References and Notes

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5. We immobilized DNA on nitrocellulose filters essentially by the method of Gillespie and Spiegelman [*J. Mol. Biol.* **12**, 129 (1965)]. Incubation was for 24 hours in 2 ml of 6XSSC (0.9*M* NaCl, 0.09*M* sodium citrate) (phage system) or for 16 hours in 1 ml of 6XSSC (mammalian system) at 67°C in the experiments where both RNA fractions were added simultaneously. Otherwise, the discs were pre-incubated for 24 hours with unlabeled RNA in 2 ml of 6XSSC, washed in 6XSSC, then incubated for an additional 24 hours with the labeled RNA in 2 ml of 6XSSC. The ribonuclease treatment and pre-ribonuclease wash were simplified by using a batch method, without filtration. After the ribonuclease treatment, the discs were washed with 50 ml of 6XSSC by filtration, the DNA-bearing side of the disc being turned downward. This method gave background values comparable with those obtained with the technique described by Gillespie and Spiegelman. Schleicher and Schuell 25-mm-type B7 filters were used; in the animal experiments, the prepared filters were cut into quarters before incubation with RNA. In the mammalian experiments, the "blank" filters carried 10 μg of *E. coli* DNA (*2*); in the T2 experiments they carried no DNA. Blank discs were subjected to the same preparative wash and vacuum drying as were the DNA-loaded filters; such discs have lower backgrounds than do untreated discs. RNA was prepared from L-cells pulsed for 45 minutes with ^{3}H-uridine and from *E. coli* cells pulsed with $^{32}PO_4$ or ^{3}H-uridine from 2 to 9 minutes after infection with T2 phage. All nucleic acids were prepared by repeated phenol extraction.
6. W. S. Riggsby, in preparation.
7. Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation. W.S.R. and V.R. are PHS postdoctoral research fellows.

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Histochemical Localization of Renal Trehalase: Demonstration of a Tubular Site

Abstract. *Trehalase activity has been localized histochemically within the cortical tubules of the human, rabbit, and mouse kidneys. The specific localization of the enzyme supports the hypothesis that trehalase, in concert with a series of other enzymes which synthesize trehalose from glucose, functions in a mechanism for the reabsorption of glucose from the glomerular filtrate.*

Trehalase, which hydrolyzes the disaccharide trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) to two glucose moieties, is found in yeast, fungi, higher plants, and several phyla of invertebrate animals, especially insects (*1*). A disaccharidase specific for trehalose has been reported in rat intestine (*2*). The finding of prominent renal trehalase activity in numerous mammalian species without detectable blood trehalose has suggested a pathway for glucose reabsorption from the glomerular filtrate (*3*). The proposed mechanism involves hexokinase, phosphoglucomutase, uridine diphosphate glucose pyrophosphorylase, trehalose-6-phosphate synthetase, trehalose-6-phosphate hydrophosphatase, and trehalase, with the overall reaction as follows (*4*):

$$2\ \text{glucose} + 2\ \text{ATP} + \text{UTP} \rightarrow$$
$$2\ \text{glucose} + 2\ \text{ADP} + \text{UDP} + \text{PP}_i + \text{P}_i$$

This hypothesis requires that trehalase be localized in the tubules of the renal cortex, the site of glucose resorption (*5*). We attempted to localize histochemically the enzyme in human, mouse, and rabbit kidneys. A human kidney was obtained from a 55-year-old white male during nephrectomy for a papillary carcinoma of the ureter. Kidneys were also obtained from a rabbit killed with pentobarbital and from a mouse killed with ether. The fresh kidney was cut into 3- to 4-mm slices containing cortex and medulla and either frozen on dry ice or fixed for 15 minutes in 2.5 percent gluteraldehyde in 0.1*M* phosphate buffer, *p*H 7.4, at 4°C followed by several changes of the phosphate buffer over 30 to 60 minutes. Sections (6 μm) cut in a cryostat were placed on coverslips coated with 0.2 percent gelatin, and incubated in a medium containing 1.8 mg of glucose oxidase, 4.5 g of Nitro Blue Tetrazolium, 2.7 mg of phenazine methosulfate, and 0.22 g of trehalose in 18 ml of 0.4*M* phosphate buffer, *p*H 6.0, at room temperature in the dark (*2*). Incubation times were from 5 to 40 minutes for the rabbit kidney, 3 hours for the mouse kidney, and 5½ hours for the human kidney. Control preparations consisted of the medium without trehalose or with sucrose substituted for trehalose.

The reduced formazan pigment, indicating the site of trehalase activity, was localized macroscopically in the cortex, prominently in the inner areas, of the mouse and human kidneys (Fig. 1). Direct enzymatic assay of the human kidney showed activities of 4.62 and 3.89 μmole of glucose produced per minute per gram of tissue (wet weight) for the inner and outer cortex, respectively (*3*). The entire cortex of the rabbit kidney was intensely stained. The larger distribution of the reduced formazan pigment in the rabbit cortex is indica-

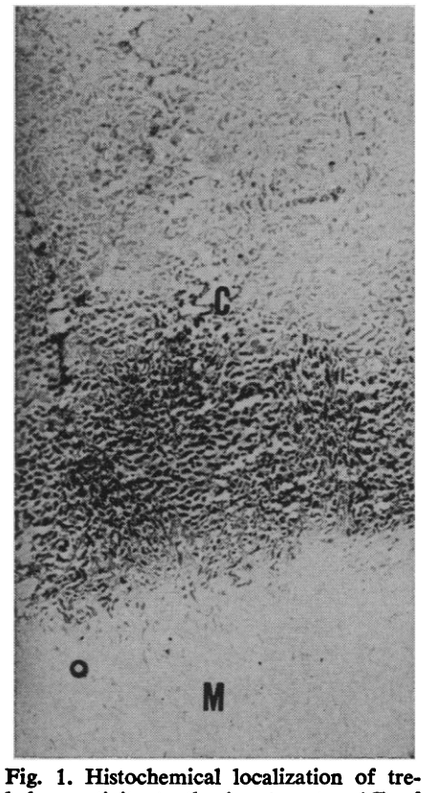

Fig. 1. Histochemical localization of trehalase activity to the inner cortex (*C*) of the human kidney. No medullary (*M*) activity is present (0.1 percent Kernechtrot counterstain, × 20).

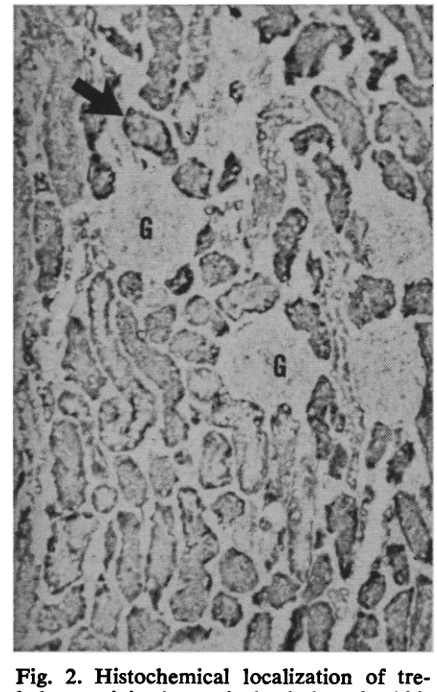

Fig. 2. Histochemical localization of trehalase activity in cortical tubules of rabbit kidney fixed in glutaraldehyde. Prominent proximal tubular staining is noted (arrow) as well as less intense staining in distal convoluted and collecting tubules. No glomerular (*G*) staining is noted (0.1 percent Kernechtrot counterstain, × 110). Control preparations showed no formazan deposition.

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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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

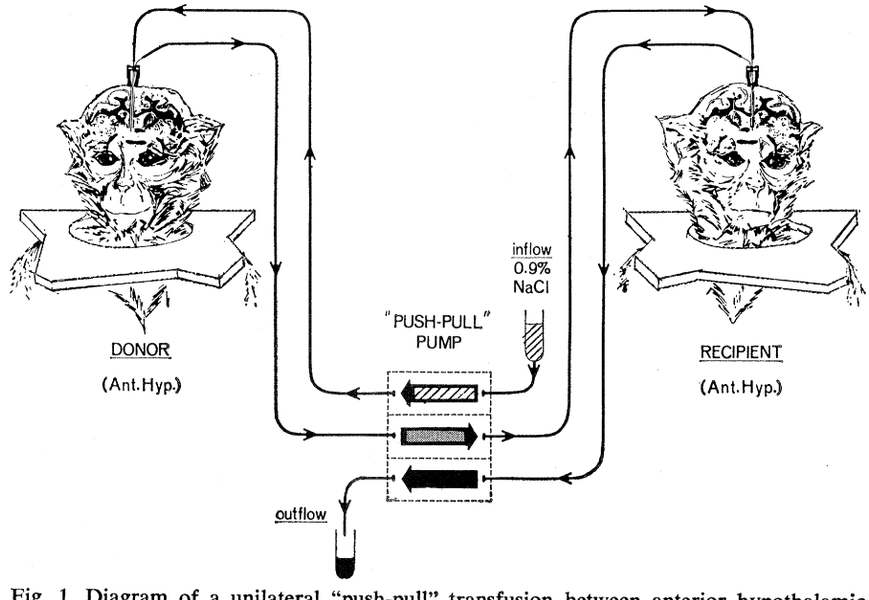

Fig. 1. Diagram of a unilateral "push-pull" transfusion between anterior hypothalamic areas of two unanesthetized monkeys. Sterile saline (*inflow*) is pumped via the "push" cannula into the donor's anterior hypothalamus (*ant. hyp.*) and withdrawn at the same flow rate via the "pull" cannula. This perfusate 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.