alter the amount of cation bound but for unknown reasons has a pronounced effect on its selectivity. This conclusion, for passive accumulation at least, is in accord with the proposal of G. N. Ling [association-induction hypothesis (*8*)] that alkali metal cation accumulation in cells occurs by the selective binding of cations to cell proteins.

My conclusion that most if not all of the cell proteins contribute to the binding of passively accumulated potassium is in contrast with the fundamental conclusion of above-cited reports (*1–4*) that the transported solute is bound to a unique protein.

This report demonstrates that *E. coli* accumulates at least one-fourth of its total complement of potassium (150 μmole/600 μmole) by the attachment of mobile cation to the fixed charges of cell protein and furthermore to proteins not residing solely in the cell membrane.

Under the experimental conditions of Table 1, the accumulated potassium in the intact cells (150 μmole/g dry weight of cells) is the molar equivalent of a 50 : 1 concentration "gradient." Thus, the accumulation of relatively large gradients can be explained with the appealing simplicity of cation binding protoplasmic polyelectrolytes without recourse to the complex concept of membrane-situated pumps. The data are consistent with the view that the ion-exchange properties of protoplasmic polyelectrolyte determines the overall ionic composition of the cell (*8, 9*).

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10. An initial study in our laboratory of the *p*H optimum of K uptake indicated that total K depletion in a medium rich in Na salts occurs at *p*H 4.0 and 9.0 after 15 minutes of incubation at 37°C. Since alkaline K depletion proved to be completely reversible, it is now used routinely to produce complete K depletion. Cells depleted of K were suspended in medium NaA (0.25 mg dry weight per milliliter) and incubated for 70 minutes at 37°C with shaking to exhaust intracellular stores of metabolizable substrate. Passive K uptake (phase I) was initiated with addition of a small amount of medium KA to achieve a final K concentration of 3 m*M*. When it was complete, glucose was added (final concentration, 0.5 percent) to initiate acitve uptake (phase II). Absence of metabolism was confirmed by performing the same experiment at 0°C and in unbuffered medium. The K uptake occurred just as readily at 0°C as at 37°C, and no acidification of the medium occurred during the 200-minute incubation at 37°C.
11. Supported by grant U-1891 from the Health Research Council of the City of New York.
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Blood-Brain Barrier: Evidence for Active Cation Transport between Blood and the Extracellular Fluid of Brain

Abstract. *The concentration gradients of Mg^{++} and K^+ in the cerebrospinal fluid system indicate that the (Mg^{++}) is higher and the (K^+) is lower in the extracellular fluid of the cerebral cortex than the concentrations of these cations in either plasma-dialysate or cisternal fluid. Such cation distribution demonstrates the existence of an active transport process across the blood-brain barrier.*

The blood-brain barrier has traditionally been regarded as a passive permeability barrier between blood and the extracellular fluid of the brain. This view has recently been challenged on the basis of results indicating that the composition of the extracellular fluid of the cerebral cortex cannot be a product of simple diffusional relationships with blood or with cerebrospinal fluid (*1*). Most strikingly, the cortical extracellular fluid seemed to have a K^+ concentration even lower than that of cisternal cerebrospinal fluid. Maintenance of such low extracellular fluid (K^+) must involve the movement of K^+ from this fluid into blood against a concentration gradient. This implies either active transport or distribution according to an electrical potential across the blood-brain barrier. Neither the chemical nor the electrical potential gradient across this barrier is known with certainty, and thus a definite distinction between these two possibilities could not be made, particularly since the concentrations of the other electrolytes (Na^+, Ca^{++}, Cl^-) studied then did not corroborate the existence of nonchoroidal active transport systems.

Our current studies revealed large (Mg^{++}) gradients in the cerebrospinal fluid, opposite in direction to the (K^+) gradients. These cannot be due to the secretory activity of the choroid plexuses alone, and indicate a net flux of Mg^{++} from blood into the cortical extracellular fluid. The results presented here show that the two cations are transported against their respective chemical gradients in opposite directions; hence, at least one of them must also be moved against the electrical potential gradient, whatever the direction of this potential. This demonstrates the existence of an active transport process at the blood-brain barrier.

Dogs (11 to 18 kg) were observed for at least 7 days, and fasted overnight. Two blood samples were taken from the saphenous vein at an interval of half an hour. Sodium pentobarbital (35 to 60 mg/kg of body weight, intravenously) was administered immediately after the second sampling, surgical anesthesia usually being achieved within 4 to 5 minutes. All samples of cerebrospinal fluid were collected within 15 to 20 minutes of induction of anesthesia, according to techniques described earlier (*1*). The P_{dial} values for dogs were calculated from the mean preanesthetic plasma concentrations on the basis of a dialysis ratio of 0.70 for Mg^{++} and 0.96 for K^+ (*2*) (Fig. 1).

Cats (2.5 to 4 kg) were anesthetized with intraperitoneal sodium pentobarbital (30 to 35 mg/kg of body weight). A blood sample was taken from the heart after the state of surgical anesthesia was reached. To estimate the plasma-ultrafiltrate electrolyte concentrations as they prevailed before anesthesia, a small dialysis sac containing dextran-saline solution was implanted into the subcutaneous tissue of the back of each cat 6 to 10 weeks before the experiment (*3*). At the time of sample collection the sacs were removed and the "in vivo dialysate" was analyzed together with the other samples.

The (Mg^{++}) and (Ca^{++}) were measured on 1 : 100 dilutions of 100-μl aliquots of the fluid samples by means of a Perkin-Elmer model 303 atomic

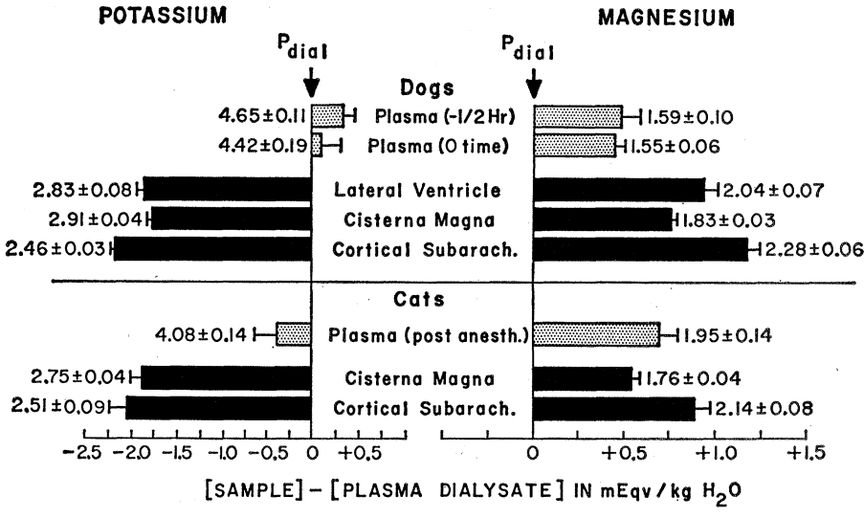

Fig. 1. Opposing (Mg^{++}) and (K^+) gradients in the cerebrospinal fluid system. The histogram represents *differences* between cerebrospinal fluid and plasma-dialysate cation concentrations. The units for the K^+ scale are one-half of the Mg^{++} units. Signs on the Mg^{++} scale are positive, that is, fluids have an excess of Mg^{++} as compared to plasma dialysate, while all the cerebrospinal fluid samples are deficient in K^+ ions, hence the differences are negative. The values represented by adjacent solid columns are significantly different from each other (paired *t*-test, $P \leq 0.05$). Abbreviations: Plasma (—½ Hr), Plasma (0 time), and Plasma (post anesth.): plasma obtained ½ hour, directly before, and shortly after induction of anesthesia, respectively. P_{dial}: concentration of cations in plasma dialysate; for cats these values were found experimentally (in vivo dialysate). Dogs: $Mg^{++} = (0.70) \times$ (mean plasma $[Mg^{++}]$); $K^+ = (0.96) \times$ (mean plasma $[K^+]$).

absorption spectrophotometer with a Ca^{++}/Mg^{++} hollow cathode lamp. Potassium was determined on the same dilutions with a K^+ Osram lamp.

The absolute concentrations of Mg^{++} and K^+ in the various fluids are presented on the histogram by the numerical values next to each column. The histogram itself represents the deviation of the Mg^{++} and K^+ concentrations from calculated (dogs) or experimental (cats) plasma dialysate values. The differences between the lengths of adjacent columns indicate the Mg^{++} and K^+ concentration gradients within the cerebrospinal system.

The (Mg^{++}) in the cat and dog plasma samples are within 5 percent of reported atomic absorption values (*4*). All (K^+) values compare reasonably well, but are in general somewhat lower than previously published values (*1*) obtained on different groups of animals by use of a less specific (flame emission) analytical technique. The concentrations of Ca^{++} as measured by atomic absorption technique are significantly lower than the previously reported (*1*) flame emission values. [Ca^{++} concentrations (in millimoles per kilogram of water): cisternal cerebrospinal fluid: dogs 1.17 ± 0.04, cats 1.13 ± 0.03; cortical subarachnoid cerebrospinal fluid: dogs 1.20 ± 0.04, cats 1.17 ± 0.04.] Because the gradients of (Ca^{++}) within the cerebrospinal fluid are small and in general agreement with those previously reported (*1*), Ca^{++} values obtained in the present set of experiments are not reported here in more detail.

The freshly secreted cerebrospinal fluid has a high (Mg^{++}) (*5*), and the ependymal walls of the ventricular system as well as the pial surfaces of the cisterna magna are permeable to electrolytes, including Mg^{++} (*6*). Therefore the simplest explanation of the (Mg^{++}) gradient between lateral ventricle and cisterna magna is diffusional loss of Mg^{++} from cerebrospinal fluid to the surrounding brain tissue or directly to blood. Direct net movement of Mg^{++} between blood and cerebrospinal fluid must be greatly restricted, however, since experimental elevation or prolonged depression of plasma Mg^{++} affects the cerebrospinal fluid (Mg^{++}) very little (*6*). It is also possible, however, that the decrease of Mg^{++}, as the cerebrospinal fluid flows from lateral ventricle to cisterna magna, is a result of the secretory activity of the choroid plexuses of the third and fourth ventricles (*5*); therefore, the direction of net movement of Mg^{++} between brain extracellular fluid and cerebrospinal fluid in this region cannot be stated with certainty. The (Mg^{++}) in the cisternal fluid is still greater than that in the corresponding plasma dialysate, so that this loss of Mg^{++}, wherever it may occur, can come about by a purely passive process.

The fact that the (Mg^{++}) in the cortical subarachnoid fluid is significantly higher (by some 25 percent) than its concentration in cisterna magna is a clear demonstration of accumulation against a concentration gradient (*7*), the (Mg^{++}) in both of these regions being well over the plasma ultrafiltrate, or even the total plasma Mg^{++} concentration. The ultimate source of the accumulated Mg^{++} must, of course, be the blood, and since there are no choroid plexuses or other known secretory structures (*8*) between the cisterna magna and the parietal subarachnoid space, direct transport of Mg^{++} from blood to cerebrospinal fluid could not account for an appreciable fraction of the observed Mg^{++} accumulation. One might argue that the transport is associated with the walls of blood vessels which run in the subarachnoid space. If there were indeed such a function at these sites, it should be regarded as part of the blood-brain barrier, since there is no morphological discontinuity between parts of these vessels that run on the surface and parts of these same vessels that penetrate into the cortex.

If the site of accumulation of Mg^{++} is indeed the blood-brain barrier, the high Mg^{++} in the cortical subarachnoid fluid must reflect diffusional exchanges between cerebrospinal fluid and extracellular fluid across the pia, similar to the exchanges that were shown to take place across the ependymal walls of the ventricular system (*9*). Even though some authors (*10*) feel that the pia may have permeability characteristics different from those of the ependyma, overwhelming evidence indicates that there is a relatively free exchange between the cerebrospinal fluid and the underlying cortex. Penetration from subarachnoid space into cerebral cortex has been demonstrated quantitatively for various extracellular tags (*11*) and for electrolytes (*12*). Furthermore, the ease of penetration of topically applied substances, including Mg^{++} and K^+ (*13*) across the pial surfaces can be deduced from their rapid physiological and pharmacological effects on the underlying cortex.

The concentration gradients between

cerebrospinal fluid and extracellular fluid must depend on the permeability characteristics of the pial surface and on the time the cerebrospinal fluid is exposed to the cortical surface. While kinetic studies will be required to establish these parameters in quantitative terms, the qualitative considerations presented here are sufficient for the present argument. In fact, if the equilibrium were incomplete, the (Mg^{++}) in the cortical extracellular fluid would have to be even higher and the (K^+) even lower than in the parietal subarachnoid fluid. Thus, until the rates of these ionic exchanges are established, the (Mg^{++}) and the (K^+) of the subarachnoid fluid must be assumed to reflect the minimal (Mg^{++}) and the maximal (K^+) of the extracellular fluid of the underlying cortex.

Maintenance of such low extracellular (K^+) and high (Mg^{++}) must, of course, under steady-state conditions, reflect a net movement of K^+ from cortical extracellular fluid to blood and a net flux of Mg^{++} from blood into this fluid; thus both Mg^{++} and K^+ move against a chemical concentration gradient. Since both of these ions carry positive charge and their net fluxes are in opposite directions, either Mg^{++} or K^+ must be transported against an electrochemical gradient regardless of the electrical potential (*14*) across the blood-brain barrier.

In conclusion, evidence given indicates that the local environment of the cells of the mammalian cerebral cortex has a low K^+ and high Mg^{++} concentration, even as compared with the concentrations of these cations in cisternal cerebrospinal fluid. Such concentration could not be maintained in the extracellular fluid of the brain by a simple combination of the known secretory activity of the choroid plexuses and a passive diffusional barrier at the blood-brain barrier. Rather, these results demonstrate the existence of an active transport function across the blood-brain barrier.

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14. The potential of the cerebrospinal fluid is about 5 mv positive with respect to blood [D. Held, V. Fencl, J. R. Pappenheimer, *J. Neurophysiol.* **27**, 942 (1964); J. W. Severinghaus, R. A. Mitchell, B. W. Richardson, M. M. Singer, *J. Appl. Physiol.* **18**, 1155 (1963)]. Since the pial and ependymal surfaces of the brain cannot be expected to maintain a large potential gradient, it could be assumed that the extracellular fluid of the cortex is also positive with respect to blood. Such potential would favor the observed K^+ distribution and would oppose the indicated Mg^{++} flux. On this basis we could conclude the Mg^{++} is actively transported across the blood-brain barrier, whereas the movement of K^+ is passive. Such a conclusion, however, is premature since neither the concentration nor the potential gradient directly across the blood-brain barrier is known with certainty. In fact, it is unlikely that the potential across the blood-brain or the pia could be sufficient (*1*) to maintain the very low apparent K^+ distribution ratio (less than 0.6) between blood and extracellular fluid. Thus the possibility that the local concentration of both K^+ and Mg^{++} are maintained by active transport processes at the level of the blood-brain barrier cannot be ruled out at present.
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Forebrain Temperature Activates Behavioral Thermoregulatory Response in Arctic Sculpins

Abstract. *Arctic sculpins of the genus* Myoxocephalus *adapted to water at 5°C escaped from warm water at 20°, 16°, and 12°C when their deep-body temperatures increased from an initial 5°C to about 8°C. Heating parts of the forebrain with water at 25°C circulating through a pair of thermodes astraddle rostral parts of the forebrain shortened the time spent in the warm water and lessened the increase in deep-body temperature before exit from the warm water. Cooling the forebrain to about −1°C caused a large increase in the body temperature and sometimes suppressed the escape from the warm water.*

Vertebrates of all classes can regulate internal temperature. Interest in this regulatory process lies both in the details of the behavioral and physiological responses available to each species, and in the mechanism that activates these responses. A network of neurons located at several sites in the body transduce temperature to neural activities which are combined in some way to activate responses. The temperature transduction, nervous integration, and activation of appropriate thermoregulatory responses appear to minimize deviation of the internal body temperature from a preferred temperature adapted by each species. More is known of body temperature regulation for several species of mammals (*1*) than for fish (*2*) and reptiles (*3, 4*). For all species, the nerve network serving as the controlling system still has to be treated as a black box, an approach that we used to study the activation of a behavioral response to a thermal stress applied to a fish.

Arctic sculpins of the genus *Myoxocephalus* were transferred from water at 5°C to warm water, from which they could escape by swimming back to the 5°C water through a narrow channel normally closed by a clear plastic gate which was manually opened as the fish approached. The deep-body temperature was continuously measured as it increased to the body temperature at time of exit (Fig. 1). A thermocouple was inserted into a plastic reentrant tube implanted into the dorsal muscles, penetrating obliquely to the rib cage about 1 cm below the skin. When fishes 1 and 2 were placed in water at 20°C, their body temperatures had increased to 8.9° ± 0.7° and 6.9° ± 0.6°C, respectively, at the time of exit from the warm water. Fishes 3 and 4 left 16°C water at body temperatures of 8.4° ± 0.8° and 8.3° ± 1.1°C, respectively. Fish 4 also left 12°C water at 8.2° ± 1.0°C. The temperature of the warm water did not appear to affect the exit body temperature.