peptidase and the γ-glutamyl cycle. Supporting this idea are the observations that methionine, a good substrate for the transpeptidase (*12*), is rapidly taken up by brain (*18*) and that glycine and aspartate, which are poor substrates (*5*), are hardly taken up at all (*18*). Both the transpeptidase (*22*) and amino acid uptake (*23*) are sensitive to monovalent cations. Also consistent with this idea is the localization of the enzyme in the endothelium of brain capillaries, which is the anatomical site of the blood-brain barrier (*24*). Finally, brain contains significant amounts of the three intermediates of the γ-glutamyl cycle—glutathione (*25*), pyrrolidonecarboxylic acid (*26*), and γ-glutamyl amino acids (*27*). It may be relevant that signs of brain damage were manifest in a patient excreting large amounts of pyrrolidonecarboxylic acid (*10*) and by two others with another block in the γ-glutamyl cycle, namely, a deficiency in the γ-glutamylcysteine synthetase (*11*).

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Regulation of Amino Acid Transport in Kidney Cortex of Newborn Rats

Abstract. *After incubation at 37°C the subsequent uptake of α-aminoisobutyric acid, cycloleucine, glycine, and* L-*proline by newborn (as compared to adult) rat kidney cortex slices is enhanced. The effect is abolished by the presence of cycloheximide, actinomycin D, and high concentrations of the above-mentioned amino acids in the medium during the 37°C incubation prior to measurement of uptake. The data suggest that there is an adaptive control mechanism which is expressed on incubation at 37°C and which can regulate amino acid transport in newborn rat kidney cortex.*

The examination of membrane transport systems in developing rat kidney cortex has aided in distinguishing the separate nature of the process for sugars and amino acids (*1*) and has served to delineate differences in the transport mechanisms of several amino acids (*2*). During further experiments with the newborn rat kidney cortex as a model system to explore the responsiveness in vitro of the tissue to hormonal stimulation, we have observed that merely incubating the cortical slices in buffer at 37°C enhances their ability to accumulate some neutral amino acids. We now report observations that indicate the presence of a time-dependent regulatory process for amino acid transport in renal cortical cells of newborn but not in those of adult rats, and that this process is associated with concomitant protein synthesis.

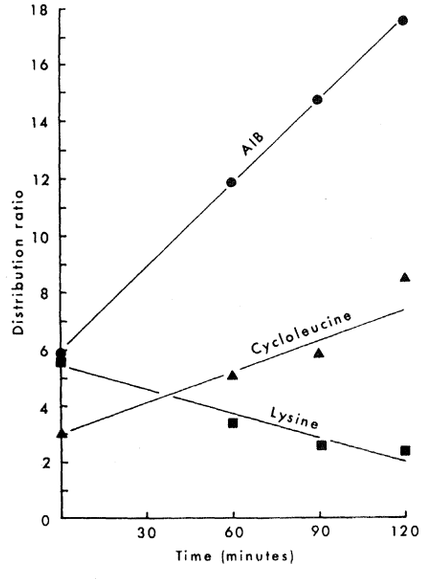

Fig. 1. The effect of the length of preliminary incubation on the subsequent uptake of amino acids by newborn rat kidney cortex slices. The abscissa is the duration of the incubation in buffer alone prior to addition of substrates for the 1-hour incubation to measure the uptake designated by the distribution ratio, that is, the number of counts per minute per milliliter of intracellular fluid to that per milliliter of medium.

The technique for determining the in vitro uptake and intracellular concentration of ^{14}C-labeled amino acids and sugars in kidney cortex slices from newborn and adult Sprague-Dawley rats has been described (*1–3*). The uptake is expressed as the distribution ratio—the ratio of the number of counts per minute per milliliter of intracellular fluid to the number per milliliter of medium. For all the substrates tested except proline, which is rapidly metabolized, the ratio is indicative of a concentration gradient. The total tissue water was 76 percent of the wet tissue weight, and the extracellular space of newborn cortical slices, as determined by inulin penetration, was 20 percent of the wet tissue weight. The experiment consisted of incubating the slices

in 2 ml of Krebs-Ringer bicarbonate buffer, *p*H 7.35, containing ^{14}C-labeled 0.065 m*M* amino acid or 2 m*M* α-methyl-D-glucoside (0.1 μc/ml) either immediately after the slices were prepared or after incubation for as long as 120 minutes at 37°C in the buffer alone.

A 60-minute incubation at 37°C of newborn kidney cortex is associated with a subsequent increase in the distribution ratio of α-aminoisobutyric acid (AIB) and cycloleucine, both model nonmetabolizable amino acids, as well as that of glycine and L-proline. The uptake of L-valine and L-lysine is significantly decreased, whereas that of α-methyl-D-glucoside, a nonmetabolizable model sugar for the glucose-galactose transport system, is unaffected. The temperature of the medium during the preliminary incubation phase appeared critical in that there was no enhanced uptake of AIB or cycloleucine when the preliminary incubation was carried out at room temperature. Kidney cortex slices from adult rats (44 days old) did not respond to preliminary incubation at 37°C by an increase in the distribution ratio (for AIB 3.01 ± 0.18 as compared to 2.83 ± 0.16; 12 determinations), thus indicating the effect is a property of young tissue.

The concentration of amino acids in the experiments shown in Table 1 is in the physiological range. When the substrate concentration was raised to 5 m*M*, there was no enhanced uptake of AIB, cycloleucine, glycine, or proline even though the tissue had been incubated in buffer at 37°C for 60 minutes. Since there appear to be at least two systems, for transport of these amino acids, distinguished by different K_m (Michaelis constant) values (*1, 4*), our data indicate that it is the low K_m system only which is affected.

Figure 1 shows the changes in amino acid uptake with increasing time of the 37°C preliminary incubation in buffer. The AIB and cycloleucine distribution ratios increase progressively, and after 2 hours are two- to threefold higher than those of slices not subjected to preliminary incubation. The uptake of lysine continues to fall, and after 120 minutes the distribution ratio is half that of slices receiving preliminary incubation.

In order to ascertain the etiology of the enhanced uptake of AIB, we performed the experiments shown in Table 2. First, the addition of cycloheximide at a concentration which almost totally inhibits protein synthesis in the preliminary incubation phase obliterates the increase usually seen. Second, actinomycin D significantly decreases the enhanced AIB uptake, but not to the same extent as cycloheximide. Third, the presence of 2 m*M* AIB or glycine in the medium during the hour at 37°C prior to measuring the uptake of [^{14}C]-AIB also eliminates the expected enhancement of uptake.

Our results lead us to believe there is an adaptive control mechanism in newborn rat kidney cortex expressed on incubation of the tissue at 37°C, and that this mechanism can regulate the entry of certain amino acids into tubule cells. On the basis of the assumed actions of cycloheximide and actinomycin D, it is reasonable to conclude that protein synthesis is involved both at the transcription and translational levels. The exogenous effects of amino acids suggest that a derepression-repression mechanism may be operative. It may be inferred from our findings that the synthesis of new "carrier" proteins takes place and that continued examination of the system could lead to identification of these proteins.

The enhancement of amino acid uptake by prior incubation in buffer at 37°C has been reported for chick embryo heart cells (*5*), immature rat uterus (*6*), and human placenta (*7*). The amino acids whose uptake is increased belong to a group with common transport characteristics designated by Oxender and Christensen (*8*) as the A system. It seems that the effect is a general property of embryonic, immature, or developing tissue which our results indicate is not present in tissues from the adult animal. The finding that the uptake of L-valine and L-lysine is diminished by prior incubation underscores the separate nature of the transport processes for various amino acids and indicates the presence of regulatory mechanisms other than those described here for α-aminoisobutyric acid, cycloleucine, glycine, and L-proline.

Table 1. The effect of preliminary incubation (PI) at 37°C on subsequent uptake of various amino acids and α-methyl-D-glucoside by newborn rat kidney cortex slices. To measure uptake, three newborn kidney cortex slices (5 to 10 mg) were incubated at 37°C for 1 hour in Krebs-Ringer bicarbonate buffer, *p*H 7.35, containing ^{14}C-labeled (0.1 μc/ml) 0.065 m*M* amino acid or 2 m*M* α-methyl-D-glucoside after which the distribution ratio, the ratio of the number of counts per minute per milliliter of intracellular fluid to that per milliliter of medium, was determined. For preliminary incubation, the tissue was placed in medium without substrate and incubated 1 hour at 37°C. At that time the flasks were opened and the radioactive substrates were added for measurement of uptake. The distribution ratios represent the means ± S.E. of six determinations.

Substrate	Distribution ratio	
	No PI	PI
α-Aminoisobutyric acid	6.87 ± 0.71	11.85 ± 0.77*
Cycloleucine	2.84 ± 0.11	4.96 ± 0.54†
Glycine	8.18 ± 0.38	10.18 ± 0.23*‡
L-Proline	5.57 ± 0.43	8.21 ± 0.53†
L-Valine	5.60 ± 0.53	3.51 ± 0.41§
L-Lysine	5.78 ± 0.42	4.08 ± 0.47‖
α-Methyl-D-glucoside	1.26 ± 0.06	1.27 ± 0.13

* $P < .001$ higher. † $P < .01$ higher. ‡ Only five determinations. § $P < .01$ lower. ‖ $P < .05$ lower.

Table 2. Effect of addition of cycloheximide, actinomycin D, and amino acids during preliminary incubation on the uptake of α-aminoisobutyric acid (AIB). The incubation conditions were as described for Table 1, except that after the preliminary incubation in buffer at 37°C the tissues were removed, washed in 0.9 percent saline, and placed in new flasks containing the radioactive substrates for 1 hour to measure uptake. The distribution ratio is the mean ± S.E. of eight determinations.

Substance added	Concentration (μ*M*)	Distribution ratio
None	0	12.80 ± 0.62
None (no preincubation)	0	6.48 ± 0.48*
Cycloheximide	50	5.91 ± 0.32*
Actinomycin D	8	9.06 ± 0.48†
AIB	5	6.40 ± 0.92*
Glycine	5	9.18 ± 0.93‡
Proline	5	6.08 ± 0.47*

* $P < .001$. † $P < .01$. ‡ $P < .02$.

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