ENERGY- AND SODIUM-DEPENDENT UPTAKE OF INOSITOL BY KIDNEY CORTEX SLICES

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During studies on the biosynthesis of inositol in kidney slices (Hauser and Finelli, 1963) we noted that only a small percentage of the free tissue inositol appeared in the media during storage or incubation of the slices. An almost complete reabsorption of inositol by the kidney tubules of the dog under physiological conditions has been reported by Perlès, Colas and Blayo (1960). These observations, coupled with the known large concentration gradient between blood and all other tissues which have been examined (see e.g. Dawson and Freinkel, 1961) led us to believe that a transport mechanism might be operative. Experiments with 2-^{3}H-inositol revealed an accumulation of radioactivity in cortical slices of rat kidney substantially higher than would be expected from free diffusion alone. This accumulation was affected by the concentration of Na^{+} as well as by the presence of ouabain or of metabolic uncouplers or inhibitors in the incubation medium.

Materials and Methods. 2-^{3}H-inositol was obtained from the New England Nuclear Corp., Boston, Mass. 200 to 300 mg of cortical kidney slices from adult rats were incubated in 3.0 ml of Krebs-Ringer bicarbonate medium, pH 7.4, in 25 ml Erlenmeyer flasks for 45 min at 37^{o} with shaking in an attempt to decrease the high endogenous inositol content of the tissues. The slices were then blotted, weighed and transferred into 3.0 ml of fresh

medium containing 1 μc (17 μM) 2-^{3}H-inositol. The flasks were gassed briefly with 95% O_2-5% CO_2 at 0, 20, and 40 min. After 60 min the medium was removed and the slices rinsed rapidly with two 10 ml portions of ice-cold water. They were homogenized in 3.0 ml 0.19 M $ZnSO_4$ followed by 3.0 ml of 0.3 M $Ba(OH)_2$. Media were similarly deproteinized and 0.5 ml aliquots of the deproteinized supernatant solutions of both media and slice extracts were counted in a liquid scintillation counter. The scintillation fluid described by Silbert (1964) was used. Inositol was determined by microbiological assay (Campling and Nixon, 1954).

$$R, \frac{\text{counts/min-ml intracellular fluid}}{\text{counts/min-ml extracellular fluid}}, \text{ the distribution ratio}$$

of radioactivity, was calculated as indicated by Fox, Thier, Rosenberg and Segal (1964), using a value of 26% of the weight of the slices as the extracellular fluid volume in normal Krebs-Ringer bicarbonate media.

Results and Discussion. When kidney slices were incubated with 2-^{3}H-inositol under standard conditions, including a period of pre-incubation in the absence of labeled inositol as outlined above, an average distribution ratio of radioactivity greater than 7 was obtained (Table I, 18 experiments). Without pre-incubation R fell to 2.32. These results indicated an active intracellular accumulation of radioactivity, which was presumed to be an energy-requiring process. However, neither the addition of glucose nor of ATP increased R, which, in fact, tended to be lower in the presence of glucose than without it (Table I). Although an external supply of energy sources was unnecessary, the accumulation was abolished in the cold or by anaerobiosis and substantially reduced by inhibitors and uncoupling agents (Table I). Similar results have repeatedly been reported in experiments on the active

TABLE I

Inositol Uptake as an Energy-Requiring Process

Conditions	Distribution Ratio
Standard	7.13
1 X 10^{-2}M glucose	5.41
5 X 10^{-3}M ATP	6.81
0°	0.14
Gas phase: N_2	0.60
2 X 10^{-3}M NaCN	1.07
2 X 10^{-3}M ouabain	2.76
5 X 10^{-4}M 2,4-dinitrophenol	3.25
5 X 10^{-4}M p-chloromercuribenzoate	3.80
1 X 10^{-3}M NaN_3	5.00

TABLE II

Effect of Altering the Na^+ and K^+ Concentrations of the Medium

Conditions	Distribution Ratio
Standard	7.13
12 mM K^+	7.29
no K^+	2.40
Na^+ replaced by K^+	<0.5
Na^+ replaced by choline	0.2
Na^+ replaced by Tris	<0.4

Standard incubations, as described under "Methods," in Krebs-Ringer bicarbonate media, Na^+ 144 mM, K^+ 6 mM. Pre-incubations in media of the same ionic composition as subsequent incubations.

transport of a variety of substances such as galactose (Krane and Crane, 1959) and amino acids (Rosenberg, Blair and Segal, 1961).

Physiological Na^+ concentrations were first found to be essential for active transport by Riklis and Quastel (1958) and have been shown to be required for sugar transport by intestine (Bihler and Crane, 1962), kidney cortex slices (Kleinzeller and Kotyk, 1961) and striated muscle (Parrish and Kipnis, 1964). To this incomplete list may now be added the accumulation of radioactivity of 2-^{3}H-inositol in kidney slices. As Fig. 1 shows, progressive replacement of Na^+ with choline$^+$ leads to a drop in R until the intracellular accumulation of radioactivity is abolished at about 20 mM Na^+. An increase in Na^+ to 1.5 times the physiological concentration is without effect. Substitution of Tris$^+$ or of K^+ for

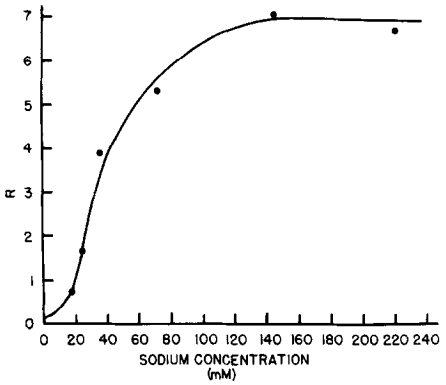

Figure 1. The dependence of inositol uptake on the Na^+ concentration of the medium. The ionic composition of the medium in which pre-incubation was carried out was identical with that used in the subsequent incubation with labeled inositol. R = distribution ratio.

TABLE III

Distribution of Radioactivity

	Activity in Extract	Activity in Inositol	%	Specific Activity of Inositol
	counts/minute			counts/min./μg.
Slices	61,160	58,160	95.2	786
Medium	152,900	58,500	38.2	4697

Incubation: standard conditions for two hours.
Inositol was isolated from paper chromatograms of deionized, deproteinized extracts. Solvent: ethanol-acetic acid-water, 8:1:1 (v/v).

Na^+ has the same consequences as replacement with $choline^+$ (Table II). Although a dramatic alteration in the functioning of the kidney slices is seen under these conditions, no permanent damage is inflicted. If the preincubation of the slices is carried out in Na^+-free buffer, followed by incubation with labeled inositol in medium of normal Na+ level, R is not lowered (R=7.87).

That the presence of K^+ is important is shown by the marked reduction of R when K^+ is omitted from the incubation medium. It can, however, be added at twice the concentration usually present in Krebs-Ringer bicarbonate medium without altering R (Table II). The requirement for K^+ for optimal operation of this system is analogous to findings for the active transport of galactose (Kleinzeller and Kotyk, 1961) and amino acids (Fox, et al., 1964) in the same tissue.

The sensitivity of Na^+-requiring transport systems to cardiac glycosides has been amply documented. Addition of 2 mM ouabain during the incubation under otherwise standard conditions lowers R to about one-third (Table I). The concentration of the glycoside is comparable to that used by Fox, et al. (1964) to reduce the accumulation of amino acids by kidney slices (0.8 mM ouabain, about 50% reduction). No diminution of R is observed below 0.3 mM ouabain.

The main difficulty in attempting to interpret these findings stems from the fact that inositol is not an inert substance, but rather is actively metabolized by kidney tissue (Hauser and Finelli, 1963). The initial catabolic step is a cleavage of the ring with the formation of glucuronic acid (Charalampous and Lyras, 1957). A large proportion of the radioactivity recovered from the media at the end of the present experiments was contained in substances other than inositol, notably glucose. However, virtually all of the radioactivity in the slices could be clearly separated on paper from glucose, glucuronic acid and glucuronolactone and be isolated as inositol (Table III). At the end of a standard incubation, the ratio of inositol concentration in intracellular to that in extracellular fluid is about 150 to 200. The possibility that a basal inositol pool may exist, which exchanges only slowly with extracellular inositol and is not readily metabolized, is under study; such a pool was

demonstrated for glutamate in Ehrlich ascites cells by Heinz, Loewe, Despopoulos and Pfeiffer (1964). Further experiments, which will be reported subsequently, are also in progress with inhibitors in an attempt to separate transport from metabolic transformations and to identify and quantitate the metabolic products in the medium.

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REFERENCES

Bihler, I., and Crane, R.K., Biochim. Biophys. Acta, 59, 78 (1962)
Campling, J.D., and Nixon, D.A., J. Physiol. (London), 126, 71 (1954)
Charalampous, F.C., and Lyras, C., J. Biol. Chem., 228, 1 (1957)
Dawson, R.M.C., and Freinkel, N., Biochem. J., 78, 606 (1961)
Fox, M., Thier, S., Rosenberg, L., and Segal, S., Biochim. Biophys. Acta, 79, 167 (1964)
Hauser, G., and Finelli, V.N., J. Biol. Chem., 238, 3224 (1963)
Heinz, E., Loewe, U., Despopoulos, A., and Pfeiffer, B., Biochem. Z., 340, 487 (1964)
Kleinzeller, A., and Kotyk, A., Biochim. Biophys. Acta, 54, 367 (1961)
Krane, S.M., and Crane, R.K., J. Biol. Chem., 234, 211 (1959)
Parrish, J.E., and Kipnis, D.M., J. Clin. Invest., 43, 1994 (1964)
Perlès, R., Colas, M.-C., and Blayo, M.-C., Rev. Franc. Etudes Clin. Biol., V, 31 (1960)
Ricklis, E., and Quastel, J.H., Can. J. Biochem. Physiol., 36, 347 (1958)
Rosenberg, L.E., Blair, A., and Segal, S., Biochim. Biophys. Acta, 54, 479 (1961)
Silbert, J.E., J. Biol. Chem., 239, 1310 (1964)