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# NEUROGLIA AND SPINAL FLUIDS

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SUMMARY

It is quite usual to see clear fluid collecting continuously on the surface of the spinal cord of 'functionally decapitate' cat preparations which have no functioning choroid plexus. The activity of potassium and calcium ions in interstitial fluid of these decapitate spinal cords is equal to that in spinal cords of cats with intact choroid plexus function, similar to normal cerebrospinal fluid, and different from blood plasma. It follows that fluid is being formed within the spinal cord by a process different from ultrafiltration. Endothelial cells seem likely to form this interstitial fluid by actively transporting $Na^+$, with water following by osmotic force. Potassium and other ion concentrations may be adjusted by independent transport processes. A role could be attributed to glial cells in these transports if exchange of material could be demonstrated between glia and endothelium.

## THE FORMATION OF THE SPINAL FLUID

During experiments in which the arches of the lumbar vertebrae are removed in order to expose the spinal cord, it is quite common to see clear fluid accumulating on the surface and around the cord and collecting in the 'gutters' formed by the cut edges of the vertebral canal. To prevent shunting of electric signals recorded from spinal roots by wire electrodes in these 'gutters', it is often necessary to remove this fluid from time to time and to continue doing so during many hours of experimentation. This is so even in functionally 'decapitate' spinal cords where the main arteries supplying the head have been tied or clamped. In these cases the fluid is not being secreted by the choroid plexus, and it is not running down the surface of the cord, but is actually originating within at a pressure at least equal to that exerted by the few centimetres of mineral oil customarily covering the spinal cord. There would be nothing remarkable about this observation were it not for the unresolved debate: whether all the cerebrospinal fluid (CSF) is being formed within the cerebral ventricles, or whether some is being added through the cerebral and spinal pia-glial membrane (Sato *et al.* 1972; Lux & Fenstermacher, 1975; Pollay, 1977; Bradbury, 1979).

In the interstitium of a 'decapitate' spinal cord, the activities of potassium and calcium ions ($[K^+]_o$ and $[Ca^{2+}]_o$) are normal, that is to say they are similar to ion activities in CSF and interstitial fluid of intact brain and spinal cord, and they are unlike those in blood plasma (Katzman & Pappius, 1973; Somjen & Lothman, 1974; Lothman & Somjen, 1975; Somjen, 1980). The interstitial fluid of 'decapitate' spinal cords, and hence presumably also the fluid that is seeping through the surface of

these cords, in therefore not a pathologic exudate (formed by opening up the blood–spinal cord barrier), but is the normal *milieu intérieur* of the spinal tissue. The ionic composition of this fluid is evidently under active homeostatic control. These observations do not prove that spinal tissue is secreting spinal fluid under normal conditions in intact organisms. However, they demonstrate that some tissue elements within the spinal cord are capable of doing so.

The next question then is, which cells are responsible for this secretory activity? The transfer of fluid must by necessity occur through the endothelial wall. Observations reported by three different groups of investigators made it plausible that endothelial cells of CNS capillaries, unlike other endothelium, may be capable of actively transporting ions (Oldendorf, Cornford, & Brown, 1977; Eisenberg & Sudith, 1979; Betz, Firth & Goldstein, 1980). The simplest hypothesis would be that endothelial cells transport sodium and/or chloride from blood into brain, with water following, coupled to the salt by osmotic force. The variable and regulated transfer of water across the blood–brain barrier lends indirect support to this concept (reviewed by Raichle, 1981).

## THE ROLE OF GLIAL CELLS IN THE TRANSPORT OF IONS

For many years there has been much discussion of the possible role of neuroglia in the regulation of ion levels, especially of $[K^+]_o$. Before the endothelial cells lining the CNS capillaries were credited with the capacity of active transport, glial cells seemed to be the ones most likely to move solutes across the blood–brain barrier. Many of the old arguments favouring this point of view remained valid, and some new ones have recently been added. It is, therefore, appropriate to examine critically the question whether, besides endothelial cells, glial cells may play such a part. Several papers in this review volume will deal with the more general topic of interstitial ion regulation. Therefore only a brief summary will be given here of those arguments bearing on the specific problem of the transfer of ions across the blood–brain barrier. First we will list the arguments which are in favour of such a role by glia, then we will examine the difficulties of the theory.

(1) *The morphological argument.* Golgi was the first to notice that glial endfeet are much more frequently in contact with capillaries than are neuronal processes. This fact has never been in dispute (see, for example, review in Bradbury, 1979).

(2) *The ontogenetic argument.* Glial cells are first cousins of ependyma, and the ependymal lining of the choroid plexus is demonstrably capable of the regulated active transport of ions. If ependyma can do it, then why not glia?

(3) *Systems analytic arguments.* Ion activity in the interstitial spaces of the CNS is regulated independently from the extracellular fluids of the rest of the body. The CNS must, therefore, have its own ion-sensing and regulating mechanism. Neurones, while obviously regulating ion content and composition of their own cytoplasm, are not in a good position to control their extracellular environment. Membrane-bound Na–K activated ATPase, for example, is strongly stimulated by an increase of $[Na^+]_i$, and much less by an increase of $[K^+]_o$. Indeed, if it were not so, neurones would vigorously gather $K^+$ ions every time one of their neighbours discharged $K^+$ into their shared environment. The result might be that inactive neurones in the midst of a gro

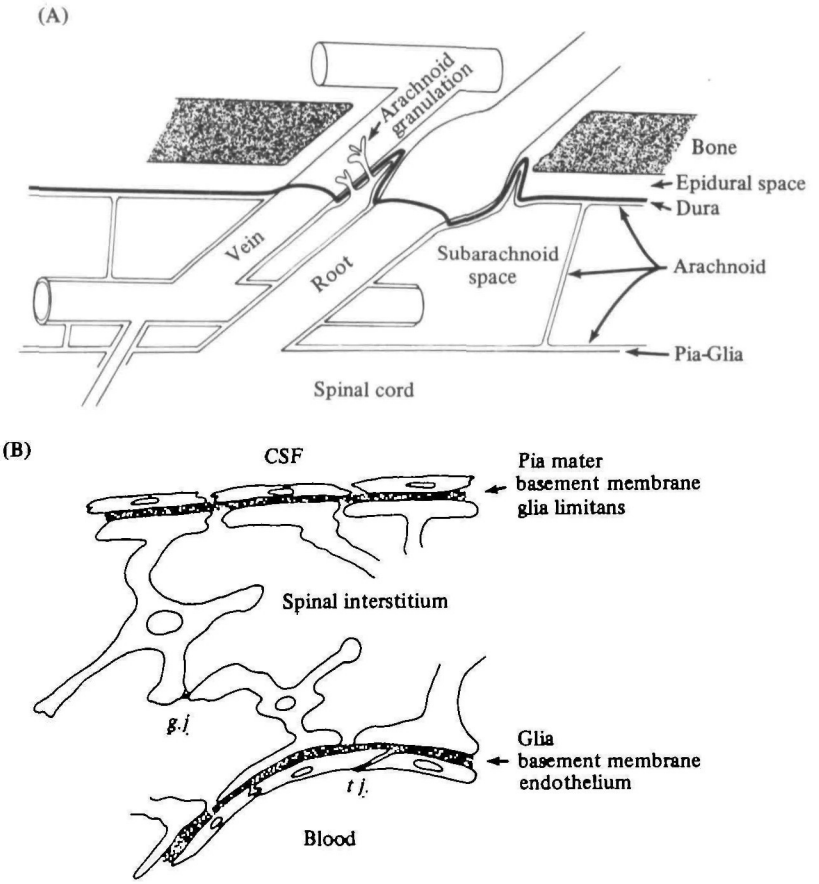


Fig. 1. Schema of extracellular fluid spaces of the spinal cord. (A) Relationship of the outer coverings of the cord. Cerebrospinal fluid fills the subarachnoid space. (B) Diagram of cell layers at CSF–spinal interstitium and blood–cord interfaces. Glial cells are believed to be connected at random by gap junctions (*g.j.*) which allow material to pass from the cytoplasm of one cell to that of another. Endothelial cells of blood capillaries are joined by complete seams of tight junctions (*t.j.*) which act as an effective barrier to diffusion; the glial endfeet forming the glia limitans membranes are not so joined, and it has been shown that macromolecules can move freely between them. The pia mater and its basement membrane present only a slight hindrance to diffusion. Note that these diagrams have not been drawn to scale; intercellular spaces are especially exaggerated.

of excited ones would accumulate an unwanted excess $[K^+]_i$. The overall regulation of $[K^+]_o$ is therefore probably the function of cells other than neurones, and glia is a likely candidate.

(4) *The argument derived from the dependence of glial membrane potential on* $[K^+]_o$. Unlike that of neurones, the membrane potential of glial cells is a linear function of the log of $[K^+]_o$ over the entire range of potassium activities found in CNS under normal as well as under pathologic conditions in invertebrates (Kuffler & Potter, 1964), amphibia (Kuffler, Nicholls & Orkand, 1966) and also in mammals (Lothman & Somjen, 1975). This uniform sensitivity could conceivably make glial cells good sensors [redacted] a central kalio-stat.

(5) *Active responses of glial cells to raised* $[K^+]_o$. According to Hertz (1966; also Hertz, Dittmann & Mandel, 1973) the oxidative metabolism of glial cells increases more than that of neurones when $[K^+]_o$ is raised. Using a different technique, Orkand, Bracho & Orkand (1973) found the same pattern with reactive glial cells of amphibia. While these data gave no hint concerning the nature of the work stimulated by the $\Delta[K^+]_o$ and requiring extra energy, more recently Hertz (1978) has also shown that glial cells actively take up $K^+$ ions when $[K^+]_o$ is being raised.

At this point we should re-emphasize the distinction between the regulation of the distribution of $K^+$ ions within the CNS, and their transport between CNS interstitium and blood. The much-discussed 'buffering' function of glia concerns the blunting of the impact of transiently disturbed distribution of $K^+$ ions within CNS tissue (see e.g. Gardner-Medwin, 1981). At issue here is, however, the related but distinct question, whether glia is taking part in the process which (*a*) keeps $[K^+]_o$ (and other ion activities) different in CNS interstitium from blood plasma under normal conditions and (*b*) also defends CNS ion activity levels against pathological fluctuations in extracellular fluid of the rest of the body.

The difficulty of attributing to neuroglia the ability to transport solutes from CNS interstitium into blood is summarized in Fig. 1 B. Available histological and ultrastructural observations (reviewed by Peters, Palay & Webster, 1970; Bradbury, 1979; Varon & Somjen, 1979; see also Landis & Reese, 1981) indicate that there are specialized contacts between glial cells which could account for the transfer of material from the cytoplasm of the one to that of the other. There are, however, no such contacts described between glial and endothelial cells (see also Landis & Reese, 1981). Supposing that the network of glial cells could transport material from intercellular spaces to the vicinity of capillaries and then release it into the pericapillary space, chances seem greater that it would leak through the gaps between glial endfeet and diffuse back into the interstitium, than that it would find its way into the blood. There are two ways, however, in which such back-diffusion could be prevented. First, if active transport by the endothelium from CNS into blood is intense enough to create a zone of low concentration in the immediate vicinity of the capillaries, this would act as a diffusional 'sink' into which glia could deposit what it had collected elsewhere. The second alternative would be if specialized junctions between glial and endothelial cells were discovered. Functional contacts should therefore be sought in cultures containing both glial and endothelial cells; and morphologic junctions in electron micrographs.

## CONCLUSIONS

Three points have been made in this short review of a very limited problem. Two are positive assertions, the third raises a question. First, spinal tissues are apparently capable of forming a fluid independently from the CSF issuing from the cerebral ventricles. Secondly, ion activities in this fluid are regulated independently from blood plasma and, at least as far as $[K^+]_o$ and $[Ca^{2+}]_o$ are concerned, are essentially identical with those in normal CSF. Thirdly, while capillary endothelium is the most likely tissue to secrete the bulk of this fluid, glia may have a role both as the sensor and as the regulator of the central kalio-stat.

I have limited my remarks to the spinal cord because evidence concerning the first two conclusions is clear only for this part of the CNS. Obviously, the processes which form interstitial fluid in the brain may well be similar to, but they need not be identical with, those in the spinal cord. After all brain tissue is located nearer to the source of the bulk of CSF in the ventricles than is the spinal cord.

## REFERENCES


BETZ, A. L., FIRTH, J. A. & GOLDSTEIN, G. W. (1980). Polarity of the blood–brain barrier: distribution of enzymes between the luminal and antiluminal membranes of capillary endothelial cells. *Brain Res.* **192**, 17–28.

BRADBURY, M. (1979). *The Concept of the Blood–Brain Barrier*. Chichester: John Wiley.

EISENBERG, H. M. & SUDITH, R. L. (1979). Cerebral vessels have the capacity to transport sodium and potassium. *Science, N.Y.* **206**, 1083–1085.

GARDNER-MEDWIN, A. R. (1981). Possible roles of vertebrate neuroglia in potassium dynamics, spreading depression, and migraine. *J. exp. Biol.* **95**, 111–127.

HERTZ, L. (1966). Neuroglial localization of potassium and sodium effects on respiration in brain. *J. Neurochem.* **13**, 1373–1387.

HERTZ, L. (1978). An intense potassium uptake into astrocytes, its further enhancement by high potassium, and its possible involvement in potassium homeostasis at the cellular level. *Brain Res.* **145**, 202–208.

HERTZ, L., DITTMANN, L. & MANDEL, P. (1973). $K^+$ induced stimulation of oxygen uptake in cultured cerebral glial cells. *Brain Res.* **60**, 517–520.

KATZMAN, R. & PAPPIUS, H. M. (1973). *Brain Electrolytes and Fluid Metabolism*. Baltimore: Williams & Wilkins.

KUFFLER, S. W., NICHOLLS, J. G. & ORKAND, R. K. (1966). Physiological properties of glial cells in the central nervous system of amphibia. *J. Neurophysiol.* **29**, 768–787.

KUFFLER, S. W. & POTTER, D. D. (1964). Glia in the leech central nervous system: physiological properties and neuron–glia relationship. *J. Neurophysiol.* **27**, 290–320.

LANDIS, D. M. D. & REESE, T. S. (1981). Membrane structure in mammalian astrocytes. *J. exp. Biol.* **95**, 35–48.

LOTHMAN, E. W. & SOMJEN, G. G. (1975). Extracellular potassium activity, intracellular and extracellular potential responses in the spinal cord. *J. Physiol., Lond.* **252**, 115–136.

LUX, W. E. & FENSTERMACHER, J. D. (1975). Cerebrospinal fluid formation in ventricles and spinal subarachnoid spaces of the rhesus monkey. *J. Neurosurg.* **42**, 674–678.

OLDENDORF, W. H., CORNFORD, M. E. & BROWN, W. J. (1977). The large apparent work capacility of the blood–brain barrier: A study of the mitochondrial content of capillary endothelial cells in brain and other tissues of the rat. *Ann. Neurol.* **1**, 409–417.

ORKAND, P. M., BRACHO, H. & ORKAND, R. K. (1973). Glial metabolism: alteration by potassium levels comparable to those during neuronal activity. *Brain Res.* **55**, 467–471.

PETERS, A., PALAY, S. L. & WEBSTER, H. (1970). *The Fine Structure of the Nervous System*. New York: Harper & Row.

POLLAY, M. (1977). Review of spinal fluid physiology: production and absorption in relation to pressure. *Clin. Neurosurg.* **24**, 254–269.

RAICHLE, M. E. (1981). Hypotheses: a central neuroendocrine system regulates brain ion homeostasis and volume. In *Neurosecretion and Brain Peptides: Implications for Brain Function and Neurological Disease* (ed. J. B. Martin), pp. 329–336. Raven Press.

SATO, O., ASAI, T., AMANO, Y., HARA, M., TSUGANE, R. & YAGI, M. (1972). Extraventricular origin of the cerebrospinal fluid: formation rate quantitatively measured in the spinal subarachnoid space of dogs. *J. Neurosurg.* **36**, 276–282.

SOMJEN, G. (1980). Stimulus-evoked and seizure-related responses of extracellular calcium activity in spinal cord compared to those in cerebral cortex. *J. Neurophysiol.* **44**, 617–632.

SOMJEN, G. & LOTHMAN, E. W. (1974). Potassium, sustained focal potential shifts, and dorsal root potentials of the mammalian spinal cord. *Brain Res.* **69**, 153–157.

VARON, S. & SOMJEN, G. (1979). Neuron–glia interactions. *Neurosci. Res. Prog. Bull.* **17**, 1–239.