PRELIMINARY NOTE

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The influence of size of rat kidney cortex slices on the accumulation of amino acids

Although many parameters of amino acid accumulation by rat kidney cortex slices have been studied[1-4] the effect of slice size has not been examined. During the course of the investigation of developmental aspects of amino acid accumulation by rat kidney cortex it became necessary to ascertain the influence of size since only very small slices can be obtained from the cortex of newborn rats. Studies were therefore initiated to compare the uptake of amino acids by variously sized segments of adult cortical slices. This report indicates that small segments have an abnormally high extracellular space and accumulate much higher intracellular concentrations of amino acids than larger segments or whole slices.

The technique for incubation of rat kidney cortex slices in 2 ml of Krebs–Ringer bicarbonate medium (pH 7.4) to study ^{14}C-labeled amino acid accumulation[1-4] as well as the estimation of extracellular space using [^{14}C]inulin has been detailed[5]. Tissue was obtained from 140–160-g animals killed by decapitation. Slices were made with a Stadie–Riggs microtome from the pole of the kidney, the first surface slice being discarded. Some of the slices were cut with a razor blade into halves, the halves into quarters and quarters into eighths, as a pie or cake would be cut into pieces. A slice or equivalent segment from each of three animals was incubated per flask so that all data represents paired tissues. The accumulation of amino acids was calculated as described previously[1] and represented by the distribution ratio, the ratio of counts/min per ml intracellular fluid to counts/min per ml medium. [^{14}C]Inulin as well as ^{14}C-labeled amino acids were purchased from New England Nuclear Corp.

The extracellular space of various segments is shown in Table I. The integrity of the extracellular space was maintained by half and quarter slices. In more extensive

TABLE I

EFFECT OF SIZE ON ESTIMATION OF EXTRACELLULAR SPACE IN ADULT KIDNEY CORTEX SLICES

Tissues were incubated in Krebs–Ringer bicarbonate buffer (pH 7.4) at 37° containing 0.25 μC of ^{14}C-labeled inulin. Each flask contained a slice or segment of a slice from each of three animals. The segments were made from a single slice. Each value represents the mean and S.E. of 3 to 8 determinations.

Incubation time (min)	*Extracellular fluid (% of wet wt.)*			
	whole slice	*½ slice*	*¼ slice*	*⅛ slice*
15	26 ± 2	25 ± 2	27 ± 2	35 ± 2
90	27 ± 2	27 ± 2	28 ± 2	37 ± 2

studies of extracellular space of whole cortex slices using inulin previously published, the value was 26.7 ± 1 % of the wet tissue weight[5]. One-eighth slices had a significantly higher extracellular space of up to 37 % of wet weight.

The accumulation of various amino acids by half and eighth slices is shown in Table II. Significantly higher accumulation by the smaller segments is seen at the longer period of incubation for both dibasic and mono-amino-mono-carboxylic amino acids. The distribution ratios for half slices are similar to those observed on many occasions with whole slices[1–4,6]. Fig. 1 demonstrates the disparity due to size in the timed accumulation of L-lysine in whole slices and eighth slices.

TABLE II

AMINO ACID UPTAKE BY SEGMENTS OF RAT KIDNEY CORTEX SLICES

Slices were incubated in Krebs–Ringer bicarbonate buffer (pH 7.4) at 37° containing 0.4 μC of ^{14}C-labeled amino acids with final concentration 65 μM. Each flask contained a slice or segment of a slice from each of three animals. The segments were made from a single slice. Each value represents the mean and S.E. of 5 determinations. Distribution ratio is the ratio of counts/min per ml intracellular fluid to counts/min per ml media.

Amino acid	*Incubation time (min)*	*Distribution ratio*	
		½ slice	*⅛ slice*
L-Valine	30	2.67 ± 0.31	3.21 ± 0.46
	90	3.30 ± 0.36	5.35 ± 0.63*
α-Aminoisobutyric acid	30	2.81 ± 0.22	3.26 ± 0.25
	90	4.74 ± 0.34	8.39 ± 1.02**
L-Cystine	30	3.07 ± 0.27	3.59 ± 0.26
	90	5.32 ± 0.48	7.61 ± 0.58**
L-Arginine	30	4.12 ± 0.23	5.45 ± 0.63
	90	5.15 ± 0.26	9.16 ± 0.23**
L-Lysine	30	3.79 ± 0.28	3.87 ± 0.14
	90	4.61 ± 0.33	8.43 ± 0.82*

* $P < 0.02$ determined using the Student "t" test applied to paired data.
** $P < 0.01$ determined using the Student "t" test applied to paired data.

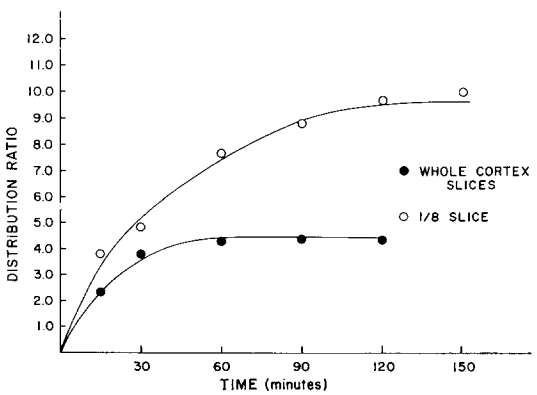

Fig. 1. The uptake of L-lysine by rat kidney cortex slices. Conditions as in Table II. Each point represents the average of triplicate determinations. The segments were made from a slice obtained from the same kidney which provided the whole slice.

Analysis of the weight and surface area of the segments has also been carried out. In the studies shown in Table II the half slices averaged 5.76 mg (range 4.70–8.00) and the eighth slices 1.29 (range 0.94–1.71 mg) for 135 segments each group. The surface area of segments was determined in a separate experiment by tracing them on standardized paper (9.39 mg/cm^2), cutting the outlines and weighing the paper on a Cahn microbalance. In six determinations, half slices had an area of 0.262 cm^2, and eighth slices 0.061 cm^2 (one surface). The average ratio of weight to surface area (one side) for half slices was 23.3 mg/cm^2 and eighth slices 21.2 mg/cm^2, thus indicating that the relationship of weight to surface area was not the prime factor influencing the higher uptake by the smaller segments.

The explanation for the higher accumulation of amino acids by a segment of kidney cortex slices weighing approx. 1 mg is open to speculation. Recently, it has been shown that intracellular accumulation by Ehrlich ascites cells is higher when the studies were carried out in dilute suspensions[7] and that the concentration of cells per unit volume is a factor in transport. It is difficult to extrapolate this phenomena to uptake by intact tissue. Perhaps a more likely explanation resides in the recent observation that isolated rabbit kidney tubules have a much higher accumulation of amino acids than cortex slices[8]. It could well be that minute pieces of a slice behave as isolated tubules.

The present observations suggest that careful attention should be paid to the influence of size on amino acid uptake by kidney fragments, especially in studies involving fetal and newborn rat kidney where slices may weigh in the 1-mg range. The importance of the size of intestinal segments on sugar accumulation has been indicated previously[9].

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