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Extracellular Space and Electrolyte Distribution in Cortex and White Matter of Dog Brain in Cold Induced Oedema*

By

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With 3 Figures

Summary

24 hours after a circumscribed cold injury of the cortex dog brains were perfused from the lateral ventricle and the frontal subarachnoidal space to the cisterna magna with an artificial CSF containing trace amounts of ^{35}S-labelled thiosulphate. Simultaneously the extracellular tracer was administered intravenously. Extracellular fluid volume was estimated and found to be increased from 10 to 15% in the oedematous cortex and from 10 to 27% in the oedematous white matter. The actual size of ECS in oedematous white matter, however, must be larger as indicated by the relative alterations of thiosulphate distribution, tissue water, sodium and chloride. Apparently a small part of the fluid accumulation affects the cellular compartment in oedematous white matter. It may be concluded from the close spatial correspondence of the spreading of I^{131} albumin and Evans blue, the increase in water and sodium content, and the enlargement of the TSS that the dilated extracellular channels are filled with a plasma like oedema fluid, derived from blood.

The oedema resulting from a local cold injury to the cortex produces an experimental model bearing similarities to the oedema following traumatic brain injury. This model has often been used to study the disturbance of blood-brain barrier to various compounds[3, 6, 11, 13, 14] as well as the morphological[3, 4, 5, 15, 22] and chemical changes[3, 6, 16] that occur in oedematous tissue. In contrast, the sequence of events occuring during the formation of oedema and the physiochemical forces (in terms of diffusion and driving forces) involved in the formation and resolution of brain oedema are not adequately known. Of

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major importance for such studies is a knowledge of the size and the changes of the extracellular space (ECS) in this type of oedema.

The present investigation was undertaken in an attempt to estimate the changes in the extracellular and intracellular fluid compartment as well as the distribution of electrolytes in these fluid spaces in cold injury oedema. Estimation of the size of extracellular space (ECS) was carried out with the aid of ventriculocisternal and subarachnoido-cisternal perfusion, a technique which prevents the *sink action* of the CSF[8, 19, 23]. In a previous study ECS in normal dogs brain was estimated with ^{35}S-thiosulphate as an extracellular marker and was found to range between 10 and 14% in 5 discrete brain areas[10, 19]. The permeability of blood-brain barrier (BBB), and the relationship of the spread of protein-rich oedema fluid to the enlargement of the extracellular space were examined using Evans blue and I^{131} albumin.

Materials and Methods

Adult mongrel dogs of both sexes weighing between 6 and 10 kg were used. In each animal a cold lesion (13) was produced under hexobarbital anaesthesia (50 mg/kg). A 15 mm trephine opening was made over the right middle suprasylvian gyrus, leaving the dura intact. A metal cylinder with a brass rod (10 mm diameter) at its base, cooled by a mixture of acetone and liquid nitrogen to — 56 °C, was applied to the intact dura for 40 seconds. Thereafter Evans blue was injected intravenously (1 ml/kg, 2% in physiological saline). 6 dogs received I^{131} serum albumin (20–25 μ Ci/kg) i.v. 24 hours later the animals were anaesthetized with pentobarbital (25 mg/kg), intubated, paralysed (Imbretil, 0.6–1.2 mg), and artificially ventilated by means of a Starling pump. Tidal volume was adjusted to keep arterial pO_2 and pCO_2 in normal range. Arterial blood pressure in the femoral artery was recorded with a Statham pressure transducer. To prevent renal elimination of the extracellular marker the animals had both renal pedicles ligated. A bilateral craniotomy was performed to avoid tonsillar herniation during ventricular perfusion. Polyethylene catheters (1.55 mm and 0.8 mm diameters respectively) were inserted into the lateral ventricles and the frontal subarachnoid spaces of both hemispheres as well as into the cisternae magnae. Ventriculo-cisternal perfusion (1.0 ml/min) and subarachnoido-cisternal perfusion (0.5 ml/min) was performed by means of perfusion pumps using an artificial CSF (7). Perfusion pressure was kept below 150 mm H_2O.

S^{35} sodium-thiosulphate (inner labelled, mean spec. radioactivity 23.0 m Ci/mM; Radiochemical Ctr., Amersham) as well as unlabelled sodium-thiosulphate (Thilo & Co., Dortmund) as a carrier were injected intravenously (ratio 1 : 4) and were added to the artificial CSF in the same concentration as in the plasma (to prevent the sink action of the CSF). The osmolarity was individually adjusted to the osmolarity of CSF by addition of D-glucose. Samples of plasma and cisternal outflow were taken at intervals to determine the S^{35}-activity. After perfusion times of 90, 180, 270 and 390 minutes the animals were sacrificed and duplicate tissue samples for the determination of water and electrolyte content, S^{35}-activity,

and I^{131}-activity were removed from cortex and white matter of the control and injured hisempheres. Samples from the injured hemispheres were taken from the faintly stained cortex surrounding the lesion and from the underlying blue-stained white matter as well as from remote unstained cortex and white matter. Additional samples were excised from caudate nucleus, medulla and cerebellum.

The S^{35}-radioactivity of brain, plasma and cisternal outflow was determined by liquid scintillation as described previously. Quench corrections were made using a technique of internal standardization. Values of plasma were corrected for plasma water [10, 9]. The thiosulphate space was computed by the equation.

$$\text{space } \% = \frac{\text{cpm/mg tissue}}{\text{cpm/}\mu\text{l of } \frac{\text{cisternal outflow} + \text{plasma water}}{2}} \times 100$$

For determination of I^{131} activity tissue and plasma samples (ca. 30–100 mg) were dissolved in 0.2 ml 20% H_2O_2 and 0.2 ml 70% $HClO_4$ diluted with 2 ml H_2O. The gamma radioactivity was measured in a well scintillation counter (Baird-Atomic) and corrected by an internal standard. I^{131} space was computed from the concentration in tissue and in plasma.

Water, sodium, potassium and chloride contents in tissue, CSF, plasma and perfusion medium were determined as described previously [10, 18].

Intracellular electrolyte concentration (Ci) was calculated according to

$$\text{Ci [m Eq/L]} = \frac{\text{El}_i \text{ [m Eq/kg d. wt.]}}{\text{H}_2\text{O}_i \text{ [L/kg d. wt.]}}$$

where El_i and H_2O_i are the intracellular electrolyte and water content, derived as the difference between total tissue content and the computed extracellular electrolyte or water content ($El_i = El_t - El_e$). For calculation of El_e in normal brain the volume of ECS and the CSF electrolyte concentration was used. El_e in the oedematous tissue was computed under the assumption that the increase in ECS is derived from plasma and mixes with the brain extracellular fluid. Therefore the mean values of the Na^+, Cl^- and K^+ concentrations in plasma, water and CSF were taken.

Appendix. It must be mentioned that all calculations of fluid spaces and extra- and intracellular electrolyte concentrations in vasogenic brain oedema are probably underestimated. If the oedema fluid is derived from plasma, the dry weight of oedematous tissue must rise in proportion to the weight of plasma solids present. This is masked when the data are expressed in terms of wet weight and disregarded when expressed as an unit of dry weight. This is shown by the following example: If 100 g of plasma are added to a control tissue (400 g water, 100 g dry weight) the total final weight of this oedematous tissue will be 600 g, the final water content 492 g and the final solids 108 g, assuming a figure of 92 g per 100 g for plasma water. The percentage water content of the control tissue would be 80% and the percentage water content of the oedematous tissue 82%. Now, if the water content of the oedematous tissue is recalculated from the percentage water content, a figure of 455 g/100 g dry weight is obtained. This is the usual way of recalculating data. A similar error is made when

data of electrolytes are expressed in terms of unit dry weight. The error is even magnified since the error made in both recalculations is of different magnitude.

Results

Water and electrolyte content in brain: 24 hours after the cold injury sodium and chloride content in the bluish stained cortex of the damaged hemisphere had increased and the potassium content had decreased. Water content remained unchanged (Table 1). In the deeply blue-stained white matter underlying the lesion a significant increase of water content of about 8% and a significant rise in the sodium and

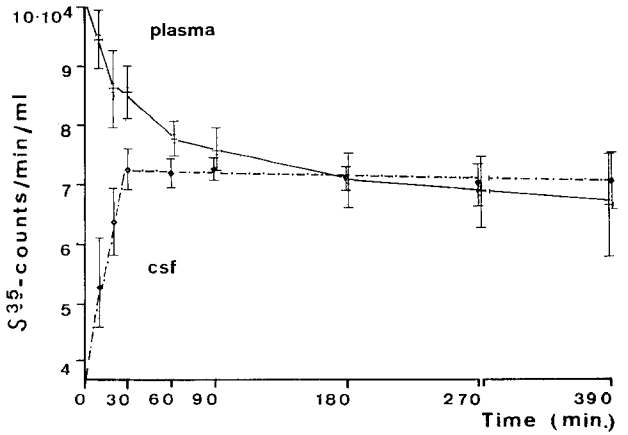


Fig. 1. ^{35}S-radioactivity in CSF and plasma plotted on the ordinate as a function of perfusion time

chloride content were observed. Values in the unstained cortex and white matter of the damaged hemisphere did not differ from values in the control hemispheres. All values in the control hemisphere as well as in each caudate nucleus, medulla and cerebellum corresponded to normal values[10, 18, 19]. These results are in agreement with previous observations of Pappius and Gulati[16]. Electrolyte concentrations and osmolarities of plasma, CSF and perfusion medium are given in Table 2.

S^{35}-*thiosulphate in plasma, CSF and brain:* A steady state was achieved within approximately 30–60 min in the cisternal effluent and within 120 min in the plasma (Fig. 1). Concentration differences between perfusion fluid and cisternal effluent ranged between 3 and 6%.

In the brain cortex the thiosulphate space remained constant after 180 min. The *mean* thiosulphate space (average of 180, 270 and 390 min) amounted to 10.2 ± 2.8% in the control hemisphere, to 15.0 ± 0.08%

Table 1. *Water and Electrolyte Content of 9 Areas of Dog Brain After a Local Cold Injury*
Mean values ± S.E.D. were obtained from the 90, 180, 270 and 390 min perfusion groups. Water is expressed as g/100 g wet weight; electrolytes as mEq/kg dry weight. *** = significantly different from controls at $p < 0.001$

		Water	Sodium	Potassium	Chloride
Cortex	control	80.0 ± 0.3 (19)	276.0 ± 7.8 (19)	537.3 ± 10.3 (19)	223.0 ± 5.0 (19)
	blue-stained	80.4 ± 0.3 (19)	338.3 ± 12.2 (19)***	482.2 ± 13.5 (19)***	279.5 ± 12.6 (19)***
	unstained	80.4 ± 0.3 (19)	280.0 ± 10.2 (19)	539.0 ± 6.4 (19)	220.2 ± 6.7 (19)
White matter	control	67.8 ± 0.4 (19)	166.3 ± 4.4 (19)	267.7 ± 7.6 (19)	138.2 ± 4.6 (19)
	blue-stained	75.9 ± 0.4 (19)***	344.7 ± 12.0 (18)***	277.1 ± 5.6 (19)	293.8 ± 12.2 (19)***
	unstained	68.2 ± 0.4 (19)	171.1 ± 5.7 (19)	269.2 ± 5.9 (19)	148.2 ± 7.8 (19)
Caudate nucleus		80.0 ± 0.3 (16)	265.8 ± 7.0 (16)	510.3 ± 13.0 (16)	209.9 ± 9.8 (16)
Medulla		69.7 ± 0.5 (19)	170.2 ± 5.8 (19)	295.5 ± 8.5 (19)	134.1 ± 4.2 (19)
Cerebellum		78.8 ± 0.3 (19)	234.2 ± 7.4 (19)	475.5 ± 16.7 (19)	171.5 ± 7.1 (19)

Table 2. *Electrolyte Concentrations and Osmolarity in Plasma, CSF and Perfusion Fluid*
Mean values ± S.E.D. are given. Number of animals indicated in brackets

	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)	Osmolarity (m osmol/l)
Plasma	157.6 ± 3.1 (18)	3.7 ± 0.18 (18)	120.0 ± 1.7 (17)	292.7 ± 2.8 (18)
CSF	162.4 ± 2.6 (17)	3.2 ± 0.10 (17)	132.5 ± 2.8 (16)	291.0 ± 3.4 (18)
Perfusion fluid	161.4 ± 2.6 (19)	3.2 ± 0.10 (19)	135.9 ± 2.4 (18)	287.1 ± 2.5 (18)

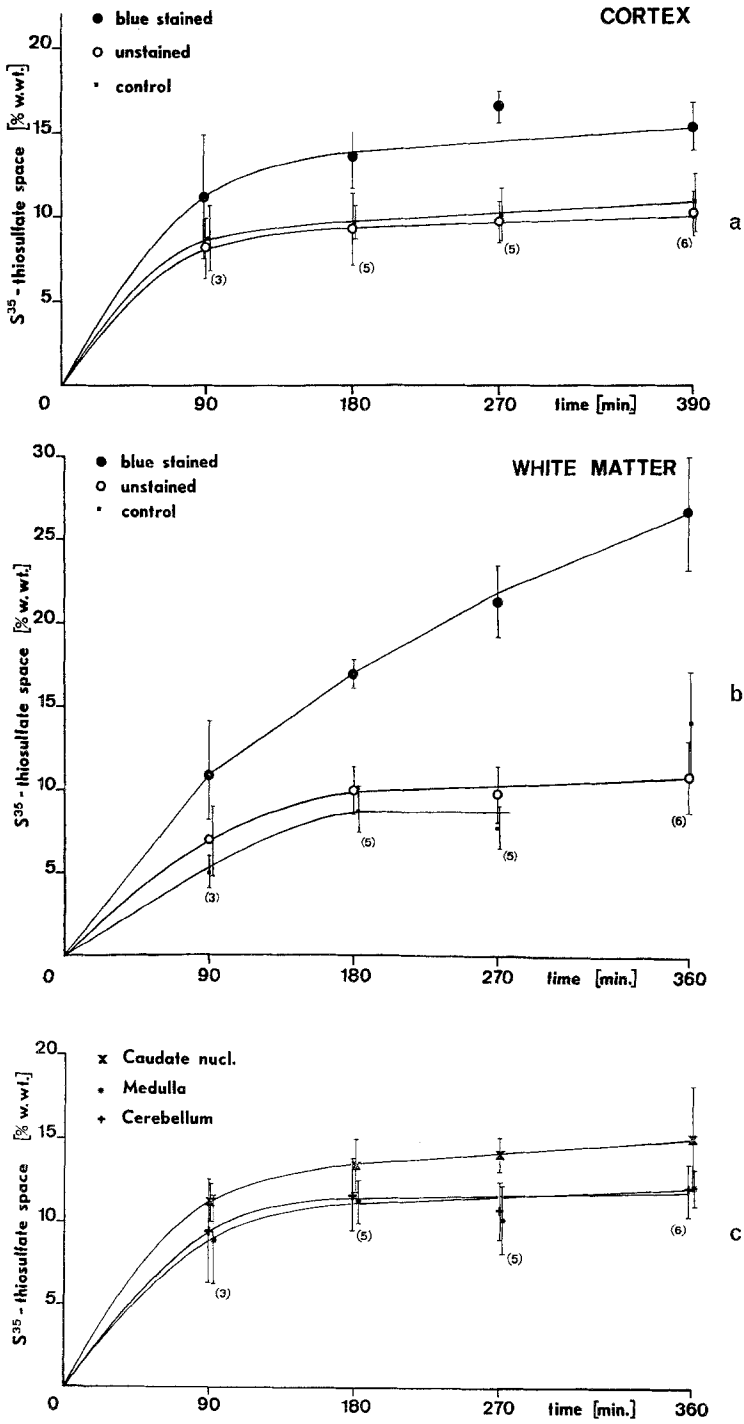
in the bluish-stained cortex, and to 9.7 ± 0.9 in the unstained cortex of the undamaged hemisphere (Fig. 2a).

In the white matter of the control hemisphere the *mean* thiosulphate space (180–390 min) amounted to 9.6 ± 1.2%. The value of 12.2 ± 2.8 after 390 minutes is markedly elevated in comparison to figures obtained after 180 and 270 minutes as a result of one high value (21.5%). The *mean* thiosulphate space of the unstained white matter was 10.2 ± ± 1.04%. In the blue-stained white matter the thiosulphate space was increased to 26.6 ± 3.3% after 390 minutes. At this time, however, a steady state was not yet established (Figs. 2b, c) and the actual figure presumably is larger. The thiosulphate space of the caudate nucleus, of medulla and of cerebellum remained in the normal range[10, 18, 19].

I^{131} *serum albumin in brain:* RISA spaces of 0.7% and 0.6% respectively were found in the grey and white matter of the control hemispheres. The RISA space rose to 3.2% in the blue-stained grey matter and to 4.6% in the blue-stained white matter. In the unstained grey and white matter small increases to 1.1% and 2.1% were noticed.

Fluid spaces in brain oedema: In order to evaluate whether the alterations in the water content correspond to the increase in ECS, the quantitative changes in the thiosulphate space and the non-thiosulphate space were computed (Fig. 3). Total fluid space in the oedematous white matter increased from 2050 ± 67 to 3076 ± 91 g/kg dry weight ($p < 0.001$) and ECS from 375 ± 74 to 1075 ± 120 g/kg dry weight ($p < 0.002$). Expressed as a percentage of the total fluid increase the expansion of the extracellular compartment accounts only for about 70%. Consequently about 30% of the total fluid increase should take place in the cellular compartment. It is likely that extracellular fluid enlargement is somewhat larger and intracellular fluid increase smaller since thiosulphate failed to achieve a steady level in oedema even after 6–7 hours of perfusion. In oedematous cortex fluid accumulation is confined to the ECS, this being increased from 537 ± 84 to 765 ± 48 g/kg

Fig. 2. Thiosulphate space in dog brain following cold induced oedema. Thiosulphate space is plotted on the ordinate as a function of the time (abscissa). Figures in parentheses under each point indicate the number of animals studied. a thiosulphate space in cortex of control hemisphere and in blue stained and unstained cortex of the injured hemisphere, b thiosulphate space in white matter of control hemisphere and of blue-stained and unstained areas of injured hemisphere, c thiosulphate space in unstained caudate nucleus, medulla and cerebellum


CORTEX
blue stained
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S35 - thiosulfate space [% w.wt.]
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WHITE MATTER
blue stained
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S35 - thiosulfate space [% w.wt.]
time [min.]
b
Caudate nucl.
Medulla
Cerebellum
S35 - thiosulfate space [% w.wt.]
time [min.]
c

Fig. 2

dry weight ($p < 0.02$) while total fluid space remained largely unchanged.

Intracellular electrolyte concentrations: Assuming an ECS of 26.6%, intracellular sodium concentration increased in the blue-stained white matter while potassium decreased. Intracellular concentrations within

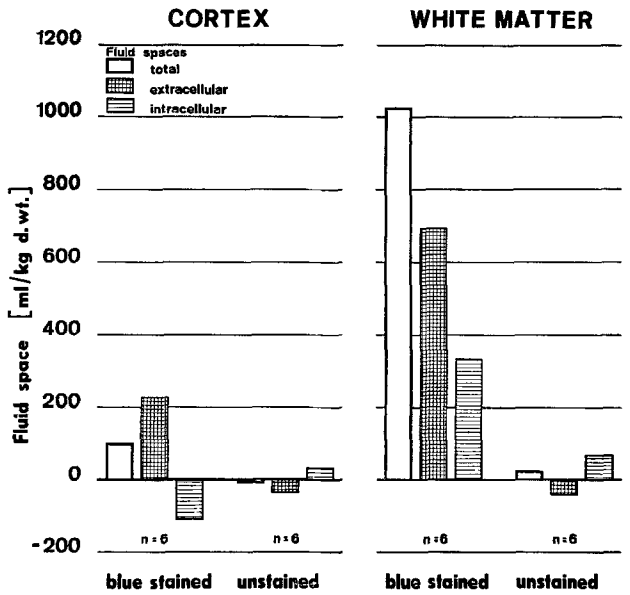

Fig. 3. Fluid spaces in cortex and white matter of dog brain following cold induced oedema. Changes in total fluid space, in extracellular and intracellular space are given as differences between control hemisphere and blue-stained or unstained areas of the damaged hemisphere. Values are derived from the 390 min perfusion group

the unstained grey and white matter remained unaltered. By comparing the above changes on the basis of the dry weight of the tissue it becomes apparent that the cellular increase of sodium surpasses the potassium loss, thus resulting in a cation increase in the cellular compartment (Table 3).

Discussion

After the local application of a cold injury a sharply demarcated necrosis develops in the cerebral cortex resulting in a disturbance of vascular permeability with a leakage of plasma contents in the lesion and regions immediately adjacent[3, 5, 6, 13, 14]. The spreading of the oedema fluid has been demonstrated in the cortical vicinity and in the subjacent white matter[3, 4, 13, 14].

In the present study a ventricular and subarachnoidal perfusion with an extracellular marker was performed on both hemispheres to enable simultaneous measurement of ECS in control and damaged hemispheres. The thiosulphate spaces (TSS) in the blue-stained areas of the grey and white matter were found to be enlarged by 5% and 17% respectively. Since the TSS is considered to be a measure of the ECS, the results are in good agreement with electron microscopical findings in this type of oedema. Electron-micrographs of the oedematous white matter showed a marked widening of extracellular spaces and to a smaller degree also swelling of astrocytic processes[4, 15, 22]. At the same time, in the affected cortex the extracellular spaces were only dilated in an area immediately adjacent to the lesion[5]. In the perifocal area the accumulation of fluid was confined to cells, particularly to astrocytes[3, 5]. As well as in white matter, the dilated ECS was now filled with a plasma-like proteinaceous material[4, 5, 15]. Estimation of the ECS after intravenous administration of extracellular markers, which yields satisfactory results in the body organs, leads to erroneously low data in the brain due to the presence of the BBB and the *sink function* of the CSF[1, 8, 23]. By using ventricular and subarachnoidal perfusions identical marker concentration can be achieved in the blood and in the brain extracellular fluid[8, 19]. Whether there is still another mechanism which affects the marker concentrations in the brain extracellular fluid is a matter of discussion. Ahmed and Van Harreveld[1] have recently suggested that transport of the marker exists from the ECS to the blood across the capillaries and they presented evidence that this transport can be inhibited or saturated. Therefore in the present studies unlabelled thiosulphate was added to the artificial CSF and the plasma.

In the white matter data show a striking spatial correspondence between the blue-staining of the tissue, the rise of I^{131} albumin, the enlargement of ECS and the increase of water, sodium and chloride. In contrast, in the more distant unstained regions the remaining parameters were also unchanged exept for a small increase in RISA space. Similar findings were reported by Bakay and Haque[3] in cold injury oedema and by Katzman et al.[12] in oedema induced by implantation of purified protein derivates. The corresponding spreading of RISA and protein-bound Evans blue with the expansion of the TSS provides evidence that the enlarged ECS in the white matter is filled with an oedema fluid containing protein, probably derived from plasma.

Exact findings on the composition of the oedema fluid are not yet available. Clasen et al.[6] analysed small quantities of oedema fluid obtained by centrifuging oedematous tissue. This fluid contained a lower sodium and chloride concentration and a markedly higher potas-

Table 3. *Intracellular Electrolyte Concentration* (mEq/l) *in Cortex and White Matter of the Control Hemisphere and the Blue-Stained and Unstained Areas of the Experimental Hemisphere*

For calculation the 6 values after 390 min perfusion were used. Mean values ± S.E.D. are given. Number of animals indicated in brackets. * = significantly different from controls at $p < 0.05$. ** = significantly different at $p < 0.01$

		sodium	potassium	chloride
Cortex	Control	63.32 ± 6.3 (6)	157.18 ± 8.9 (6)	47.22 ± 3.8 (6)
	Blue-stained	74.12 ± 6.6 (6)	144.63 ± 9.0 (6)	51.49 ± 6.8 (6)
	Unstained	64.90 ± 6.0 (6)	155.80 ± 7.2 (6)	47.04 ± 1.4 (6)
White matter	Control	62.17 ± 5.8 (6)	163.22 ± 5.9 (6)	48.88 ± 6.1 (6)
	Blue-stained	80.54 ± 6.6 (6)*	121.70 ± 11.5 (5)**	67.53 ± 11.1 (6)
	Unstained	66.50 ± 5.0 (6)	146.03 ± 7.1 (6)	47.00 ± 4.7 (6)

sium concentration than the plasma. Clasen et al., however, concede that the concentrations measured might not correspond to the *in-vivo* values. As demonstrated by use of fluorescent tracers[13, 14], radioactive labelled proteins[3, 14], electrophoresis and immunologic methods[6, 9]

Table 4. *Increase of Fluid and Electrolyte Content in Blue-Stained White Matter*
Comparison between the actual measured increase (a), the increase of electrolytes on an assumed ECS of 26.6% (b), and (c) on the assumption that the total fluid increase takes place extracellularly. For calculations the 6 values after 390 minutes perfusion were used

Increase of	a) Experimental data n = 6	calculated on assumed	
		b) ECS = 26.62% n = 6	c) ECS = 1026 ≙ 34.4% n = 6
Water (g/kg d. wt.)	1026.00 ± 78.18		
ECS (g/kg d. wt.)		694.50 ± 119.60	1026.00 ± 78.1
Sodium (mEq/kg d. wt.)	165.02 ± 10.57	105.63 ± 19.35	156.95 ± 15.2
Potassium (mEq/kg d. wt.)	— 2.64 ± 22.94	2.74 ± 0.55	3.98 ± 0.3
Chloride (mEq/kg d. wt.)	123.70 ± 8.25	89.17 ± 16.72	131.30 ± 12.1

protein changes in the oedematous white matter must be related to migration of serum proteins, particularly albumin, from the site of the lesion. It may be that plasma-like fluid leaking from injured blood vessels mixes with the brain extracellular fluid which probably resembles CSF in composition. The latter was assumed in the calculations of the electrolyte distribution.

The question arises whether the increase in water, sodium and chloride in the oedematous white matter can be explained exclusively by the enlargement of the TSS. If the increase of water, sodium and chloride, as derived from the experimental data, is compared with the respective values calculated on an assumed ECS of 26.6%, only approximately 68% of the water increase, 64% of the sodium increase and 72% of the chloride increase can be explained by the enlargement of the thiosulphate space (Table 4). Consequently the remaining increase of water, sodium and chloride should occupy the non-thiosulphate space, i.e. the intracellular space. However, as the white matter

TSS has not reached an equilibrium during the experimental period, the "true" interstitial space must be larger than the distribution space after 390 minutes. A longer ventriculo-cisternal perfusion did not seem to be feasible for technical reasons. The actual value for the ECS must range between 26.6% and 34.4%, a value computed on the assumption that the total fluid increase of 1026 g/kg dry weight would take place interstitially. A calculation of the sodium and chloride increase based on a hypothetical ECS of 34.4% reveals that these figures are close to the measured experimental data. Thus, it may be concluded from our experiments that the extracellular space in the white matter increases from 10% to a figure higher than 27% but lower than 34%. A similar increase of ECS in oedematous white matter was reported by Streicher et al.[21].

Apparently a fairly small part of the accumulation of the sodium, chloride and water affects the intracellular compartment. The changes certainly are smaller than indicated in Table 4 if an ECS higher than 26.6% is taken into account. However, departing from the assumption that electron microscopical findings are correct and that only glial cells swell[15, 22], it is evident that electrolyte changes in these cells will be more pronounced than the average concentrations, being however qualitatively identical.

In contrast to the white matter the thiosulphate space in the oedematous cortex remained constant after 180 min as did the enlarged interstitial space which showed an extravasation of Evans blue and a rise in I^{131} albumin space. Recent electron microscopical examinations with an improved technique of tissue fixation have shown that the extracellular space in fact is widened in the marginal cortex adjacent to the lesion and is filled with a plasmalike fluid[3, 4]. It is noteworthy that this marginal cortical area also differs biochemically from the unstained cortex. A breakdown of energy-rich phosphates, of glycogen and glucose as well as an accumulation of lactate has been shown to occur in oedematous cortex[20].

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