Sugar Transport: Effect of Temperature on Concentrative Uptake of α-Methylglucoside by Kidney Cortex Slices

Abstract. *The ability of renal cortical slices to accumulate a monosaccharide is enhanced at temperatures below 37°C. Increase in concentration gradients occurs despite a decrease in total sugar flux. The apparent explanation for this paradox is a proportionally greater inhibition of efflux.*

The effect of temperature on the rate of chemical and biochemical reactions is well known, and the examination of temperature alteration on reaction rates has contributed to our understanding of biological phenomena. This has been the case in the field of membrane transport where the decreased transport of sugars and amino acids in vitro at temperatures below 37°C has been used as evidence for the energy dependence of the process in a variety of tissues (*1*). Indeed, one of us has reported a detailed kinetic comparison of the transport of the nonmetabolizable model amino acid, α-aminoisobutyric acid, at 37° and 27°C by renal tubule cells (*2*).

In order to determine the distinctive features of monosaccharide transport in this tissue, we have studied the effect of reduced temperature on α-methylglucoside (α-MG) uptake in vitro. This sugar appears to be a nonmetabolizable model substance that shares the characteristics of the glucose-galactose transport mechanism (*3*). In experiments to compare steady state concentration gradients at 25° and 37°C, we observed a greater intracellular concentration of sugar at the lower temperature. This communication reports these observations and presents an explanation for the paradoxical enhancement of α-MG uptake at temperatures lower than 37°C.

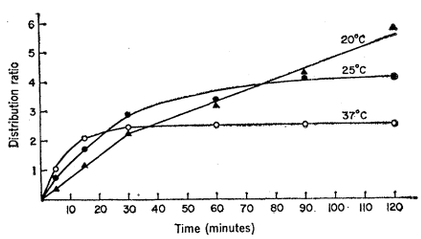


Fig. 1. Accumulation of ^{14}C-labeled α-methyl-D-glucoside by rat kidney cortex slices. Three kidney cortex slices totaling 20 to 35 mg were incubated in 30-ml plastic flasks containing 2 ml of Krebs-Ringer bicarbonate buffer (118.5 m*M* NaCl, 4.75 m*M* KCl, 2.53 m*M* $CaCl_2$, 1.2 m*M* KH_2PO_4, 1.2 m*M* $MgSO_4$, and 25 m*M* $NaHCO_3$), *p*H 7.4 as measured at room temperature (22°C), containing 2 μmole of α-MG per milliliter and 0.1 μc of label per milliliter under an atmosphere of O_2 and CO_2 (95 : 5) in Dubnoff metabolic shakers at 37°, 25°, and 20°C. Uptake is designated by the distribution ratio, the ratio of counts per minute per milliliter of intracellular fluid to counts per minute per milliliter of medium, using tissue water and inulin spaces mentioned previously.

The techniques for the determination of intracellular concentration of ^{14}C-labeled α-MG by Sprague-Dawley rat kidney cortex slices incubated in Krebs-Ringer bicarbonate buffer (*p*H 7.4) have been published (*4*). Tissue uptake is expressed as the distribution ratio—the ratio of counts per minute per milliliter of intracellular fluid to counts per minute per milliliter of medium (*5*). Since α-MG is not metabolized, the distribution ratio represents a true concentration gradient. Efflux studies were performed after steady state was reached on incubation of slices with ^{14}C-labeled α-MG, as previously reported (*6*). Total tissue water was 75 percent of the total wet tissue weight. After 20 minutes, extracellular space, determined by inulin penetration, was 22.4 percent at 20° and 25°C and was 25.6 percent at 37°C of total wet tissue weight.

Figure 1 shows the concentrative uptake of α-MG with regard to incubation time at three different temperatures. At 25°C the initial uptake rate is slower than at 37°C, but the accumulation continues until a new steady state is reached with a distribution ratio of 4 instead of 2.5. At 20°C there is a slow continuous uptake with achievement of concentration gradients higher than at 25°C. No steady state is reached at 20°C during the period observed. The differences in the uptake curves of the labeled sugar are also seen when slices are incubated with unlabeled compound to steady state conditions before addition of tracer α-MG (a net flux of 0.0220 μmole/min per 100 mg at 37°C versus a net flux of 0.0168 μmole/min per 100 mg at 25°C).

If one assumes that a closed, two-compartment system described for analysis of amino acid transport kinetics in kidney cortex slices (*2*) is applicable here, then the steady state is attained when the substrate flux into the cell is equal to efflux from the cell. In such a system, the attainment of a higher steady state concentration with a diminished influx is explainable by an even greater inhibition of efflux. The influx rate at 25°C as calculated from the 5-minute uptake value (Fig. 1) is 60 percent of the influx rate at 37°C. Efflux of ^{14}C-labeled α-MG was, therefore, examined and the results are shown in Fig. 2. The efflux rate constant was decreased from 0.105 to 0.037 per minute per 100 mg final wet weight of tissue. A solution of the parameters of the two-compartment system at 25° and 37°C is found in Table 1. The values for the rate constants λ_{IM} (movement into intracellular space from medium) and λ_{MI} (movement into medium from intracellular space) were measured independently. Each independent measurement correlated well with the value calculated from the other measured parameter. Whereas the rate constant for movement of sugar from the medium into the cell is reduced by 43.5 percent, that for movement out of the cell space is reduced by 64.8 percent. Similar results have been observed when rabbit renal cortex is substituted for rat tissue.

Despite the marked reduction in net flux from 0.0276 to 0.0156 μmole/min per 100 mg of tissue produced by low-

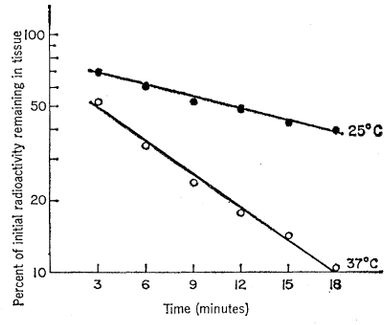
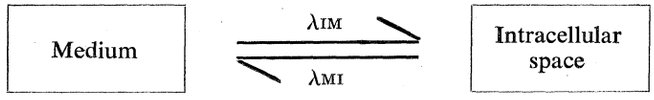


Fig. 2. Efflux of accumulated substrate from rat kidney cortex slices. Renal cortex slices were incubated for 60 minutes at 37° and 25°C in 2 m*M* and 1.4 m*M* α-MG, respectively. In all cases the media contained 0.1 μc of ^{14}C-labeled α-MG per milliliter. The substrate was varied so that the intracellular concentration at the onset of efflux was essentially the same at both temperatures, taking into account the higher concentrating ability of the tissue at 25°C. The design of the experiment has been published (*6*). At the end of the incubation the tissues were quickly removed, rinsed in saline, blotted, and transferred to flasks containing 3 ml of substrate-free buffer. At 3-minute intervals the flasks were opened and media sampled for radioactive assay. After 18 minutes, the α-MG in the tissue was determined, which, together with the amount effluxed, denotes the total in the slice at the start of efflux.

Table 1. Influence of temperature on kinetic parameters of sugar transport.



Incubation temperature (°C)	Medium pool size (μmole)	Steady state distribution ratio	Intracellular fluid pool (μmole)*	Fractional turnover rates per minute†		Net flux (μmole/min per 100 mg final wet weight)
				λ_{MI}	λ_{IM}	
37	4	2.5	0.263	0.105	0.0069	0.0276
25	4	4.0	.421	.037	.0039	.0156

* At 25°C intracellular space was found to be 52.6 percent of tissue weight. All calculations are based on 100 mg of tissue. † The rate constants are related by the equation $M \cdot \lambda_{IM} = ICS \cdot \lambda_{MI}$, where M is the medium concentration and ICS is the intracellular space.

ering the incubation temperature from 37° to 25°C, the renal tubule cells have a greater ability to concentrate α-MG. This paradox is explainable by a relatively greater inhibitory effect of temperature reduction on the sugar exit process than on the entry mechanism. These results differ from those obtained when the nonmetabolizable amino acid α-aminoisobutyric acid is affected by temperature reduction (*2*). A decrease from 37° to 27°C reduces the total amino acid flux by 60 percent; however, there is no difference in steady state gradients achieved since influx and efflux rate constants are reduced equally. In only one instance, that of cysteine transport, has a reduction of temperature resulted in an increased steady state gradient (*6*).

The phenomena reported here emphasize the importance of the efflux process in the establishment and maintenance of tissue concentration gradients. Our observations also underscore the need for analysis of tissue transport kinetics when perturbations are made in the incubation environment. This analytic requirement for amino acid and sugar uptake studies has been reviewed in depth previously (*7*). Our present knowledge of sugar transport in kidney cortex slices is derived mainly from the detailed reports of Kleinzeller and his associates (*3*, *8*) who have routinely employed a 25°C incubation temperature. Whether the latter observations reflect those at a physiological temperature remains to be determined.

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Calcium: Is It Required for Transmitter Secretion?

Abstract. *Ethanol multiplies miniature end-plate potential frequency independently of calcium ion concentrations and also multiplies calcium-dependent depolarization-evoked quantal release, to the same extent. This result implies a final common pathway, requiring little or no calcium, for both kinds of transmitter secretion. Chlorpromazine and hypertonicity act similarly to ethanol, but also depress depolarization-secretion coupling.*

Calcium ions, or divalent cations that can substitute for calcium, are essential to the process by which transmitter secretion by motor nerve terminals is accelerated by a presynaptic action potential or by presynaptic depolarization (*1*). A similar dependence on calcium has been demonstrated for a number of secretory systems, and it has been proposed that this reflects a fundamental similarity in the mechanism of such diverse secretory processes (*2*). However, it has been reported that raised osmotic pressure and black widow spider venom each cause an increase in the frequency of miniature end-plate potentials (MEPP's), which is the same in Ca^{2+}-free media as when Ca^{2+} is present (*3*). We have found that transmitter release by alcohols (*4*) is similarly independent of Ca^{2+}. Moreover, other "membrane stabilizers" (*5*) act in the same way as ethanol. The interaction of these agents and presynaptic depolarization indicates that the Ca^{2+}-independent release system shares at least its final step with the system by which transmitter is secreted in response to presynaptic depolarization. Thus, the actual transfer of transmitter quanta to the extracellular medium, as distinct from depolarization-secretion coupling, requires no Ca^{2+}.

Conventional methods were used for intracellular recording of MEPP's from end-plate regions of mouse diaphragms in vitro. To allow rapid changes of the bathing solution, we devised a superfusion system by which solution emerging from a nozzle of about 200 μm diameter flowed directly onto the surface of the muscle, keeping the solution there continuously mixed. The nozzle was connected by polyethylene tubes to reservoirs containing the various test solutions. With this system it was possible to change the solution in synaptic clefts within 5 seconds, as evidenced by changes of MEPP size when postsynaptic blocking agents or anticholinesterases were applied. To evoke transmitter release by nerve terminal depolarization, current was applied focally to end plates as described by Katz and Miledi (*6*). Using electrodes with tips of 25 to 50 μm diameter, we were able to obtain uniform polarization of the motor end plates, which are much more compact in the mouse than in the frog (*7*). Tetrodotoxin (Sankyo, 5×10^{-8} g/ml) was added to solutions to prevent action potential generation (*6*).

Increase of MEPP frequency by ethanol was found to persist in the absence of Ca^{2+}. In preparations superfused with a Ca^{2+}-free solution—calculated (*8*) free $[Ca^{2+}] < 10^{-10}M$—containing 10 m*M* ethylenebis(oxyethylenenitrilo)-tetraacetic acid (EGTA) and 0.5 m*M*