tive of the greater trehalase activity found biochemically [17.0, 10.7, and 4.3 for rabbit, mouse, and human, respectively (*3*)]. No localization of reduced formazan pigment was seen in the medulla of the three species.

The most prominent deposition of reduced formazan pigment was noted within the basilar portions of the cells of the proximal convoluted tubules. The distal convoluted tubules and cortical collecting ducts stained with much less intensity. The difference in tubular staining reactions was more apparent in unfixed sections; however, the morphological preservation was considerably improved with the glutaraldehyde fixation. Glomeruli, interstitial cortical vessels, and nuclei of tubular cells were unstained (Fig. 2).

The histochemical reaction indicates that trehalase activity is present within all the cortical renal tubules, however, with a definite increased activity in the proximal convoluted tubules. Micropuncture studies (*5*) indicate that the first half of the proximal convoluted tubule is the site of glucose reabsorption, although other tubular sites were not excluded. The possibility of artifactual staining of the distal convoluted and collecting tubules due to membrane alteration during the preparation of the cryostat sections must be considered, but this explanation appears unlikely since other adjacent structures, such as glomeruli and blood vessels, were unstained. Nevertheless, the histochemical localization of trehalase is consistent with the hypothesis that trehalase functions in the renal reabsorption of glucose.

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18 June 1968

## Temperature in the Monkey: Transmitter Factors Released from the Brain during Thermoregulation

Abstract. *When perfusate is collected from the anterior hypothalamus of a cooled donor monkey and is transfused to a corresponding hypothalamic site in a normal monkey, fever occurs in this recipient. Conversely, perfusate from a heated donor monkey lowers the recipient monkey's temperature when the same hypothalamic transfusion procedure is followed. These experiments provide direct evidence of a neurochemical "coding" within the specific anatomical region of the brain historically implicated in the control of body temperature.*

A dual neurochemical system in the hypothalamus has been postulated as the principle mechanism in the diencephalon for the regulation of body temperature (*1*). This theory was based on the finding that serotonin and a catecholamine injected directly into the brain of a cat produce hyper- or hypothermia, respectively (*2*). Similar temperature responses occur in the primate (*3*) and in other species (*4*) if these endogenous amines are injected intracerebrally. However, for the existence of a dual transmitter system for a specific function, such as thermoregulation, to be substantiated, the actual release of transmitter substances from a specific site must be demonstrated during a physiological change.

If cerebrospinal fluid (CSF) from a cooled donor monkey is transfused to the cerebral ventricle of a second monkey, the recipient often develops a fever (*5*). Because of variations in the recipient's response, due probably to the nonspecific nature of CSF, we devised a transfusion assay based on the classical physiological procedures of Dale and Loewi (*6*). Perfusate was collected from a donor monkey's anterior hypothalamus (*7*) and transfused directly to a corresponding site in the recipient.

Ten pairs of male rhesus monkeys (4 to 6 kg) were acclimated to primate restraining chairs for 2 to 3 weeks before surgery. Bilateral push-pull cannulae (*8*) were stereotaxically implanted in the anterior hypothalamic area under rigid aseptic precautions (*9*). So that body temperature could be continuously monitored, a thermistor bead attached to an amphenol connector was placed against the wall of the falx cerebri. Seven days after operation, two monkeys were placed side by side, and each animal was used as the donor in one hypothalamic transfusion and as a recipient in another.

In both control and temperature transfusion experiments, the 26-gauge injector or "push" cannula was inserted 1.0 mm below the 20-gauge outer or

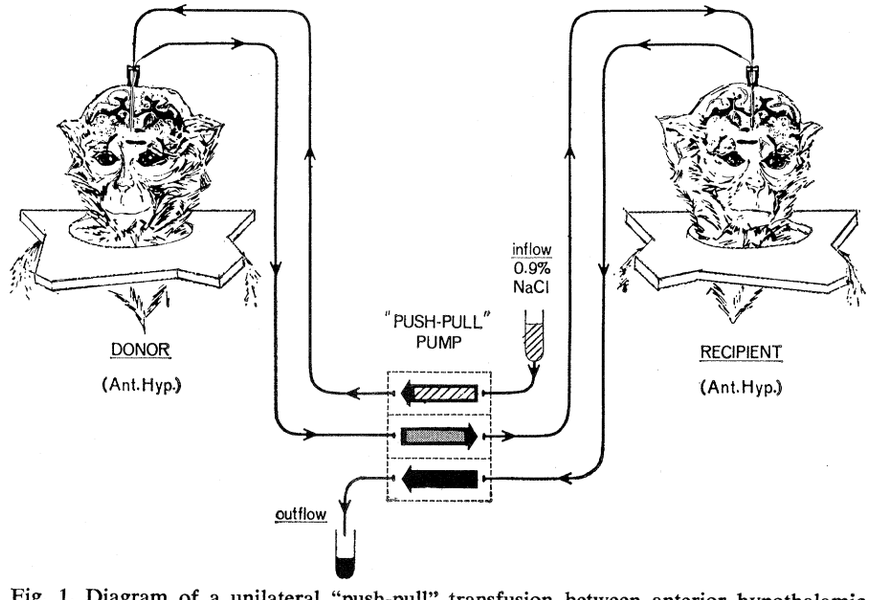


Fig. 1. Diagram of a unilateral "push-pull" transfusion between anterior hypothalamic areas of two unanesthetized monkeys. Sterile saline (*inflow*) is pumped via the "push" cannula into the donor's anterior hypothalamus (*ant. hyp.*) and withdrawn at the same flow rate via the "pull" cannula. This perfusate is then pumped by the withdrawal syringe to a corresponding hypothalamic site (*ant. hyp.*) in the recipient monkey via its "push" cannula. The perfusate is then drawn off at the same rate via the "pull" cannula to the *outflow*. The donor is either heated or cooled just before transfusion, and changes in the recipient's temperature are monitored after transfusion.

"pull" cannula, and 0.9 percent sterile saline adjusted to a *p*H of 6.5 was then perfused through the anterior hypothalamic area. Figure 1 illustrates the procedure whereby the anterior hypothalamic region of a donor monkey is linked unilaterally by the push-pull pump system to a corresponding site in the recipient. The pump consists of two calibrated 1-ml syringes mounted in opposition so that they are driven by the same motor-gear system. Within a maximum perfusion time of 20 minutes, 0.5 to 1.0 ml of perfusate was washed through the donor at a rate of 25 to 100 μl/min. As soon as a predetermined volume was collected, the transfusion of this perfusate to the recipient's anterior hypothalamus was begun. If, because of cannula occlusion, the rate of outflow during a perfusion of either donor or recipient was less than that of inflow, the transfusion experiment was terminated immediately. The extent of hypothalamic tissue washed by the push-pull perfusate was determined by substituting a 2 percent bromophenol blue solution for the saline perfusate. Postmortem histological examinations revealed that a spherical volume of tissue approximately 1.5 to 2.0 mm in diameter at its widest point was infiltrated by the perfusate; this result corresponds closely to that reported by McClennan (*10*).

To cool the donor monkey, we placed dry ice in wire-mesh containers against the inner sides of the chair chamber; within 15 minutes the chamber temperature fell to −5° to −15°C. To heat the donor monkey, a stream of warm air was blown into the chair chamber so that within 15 minutes the chamber temperature reached 50° to 55°C. In control experiments, the environmental temperature of the donor was not altered. The temperature of the perfusate, whether from a heated, cooled, or normal donor monkey, always reached the ambient level as it passed through the 90-cm length of polyethylene tubing (PE-50) connecting donor to recipient.

No apparent physiological or behavioral change occurred in the recipient monkey after a control transfusion. However, when perfusate from a cooled, shivering donor was transfused to the normal monkey's anterior hypothalamus, the recipient began to shiver vigorously usually within 30 to 90 seconds. Almost immediately, its temperature increased and continued to rise sharply for as long as 20 to 40 minutes; a gradual return to within 0.5°C of the original temperature occurred usually

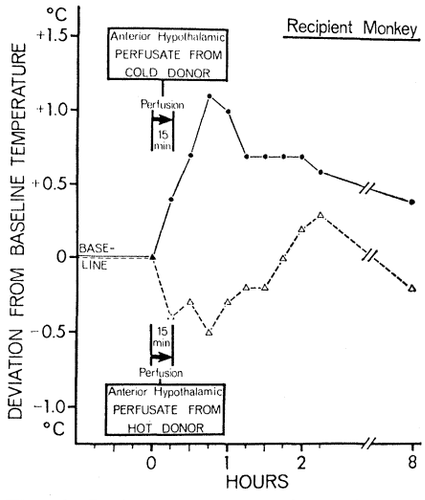


Fig. 2. Body temperature (°C) of a recipient monkey, expressed as the deviation from the temperature. Black arrows indicate the perfusion interval (15 minutes). In the first experiment (●), the recipient's anterior hypothalamus was infused with perfusate from a corresponding site in a cooled donor monkey. In the second experiment (△) 3 days later, perfusate from a heated donor was transfused to the same site.

4 to 8 hours later. On the other hand, as perfusate from the heated donor was transfused to the anterior hypothalamus of the recipient monkey, the temperature of the recipient declined immediately. The hypothermia, which lasted for less than 1 hour, was always followed by an overshoot of as much as 0.5° to 1.0°C above the original temperature, during which shivering and vasconstriction were noted. Temperature changes of a recipient monkey are shown in Fig. 2 for two representative experiments carried out 3 days apart, in which the donor was cooled in the first and heated in the second. The initial hyper- or hypothermia closely parallels each of the periods of perfusion, and in this sense the results resemble a stimulus-bound response to electrical stimulation (*11*).

In 27 experiments, these results were consistently obtained when cannulae were appropriately placed in donor and recipient, and if bilateral push-pull perfusions were carried out in both monkeys (*12*). Within 1 hour after the start of a transfusion from a cold donor, the mean temperature rise for all recipients was +0.7°C. Similarly, when perfusate was transfused from a heated donor, the mean decline for the recipients was −0.4°C. In 29 control experiments, the mean variation of the recipient's temperature was less than 0.1°C.

Within the framework of a neurochemical "coding" hypothesis proposed by Miller (*13*), it appears that two independent cellular systems may function at the same morphologic site in a primate exposed to either a hot or cold environment. If the monkey is cooled, a pharmacologically potent factor, which activates the efferent pathways involved in heat production, is apparently released from the anterior hypothalamus. When this factor is transfused to a normal monkey's hypothalamus, the animal's temperature rises. Conversely, heating the primate seems to elicit the release of a different neurochemical factor which, at the same hypothalamic site, activates the efferent systems mediating the loss of body heat. If this factor is transfused to the normal monkey's hypothalamus, the animal's temperature falls.

Thus, the anterior hypothalamus, a region which has thermosensitive and chemosensitive cells (*14*), apparently contains a dual neurochemical system which may trigger opposing efferent responses. Even though the cellular mechanisms responsible for the release of these substances are unknown, our experiments suggest that a neurochemical "coding" exists within a restricted region in the brain that functions in a specific homeostatic process—thermoregulation.

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15. Supported in part by ONR contract N00014-67-A-0226-0003 and by NSF grant GB3874. We thank Peter Curzon and Peter F. East for assistance in this investigation.

3 July 1968