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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7. The possibility that the apparent accumulation of Mg^{++} is due to binding or complexing of Mg^{++} to some constituent of the subarachnoid cerebrospinal fluid is precluded by the fact that Ca^{++} did not show similar accumulation; its concentration in all samples of this fluid is less than the plasma dialysate value.
8. The arachnoid villi are sites of drainage. The flow through them is unidirectional and, in general, no anatomical or physiological evidence indicates a secretory role for these structures [K. Welch and V. Friedman, *Brain* **83**, 454 (1960)].
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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.
15. Supported in part by PHS research grant NB 06766-01,02 from the National Institute of Neurological Diseases and Blindness and in part by a grant from the National Multiple Sclerosis Society. Berry Goozner, Ronnie Gelfond, Joan Dawson, and Erica Salvador provided assistance during various phases of this project.

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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.

A pair of thermodes 3 mm apart were also implanted astraddle the brain rostral to the optic chiasm (Fig. 2) (*5*). These were perfused with water at 25°C to heat the brain tissue between the thermodes, or they were perfused with a mixture of alcohol and water at − 1°C to cool the rostral brain tissue. When the fishes were placed in the warm water and the brain tissue was heated at the same time, the average response of each fish was to remain in the warm water a shorter time and to swim out of the warm water at a lower body temperature. The difference between the body temperature at exit in the case of the heated and the normal brain was highly significant for fishes 1 and 3 ($P < .001$ and $P < .01$, respectively); the difference was not significant for fishes 2 and 4 ($P < .07$ and $P < .2$, respectively).

Cooling the rostral brain tissue while the fishes were in the warm water either greatly increased the body temperature at which the fish swam from the warm environment, or suppressed the escape response so that the fish had to be manually removed from the warm water at the temperatures indicated.

We conclude that the temperature of some rostral part of the brain is involved in activation of a response to escape from an environment that is warmer than the optimum temperature for this species of fish. The significance of these observations is that a fish can exhibit homeothermism when not deprived of its normal behavioral response, and that the sensory inputs for this homeothermism involve the rostral part of the brain. Since homeothermism, achieved by activation of appropriate thermoregulatory responses and activated by thermoreceptors in the brain, applies also to a lizard, the dog, goat, ox, rat, rabbit, chicken, and others, it is likely that all vertebrates can regulate internal body temperatures; that is, they are homeothermic, at least some of the time.

Another inference may be drawn from the data of fishes 1 and 3. The thermodes in these fishes were implanted 3 and 10 mm, respectively, rostral to the optic chiasm so that they straddled only the olfactory tracts or olfactory bulbs, or both. Another fish, No. 5, with thermodes 9 mm rostral to the optic chiasm, also left the 20°C bath at a significantly lower exit body temperature when the olfactory tracts or bulbs, or both, between the thermodes were heated. The thermodes in fishes 2 and 4 were implanted astraddle the brain stem at the optic chiasm. At this position the thermodes could also affect the temperature of the olfactory tracts and olfactory lobes as well as tissues of the forebrain, such as the lamina terminalis and basal nuclei. Our results might suggest another role for the primitive forebrain or telencephalon. Perhaps the rudiments of temperature regulation as well as olfaction are subserved by some of its parts. Parts of the telencephalon transduce olfactory stimuli to nerve impulses and they may also transduce temperature. The forebrain has ample connections with the hypothalamus serving olfaction and perhaps also temperature regulation. The basal nuclei and other areas of the forebrain receive nerve impulses transduced from olfactory stimuli; they may also receive impulses from thermal stimuli. The basal nuclei are correlation centers receiving strong projection fiber bundles from the thalamus, even at an early stage of vertebrate evolution, and they become increasingly important as correlation centers. If the forebrain of early vertebrates served olfaction and temperature regulation as well, then the evolution of the cerebral hemispheres need not be attributed solely to olfaction (*6*).

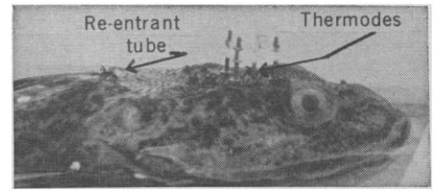

Fig. 2. Thermocouple re-entrant tube and thermodes with lower part of perfusing chamber are shown in place on fish 3 9 days after implantation (*5*). The re-entrant tube was polyethylene PE-90 tubing inserted obliquely into the muscle for 3 cm with the sealed end 1 cm beneath the surface. The thermodes were stainless tubing with an outside diameter of 1.0 mm and a wall thickness of 0.05 mm. They were spaced 3 mm apart and the sealed ends were about 1 cm below the surface so as to straddle parts of the forebrain.

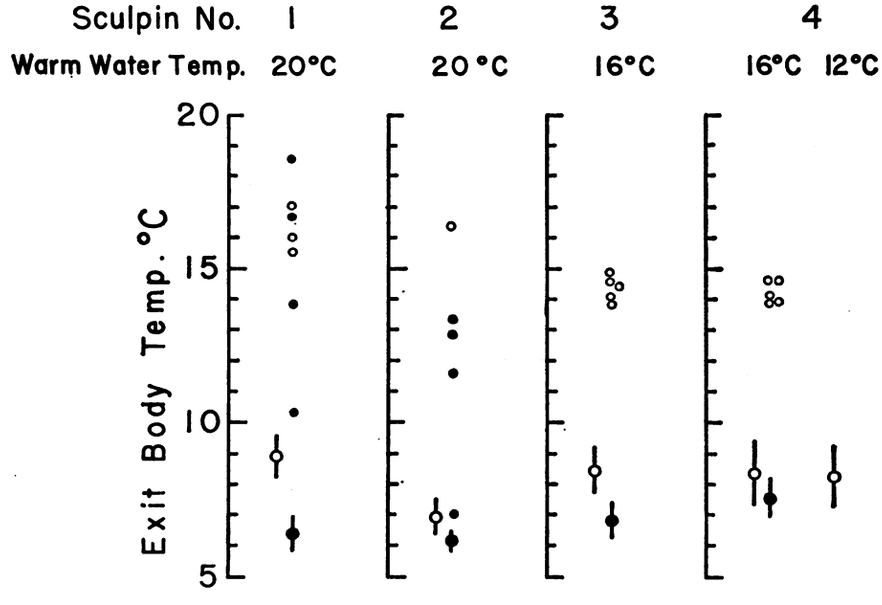

Fig. 1. Body temperatures of sculpins, *Myoxocephalus*, escaping from warm water at 20°, 16°, or 12°C to return to 5°C water to which they were adapted. The large open circles are the mean and standard deviation for the body temperatures at exit of each fish with normal brain temperature. Either five or six exits were recorded in each case. The large closed circles are the mean and standard deviation of exit body temperatures when 25°C water was circulated through the thermodes heating a part of the forebrain. Six exits were recorded. The small circles are the exit body temperatures for each exit when the forebrain was cooled (thermodes at −1°C); open circles indicate assisted escape and closed circles indicate unassisted escape. Fishes 1, 2, and 4 were *M. scorpioides*, and fish 3 was *M. scorpius*.

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Ribosomal Protein Conferring Sensitivity to the Antibiotic Spectinomycin in Escherichia coli

Abstract. *Reconstitution of 30S ribosomal particles from 16S ribosomal RNA and total proteins, or from core proteins and split proteins obtained from the ribosomes of strains of* Escherichia coli *sensitive to and resistant to spectinomycin, shows that the split protein fraction determines the response of polypeptide synthesis in virto to spectinomycin. Reconstitution of active particles in the presence of isolated split proteins allowed the identification of the single split protein responsible for spectinomycin sensitivity.*

The aminoglycoside antibiotic spectinomycin is a strong inhibitor of protein synthesis in whole cells and in extracts, and it has been demonstrated that sensitivity and resistance to this drug are properties of the 30*S* ribosomal subunit (*1*). The genetic locus conferring resistance to spectinomycin is located close to the locus for streptomycin resistance on the *Escherichia coli* linkage map; the results of mapping by conjugation (*1*), transduction (*1–3*), and complementation studies in diploids (*4*, *5*) strongly suggest that these two determinants are related to changes in two separate proteins of the 30*S* subunit.

Active 30*S* ribosomal particles have been reconstituted from split proteins and core particles (*6*), and more fundamentally from ribosomal RNA and ribosomal proteins (*7*). These ribosomal proteins have been separated and characterized (*8*, *9*). We now report identification of the ribosomal component that confers sensitivity to spectinomycin in a manner identical to the identification of the protein which confers sensitivity (*10*, *11*) and dependence (*12*) to streptomycin.

Ribosomal subunits were prepared by zonal centrifugation of extracts from a strain of *Escherichia coli* MRE600 sensitive to spectinomycin and a mutant resistant to spectinomycin derived from this strain by spontaneous mutation. Split proteins and core particles were prepared according to Meselson *et al.* (*13*). Total 30*S* ribosomal protein was obtained by lithium chloride–urea extraction of 30*S* subunits, and purified single ribosomal proteins were obtained by phosphocellulose chromatography (*8*). Reconstitution of 30*S* ribosome subunits from 16*S* ribosomal RNA and total proteins has been described by Traub and Nomura (*7*). The integrity of all reconstituted particles was confirmed by sucrose density-gradient centrifugation. Because spectinomycin shows considerable variation in its inhibitory effects against polypeptide synthesis directed by synthetic polynucleotides, we used protein synthesis directed by RNA from bacteriophage R17 or f2 as assays for the properties of 30*S* subunits.

The results of reconstitution of 30*S* subunits from 16*S* ribosomal RNA and total core and split proteins (Table 1) show that a split protein is involved in sensitivity to spectinomycin. This distinguishes the protein determinant of spectinomycin sensitivity from that of streptomycin sensitivity, because the latter is a core protein (*10*).

The results of reconstitution of 30*S* subunits from 16*S* ribosomal RNA, total proteins, and single split proteins (Table 2) show that a single protein (P_4) [formerly called B_2, see (*8*)] purified from the split protein fraction, determines sensitivity to spectinomycin in 30*S* ribosomal subunits.

In similar experiments, protein P_4, purified directly from total 30*S* ribosomal proteins (*11*), was used and the same conclusion was obtained. Previous experiments have shown that the pro-

Table 1. Sensitivity to spectinomycin of 30*S* ribosomal particles reconstituted from 16*S* RNA (optical density at 260 nm, 25 units), total proteins (TP) (750 μg), core proteins (CP30), and split proteins (SP30) (a threefold excess). The letters S or R indicate that they are derived from spectinomycin-sensitive or spectinomycin-resistant cells, respectively. The reconstituted particles or control undissociated 30*S* ribosomal particles (60 μg) were assayed for their activity in f2-bacteriophage RNA-directed incorporation of valine in the presence of 50*S* particles (120 μg) from spectinomycin-sensitive cells, and when indicated, in the presence of spectinomycin (+Spc) (6×10^{-6} mole/liter). Sensitivity of native and reconstituted particles to spectinomycin ranged between 65 and 80 percent inhibition. Samples were counted for at least 1000 counts, and experiments were repeated at least two times with independent preparations.

Reconstituted 30*S*				Control 30*S*	Incorporation (count/min)		Inhibition by Spc (%)
16*S* RNA	TP	CP	SP		Control Spc	+Spc	
				S	159	51	66
				R	280	224	20
S	S				314	112	65
S	R				169	117	30
S	R	S			255	185	27
S	R	R			249	184	26
S	R		S		269	76	72
S	R		R		186	162	15

Table 2. Sensitivity to spectinomycin (Spc) of 30*S* ribosomal particles reconstituted from 16*S* RNA (optical density at 260 nm, 25 units), total proteins (TP) (750 μg), and single split proteins (threefold excess) (*8*). Symbols and procedure are as described in the legend of Table 1; P_1, P_4, and P_6 correspond to split proteins A_1, B_2, and B_3, as described previously (*8*).

Reconstituted 30*S*			Control 30*S*	Incorporation activity (count/min)		Inhibition by Spc (%)
16*S* RNA	TP	Single SP		Control Spc	+Spc	
			S	159	51	66
			R	280	224	20
S	S			314	112	65
S	R			169	117	30
S	R	P_1 (A_1)		225	142	37
S	R	P_4 (B_2)		338	107	70
S	R	P_6 (B_3)		479	337	30