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14. I thank Mr. Ronald Howard, principal of Arbor View School, District 89, Illinois, Mr. Lloyd Cash, principal of Lanier School, Hall County, Georgia, and their staffs for their cooperation in providing subjects for this experiment. Supported by grant HD 4348 from the National Institute of Child Health and Human Development.

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Body Temperature: Possible Ionic Mechanism in the Hypothalamus Controlling the Set Point

Abstract. *The body temperature of many mammals is set at or around 37°C. The mechanism for this set point appears to depend on a constant and inherent balance between sodium and calcium ions within the posterior hypothalamus. When this region is perfused in unanesthetized cats, an extracellular excess or a normal physiological concentration of sodium ions evokes a rise in body temperature if calcium is not in the perfusate. At the same site, an excess or normal concentration of calcium ions causes the temperature to fall when sodium is absent.*

According to the monoamine theory of thermoregulation (*1*), serotonin (5-HT) and norepinephrine (NE) are released possibly as transmitters from the anterior hypothalamus in functional opposition to one another in order to control the temperature of an animal around a given set point (*2*). Acting within the same hypothalamic site, one amine activates the heat-production pathway when the animal is cold, and the other stimulates the heat-loss pathway when the animal is warm (*3*). However, the theory does not account for the mechanism in the central nervous system whereby temperature is intrinsically set and maintained in most mammals at a constant level of 37°C or thereabouts.

Recently, it was found that a solution of isotonic NaCl caused shivering and a rise in temperature when it was perfused through the cerebral ventricles of a conscious cat (*4*). By adding calcium in a normal physiological concentration to the NaCl solution, this hyperthermic response was blocked. As a result of these findings, it was suggested that the calcium level in the hypothalamus may be the physiological basis of the set point.

We now propose that the set point for body temperature is localized within the posterior hypothalamus and is determined and maintained by the inherent ratio in the concentrations of two essential cations, Na^+ and Ca^{2+}. This concept is based on experiments in which the balance between Na^+ and Ca^{2+} levels was selectively altered within specific regions of the hypothalamus.

In each of ten cats, four guide tubes were implanted stereotaxically with the use of aseptic precautions and procedures described earlier (*5*). The tip of each tube rested at a locus above the rostral or caudal parts of the hypothalamus—those regions classically implicated in thermoregulation (*6*). A polystyrene pedestal was affixed to the skull and capped so that a sterile preparation could be maintained throughout the experiments. Postoperatively, body temperature was monitored by a thermistor probe inserted to a depth of 10 cm into the colon, and the temperature of the animal was plotted continuously before, during, and after each perfusion.

To alter the ionic concentrations within the anterior or posterior hypothalamus, a double-walled concentric "push-pull" cannula (*5*) was lowered through each guide tube. The tip of the inner "push" cannula extended 1 mm beyond the end of the outer "pull" cannula, so that a sphere of tissue only 1 mm in diameter was perfused. Since inflow and outflow rates were identical, the perfusion fluid was drawn off at precisely the same rate as it was pumped in (*7*). Bilateral hypothalamic sites were perfused simultaneously at a rate of 50 μl/min for 20 to 30 minutes; the temperature of the perfusate at the cannula tip was the same as the cat's brain temperature (*8*).

When a solution containing normal physiological concentrations (*9*) of both Na^+ and Ca^{2+} (143 m*M* Na^+ and 2.6 m*M* Ca^{2+}) was perfused at sites in the posterior hypothalamus, the temperature of the cat remained unchanged. However, if a calcium-free solution containing a normal or an excess amount of Na^+ (at a concentration 13.6 m*M* to 34.0 m*M* greater than the physiological concentration) was perfused at the same site, the animal began to shiver almost immediately and its temperature rose sharply. This hyperthermic response is shown in Fig. 1 (top). Accompanying the rise in temperature were other changes associated with heat production, including vasoconstriction and piloerection. On the other hand, if the Na^+ was omitted from the perfusate and a solution containing normal or an excess in Ca^{2+} ions (at a concentration 2.6 m*M* to 13.0 m*M* greater than the physiological concentration) was perfused at the same site in the posterior hypothalamus, the temperature of the cat fell sharply. During this Ca^{2+}-evoked decline in temperature, which is illustrated in Fig. 1 (center), vasodilation, slight sedation, and a decline in respiratory rate also occurred. Data in Table 1 illustrate the average changes in temperature from the baseline level, following the localized perfusion of Na^+, Ca^{2+}, and other ions within the posterior hypothalamus.

To control for the factors of both tonicity and the action of other cations, several other solutions were used for the "push-pull" perfusions of the posterior hypothalamus. A normal Krebs solution (314 m*M*) (see Fig. 1, bot-

Table 1. Average maximum change from baseline temperature during the first 60 minutes of repeated "push-pull" perfusions of the posterior hypothalamus of unanesthetized cats. The millimolar (m*M*) values represent the ion concentration above those in a Krebs solution. The control perfusates consisted of isotonic sucrose; double isotonic sucrose; Krebs solution; Krebs solution with twice the amount of each salt; an NaCl-$CaCl_2$ solution with the ionic values of Krebs solution; and a solution containing Na^+, Ca^{2+}, and K^+ in weights equal to those in Krebs solution.

Ion	Concentration (m*M*)	Perfusions (No.)	Mean change from baseline (°C)	Standard error
Na^+	13.6 to 34.0	9	+0.68	±0.06
Ca^{2+}	2.6 to 13.0	9	−0.65	±0.14
K^+	4.8 to 23.6	6	−0.13	±0.10
Mg^{2+}	5.8	2	+0.10	±0.14
Control		6	−0.08	±0.06

tom) or an isotonic sucrose solution (9.25 percent) caused no change in temperature. Similarly, a Krebs solution in which the concentration of each of the ionic constituents was doubled (628 m*M*) or a sucrose solution twice its isotonic concentration (18.5 percent) had no observable effects on temperature or other responses when they were perfused similarly. Surprisingly, magnesium or potassium ions perfused in two to five times their normal physiological concentrations (that is, 1.2 to 5.8 m*M* Mg^{2+} or 4.8 to 23.6 m*M* K^+) found in the extracellular fluid (*9*) also caused little if any change in body temperature or other responses. In order to control for the possible effects of an excess in the chloride ion concentration, a sodium compound ($C_7H_7SO_3Na \cdot H_2O$) was used in which toluene-*p*-sulfonate served as the anion (*10*) so that the concentration of Na^+ could be elevated in the perfusion fluid without altering the level of Cl^- ions. When a solution of $C_7H_7SO_3Na \cdot H_2O$ of ionic strength similar to NaCl was perfused through the posterior hypothalamus, the hyperthermic effects produced by Na^+ ions in this form were virtually identical to those of NaCl.

There are several factors that suggest that the set point for body temperature may possibly be determined in the cat by a balance in Na^+ and Ca^{2+} concentrations in the brainstem. The posterior region of the hypothalamus is considered classically to be the area involved in heat maintenance (*6*). As our results show, the responses to the changes in ion concentrations are remarkably site-specific, and if the cannula tip was located more than 1.0 mm away from the posterior area, different responses occurred. For instance, when the Na^+ level was elevated in the anterior hypothalamus, there was often a slight decline in temperature, and Na^+ perfused in the lateral hypothalamus caused a feeding response (*11*). The Ca^{2+}, perfused through these other areas of the hypothalamus, produced either no effect or evoked sedation, hyperthermia, hypothermia, a sleeplike state, purring, or some of the other responses observed when Ca^{2+} levels are altered in the ventricular fluids and brainstem tissue (*12*).

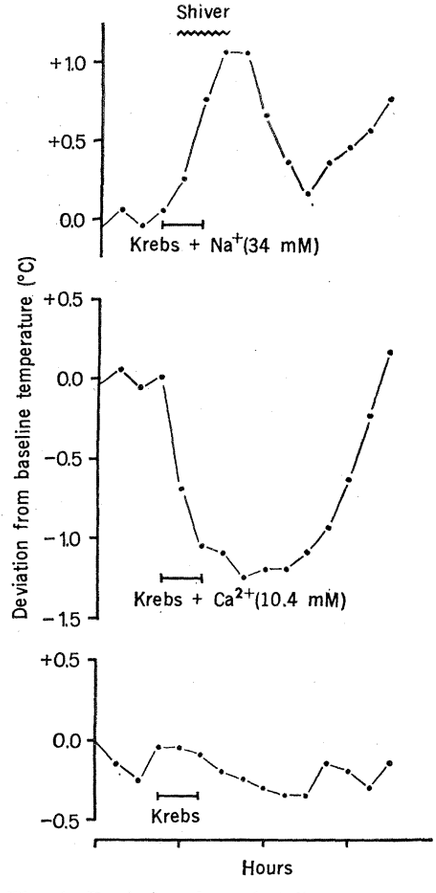

Fig. 1. Deviation from baseline temperature (°C) in a cat in which the posterior hypothalamus was perfused bilaterally three times at 48-hour intervals. |——|, denotes a 30-minute perfusion with Krebs solution having excess Na^+ at a concentration of 34.0 m*M* (top); excess Ca^{2+} at a concentration of 10.4 m*M* (center); and normal values of all salts (bottom). Shivering accompanied the Na^+ perfusion as indicated (top).

The importance of a constant ratio between ions is substantiated by the fact that temperature of the animal does not alter (i) when the local concentrations of Na^+ and Ca^{2+} are doubled simultaneously in the perfusate flowing through the posterior hypothalamus; or (ii) if the local concentrations of both ions are reduced by utilizing sucrose as the perfusion fluid. As long as the *ratio* between normal physiological levels of Na^+ and Ca^{2+} is maintained within the posterior hypothalamus, the temperature remains stable. We hypothesize here that the constancy in the concentration of extracellular ionic constituents maintains the firing rate of the neurons of the posterior hypothalamus. This steady-state discharge pattern thus keeps the set point at or about 37°C.

It has been proposed that during a bacterial-induced fever, the set point is readjusted (*13*). If this is the case, then our theory would require that within the hypothalamus either the Ca^{2+} level should be lowered or the Na^+ concentrations elevated during such a fever. In preliminary experiments, we have found that Ca^{45} levels are lower in the posterior hypothalamus during a 30- to 90-minute interval following the injection of a bacterial pyrogen into the cerebral ventricle. This corresponds to the report of Skarnes (*14*), who found that serum calcium in the rabbit falls temporarily after intravenous endotoxin. It would seem that only a slight and very transient reduction of Ca^{2+} in the blood which bathes the posterior hypothalamus would be sufficient to shift the balance between the Na^+ and Ca^{2+} ions, after which the set point is raised. During the period of Ca^{2+} deficiency, hyperthermia would occur. Our experiments seem to mimic this condition, since even a short-term imbalance between Na^+ and Ca^{2+} caused a pyrexic response. Moreover, it has been reported clinically that a hypernatremia can be associated with an intense prolonged febrile response (*15*).

Although some mammals may not possess adequate thermoregulatory capabilities at birth, their temperature set point nevertheless is approximately 37°C at parturition (*16*). This could result from the fact that the ratio between the ionic constituents in extracellular fluid is established prenatally. The reason the neonate does not also possess an adequate thermoregulatory

capacity may be related to the fact that, at birth, 5-HT and NE systems are relatively nonfunctional (*17*) within the brainstem. This could explain why regulation around this very fundamental, physiologically determined set point may not be adequate.

Finally, the cellular mechanisms by which the balance in ions causes the appropriate efferent response in the hypothalamus are not as yet known. Ratios of ionic constituents appear to govern the activity of the synaptic vesicles and the release of transmitters, and ions could mobilize intracellular cyclic adenosine monophosphate, adenosine triphosphate, and adenylcyclase (*18*). The enzyme systems appear to be linked to transmitter release, and hence the ionic balance and enzymatic activity could be intimately related. An inherent ionic balance within the framework of enzymatic activity may also provide an explanation for other homeostatic mechanisms involved in functions mediated by the brainstem.

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7. It should be emphasized that the "push-pull" perfusion used in these experiments is not similar to a microinjection in which a high concentration of a chemical substance or ion remains at a local site. By an exchange of extracellular fluid, only a temporary elevation of a given ion occurs in the region of the perfusion. This may explain the stimulus-bound nature of the responses observed.
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Synchronous Culture Production by Density Selection

Sitz *et al.* (*1*) report the synchronization of *Chlorella* cells by isopycnic banding to select cells of a given density. Their last paragraph appears to suggest that this method might be applicable to mammalian cells. This is not the case, since the density of such cells is invariant around their life cycle (*2*).

The absence of a rigid outer wall in mammalian cells probably accounts for their difference from algae, yeast, and bacteria in this respect. The mammalian cell can support no hydrostatic pressure difference, and osmotic equilibrium is maintained by the movement of water. This results in a very uniform concentration of dry matter within the cell. In unicellular plants, the rigid capsule fixes the volume, and equilibrium is maintained by the development of a difference in hydrostatic pressure. Under these conditions, a variation in density is possible, depending on the phase relation between the "nuclear" and "cell" cycles, as pointed out by Mitchison (*3*).

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It would be unfortunate if these comments by Anderson discouraged further investigation of the relation between age and density of mammalian cells. Human erythrocytes, which do not possess rigid outer walls, nevertheless become distributed in a density gradient according to age (*1*).

The conditions used in measuring the density of Chinese hamster cells (*2*) allowed an average recovery of only 24 percent of the cells taken. Apparently no attempt was made to culture the recovered cells. These cells were probably not viable after centrifugation in view of the fact that Fox and Pardee (*3*) found viability to be dependent on the presence of serum in the Ficoll gradient.

Until gradient conditions that permit complete recovery of essentially all the cells taken are used, and until the degree of synchrony of cells in subfractions taken serially from the buoyant band or bands is evaluated in cell cycle experiments, there remains the possibility that, at some age, mammalian cells attain a density that permits the separation of this age cell from the rest of the population by the isopycnic technique.

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