magna (Galeni) in confluence of sinuses (Herophili) where ^{3}H-water reaches very high concentration (Bulat et al., 2008; Bulat, 1993). Due to such rapid transventricular absorption

[3]H-water does not reach CM from LV (Bulat et al., 2008). Furthermore, when aqueduct of Sylvius is occluded in cats, the CSF pressures in isolated ventricles and CM remain equal over several hours of measurement, i.e. without the development of transmantle pressure gradient (M. Klarica, unpublished observation). In addition, it was shown in patients with communicating and noncommunicating hydrocephalus that CSF pressures in LV and CSS were equal (Stephensen et al., 2002), and that after the obstruction of shunt in some children with noncommunicating hydrocephalus, the progression of hydrocephalus does not take place (Holtzer and de Lange, 1973). All this evidence indicates that CSF formation and absorption within ventricle are in balance. A recent hypothesis (Bulat and Klarica, 2005) claims that during water filtration in arterial capillaries under high hydrostatic pressure, the plasma osmolytes are sieved (retained) so that an osmotic counterpressure is generated, which rises along capillary until it reaches at a point the level of capillary hydrostatic pressure so that water filtration is halted. When such hyperosmolar plasma is delivered to venous capillaries and postcapillary venule where hydrostatic pressure is low, the osmotic absorption of water from interstitium occurs, which leads to the normalization of plasma osmolarity (Bulat and Klarica, 2005). In such a way, a rapid turnover of water volume between capillaries and interstial fluid, including CSF, takes place (Bulat et al., 2008; Bulat and Klarica, 2005).

In conclusion, the analysis of our results and data from literature suggest that ^{3}H-inulin injected into CM, LV and CCC is characteristically distributed between variuos CSF compartments due to the process of CSF mixing produced by systolic-diastolic to-and-fro displacements of CSF volume. This process of CSF mixing and bidirectional distribution of ^{3}H-inulin is greatly accentuated by respiratory and body movements. Simultaneously ^{3}H-inulin from CSF enters rapidly perivascular CNS spaces reaching a very large surface area of capillaries and by slow diffusion across microvascular walls reaches the bloodstream to be rapidly eliminated in the urine.

4. Experimental procedures

Experiments in vivo and post mortem were performed on adult mongrel dogs (10 – 33 kg b.w.) according to the Croatian Animal Welfare Act and the approval of the institutional Committee for animal experimentation. Total number of animals used in experiments was 46. During the experiments all efforts were undertaken to eliminate the pain and suffering of the animals. In in vivo experiments ^{3}H-inulin (spec. act. 1-5 Ci/mmol; Amersham, England) was dissolved in physiological saline and injected in cisterna magna (CM), lateral brain ventricle (LV) or cisterna corporis callosi (CCC) and its distribution over time to various CSF compartments was analyzed. In some of these experiments the absorption of ^{3}H-inulin into bloodstream and its elimination in urine were followed. In post mortem experiments the volume and some dimensions of brain ventricles were determined and distances between subarachnoid CSF compartments were measured.

4.1. Experiments in vivo

Dogs were anaesthetized by inhalation of diethyl ether, initially in air-tight box and than via face mask. After the head was fixed in stereotaxic frame (D. Kopf, USA) CM was transcutaneously punctured by stainless steel cannula (0.9 mm o.d.), 300 μl of CSF removed and the same volume of ^{3}H-inulin solution injected over 10 sec in CM, the inhalation of ether discontinued and animals woke up continuing their usual activities.

In another series of experiments, ^{3}H-inulin solution was injected in right lateral ventricle (R-LV). In ether anesthesia, the head of the animal was fixed in stereotaxic frame, dorsal midline incision of the skin and muscles was performed, bone cleaned and drill hole (4 mm d.) made at coordinates 5 mm anterior to the interaural line and 8 mm lateral from the midline. Through this hole the stainless-steel cannula (0.5 mm o.d.) was stereotaxically introduced 13-17 mm below dura (Lim et al., 1960), 100 μl of ventricular fluid withdrown

and the same volume of ^{3}H-inulin solution (25 μCi) injected in R-LV over 10 sec, and cannula removed.

For the injection of ^{3}H-inulin solution in CCC method of Levin et al. (1970) was used. In ether anaesthetized dogs the head was fixed in stereotaxic frame, a burr hole (4 mm d.) was made in midline of skull 5 mm posterior to bregma and stainless steel cannula (0.5 mm o.d.) introduced stereotaxically through superior sagittal sinuses into CCC 8-10 mm below the surface of dura. After the removal of 100 μl of CSF the same volume of ^{3}H-inulin solution (25 μCi) was injected into CCC over 10 sec.

After the injection of ^{3}H-inulin into LV and CCC, bone holes in the skull were closed with dental cement, all exposed tissue surfaces covered by a thin layer of lidocaine gel, dissected skin sewed, inhalation of ether discontinued and animals woke up continuing their usual activities. At different time intervals after ^{3}H-inulin injection the animals were anaesthetized with thiopenton sodium (45 mg/kg i.p.) and femoral vein cannulated for additional application of thiopenton when required. The head of the dog was fixed in stereotaxic frame and samples of CSF (100 μl) from different CSF compartments were taken in the following order: CCC (in experiments with the injection of ^{3}H-inulin in that cistern), CSS, LV, CB and LSS. Only one CSF sample from these CSF compartments was taken in the same animal.

For obtaining CSF sample from CCC the same approach through superior sagittal sinus was used as for injection of ^{3}H-inulin (see above). For the sampling of CSF from CSS a drill hole (5 mm d.) was performed in parietal bone 5 mm anterior to interaural line and 8 mm lateral to midline and CSF was obtained by direct puncture of subarachnoid space over cortical surface (Bulat and Živković, 1978). Through that hole in parietal bone a cannula (0.5 mm o.d.) was introduced in LV by micromanipulator (13-15 mm below level of dura) (Lim et al., 1960) for CSF sampling. The CSF sample from CB was obtained via a cannula introduced

by micromanipulator through a drill hole in the parietal bone (3 mm d.), 5 mm posterior to coronal suture and 15 mm lateral to midline, until it reached skull base (Vladić et al., 2000). After the sampling of CSF from CCC, LV and CB a few drops of Evans blue were applied to mark positions of the cannulas. The CSF from CM was obtained by its transcutaneous puncture. For the sampling of CSF from LSS, a steel cannula was introduced along the anterior part of processus spinosus of the fifth lumbar vertebrae into lumbar subarachnoid space (Parker, 1972).

In animals with the injection of ^{3}H-inulin into CM and CCC after the sampling of CSF, samples of blood (1 ml) were obtained from femoral artery and superior sagittal sinus. To obtain venous blood from sinus, a drill hole was performed in the midline 5 mm posterior to bregma and sinus punctured. In addition, in animals with the injection of ^{3}H-inulin in CCC, brain surface was exposed and samples of blood from superior cerebral vein were obtained. In these two groups of experiments urine samples were obtained by transcutaneous puncture of urinary bladder.

At the end of the experiments the animals were killed by intravenous overdose of thiopenton. Positions of cannulas in CCC, LV and CB was verified in situ by brain dissection and finding of Evans blue coloration of tissue. Radioactivities of CSF, plasma and urine samples (50 μl) were measured in 5 ml of Bray's solution by liquid scintillation counter (Beckman LS 1701, USA). The results are shown as mean ± SEM and statistical analysis of plasma samples obtained from femoral artery, superior sagittal sinus and superior cerebral vein was performed by paired t-test.

4.2. Experiments post mortem

Since we were not able to find in literature data about the volume of brain ventricles and distances between various CSF compartments, we measured them in dogs post mortem. For

measurement of the volume of brain ventricles, the dog was anaesthetized by thiopenton sodium (45 mg/kg i.p.), the head was fixed in stereotaxic frame and stainless steel cannulas (1.8 mm o.d.) were introduced by micromanipulator in both lateral ventricles 5 mm anterior to interauricular line and 8 mm lateral from the midline (see above). The cannulas were fixed in position by cyanoacrylate in parietal bone hole and connected by plastic tubing to syringes containing casting material (Polyester kit; Chromos, Croatia). After that a stainless steel cannula (1.8 mm o.d.) was positioned in CM, the animal was euthanized by intravenous overdose of thiopenton and cisternal cannula open to permit a free outflow of CSF. At the same time the casting material (6-8 ml) was injected in both lateral ventricles under slight pressure over several minutes. Eight hours later, when casting material was hardened, the brain was removed from the skull, cerebral tissue removed and casts of brain ventricles isolated. The volume of lateral, third, and fourth ventricles was measured by the displacement of water volume in finely graduated measure (Table 1).

For the measurement of distances between the CSF compartments, the animal was anaesthetized by thiopenton, right carotid artery was cannulated and left carotid artery occluded, while both jugular veins were completely cut to induce free bleeding. At the same time brain perfusion through right carotid artery was started with 200 ml of physiological saline, thereafter with 200 ml of 70% of ethanol and 500 ml of 10% formalin solution under the pressure of 100 mmHg. One hour after the brain perfusion, the cerebral tissue was hardened and the brain was removed from the cranial cavity. Distances between cerebral cisterns were measured by a flexible plastic tubing, while the distance between CM and spinous process of fifth lumbar vertebra was measured over the skin of the animal. Thereafter, the brain ventricles were opened to measure some of their anatomical dimensions (Table 2).

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Figure legend

Fig. 1. Concentrations of ^{3}H-inulin in CSF (dpm/50 μl) of cisterna magna (CM), cisterna basalis (CB), lateral ventricle (LV), cortical subarachnoid space (CSS) and lumbar subarahnoid space (LSS) at different time intervals (hours) after ^{3}H-inulin injection into CM. Results are shown as mean and SEM (n = 4).
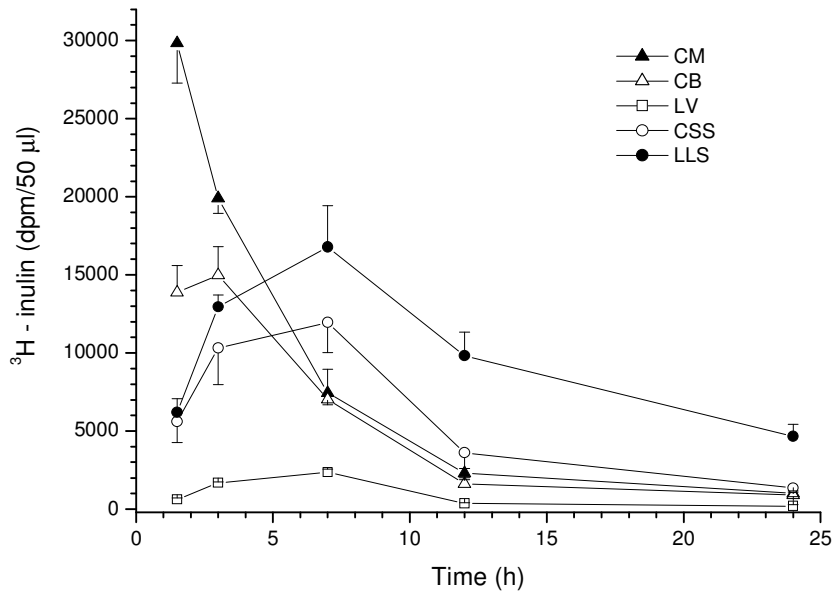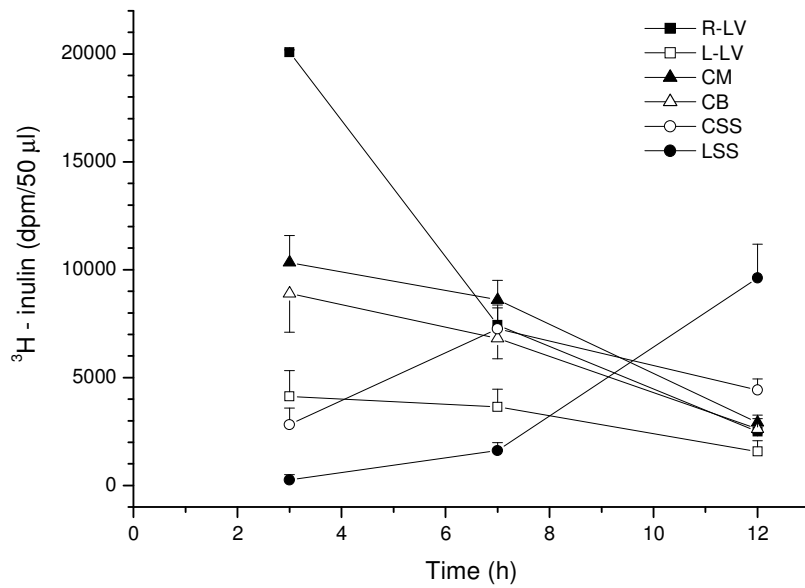
Fig. 2. Concentrations of ^{3}H-inulin in CSF (dpm/50 μl) of right lateral ventricle (R-LV), left lateral ventricle (L-LV), cisterna magna (CM), cisterna basalis (CB), cortical subarachnoid space (CSS) and lumbar subarachnoid space (LSS) at different time intervals (hours) after ^{3}H-inulin injection into R-LV. Results are shown as mean and SEM (n = 4).
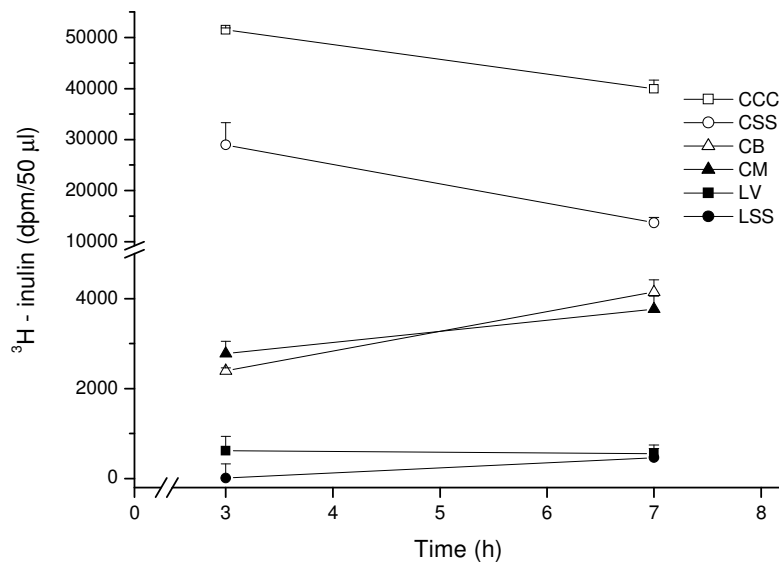
Fig. 3. Concentrations of ^{3}H-inulin in CSF (dpm/50 µl) of cisterna corporis callosi (CCC), cortical subarachnoid space (CSS), cisterna basalis (CB), cisterna magna (CM), lateral ventricle (LV) and lumbar subarachnoid space (LSS) at different time intervals (hours) after ^{3}H-inulin injection into CCC. Results are shown as mean and SEM (n = 4).

Fig. 4. Concentrations of ^{3}H-inulin (dpm/50 μl) in plasma of superior sagittal sinus (SSS), femoral artery (FA) and urine at different time intervals (hours) after ^{3}H-inulin injection into cisterna magna. Results are shown as mean and SEM (n = 4).
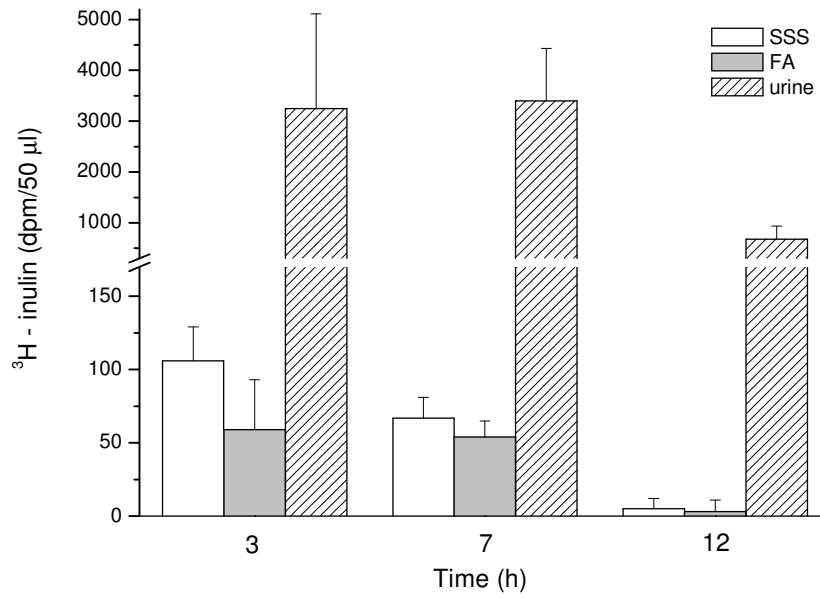
Fig. 5. Concentrations of ^{3}H-inulin (dpm/50 µl) in plasma of superior sagittal sinus (SSS), femoral artery (FA) and urine at 3 and 7 hours after injection of ^{3}H-inulin into cisterna corporis callosi (n = 4), as well as in plasma of superior cortical vein (SCV, n = 3) at 7 hours. Results are shown as mean and SEM.
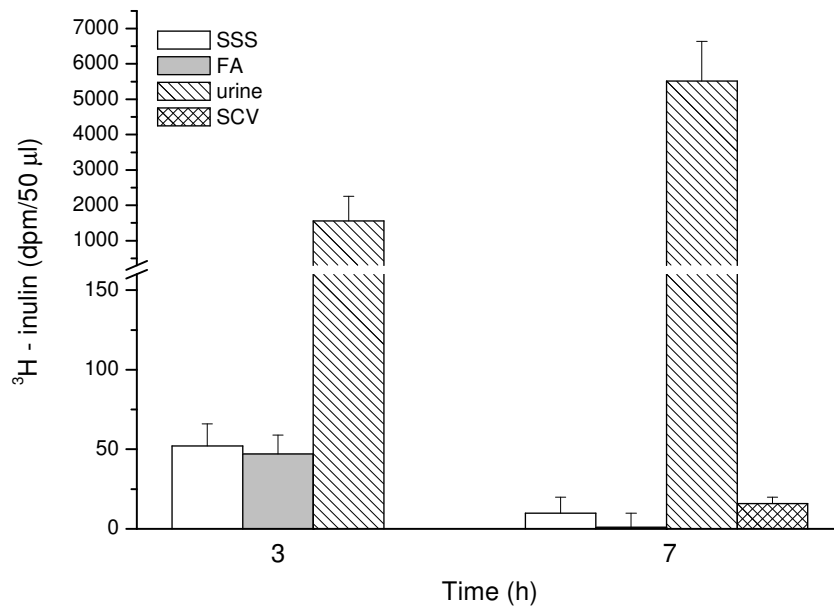
Fig. 6. Concentrations of ^{3}H-inulin (dpm/50 μl) in CSF of cisterna magna (CM) and right lateral ventricle (R-LV) at 3, 7 and 12 hours after its injection either in CM or R-LV, respectively. Data for CM are taken from Fig. 1. and those for R-LV from Fig. 2., respectively. The results are shown on semi-log scale.