Yale University
EliScholar – A Digital Platform for Scholarly Publishing at Yale

Yale Medicine Thesis Digital Library | School of Medicine

1970

Effects of tissue and media amino acid pools on transport of amino acids by rat kidney cortex slices

William G. Koehne
Yale University

Follow this and additional works at: http://elischolar.library.yale.edu/ymtdl

Recommended Citation

Koehne, William G., "Effects of tissue and media amino acid pools on transport of amino acids by rat kidney cortex slices" (1970). *Yale Medicine Thesis Digital Library*. 2809.
http://elischolar.library.yale.edu/ymtdl/2809

This Open Access Thesis is brought to you for free and open access by the School of Medicine at EliScholar – A Digital Platform for Scholarly Publishing at Yale. It has been accepted for inclusion in Yale Medicine Thesis Digital Library by an authorized administrator of EliScholar – A Digital Platform for Scholarly Publishing at Yale. For more information, please contact elischolar@yale.edu.

T113
+Y12
3058

YALE UNIVERSITY LIBRARY
3 9002 06584 5381

EFFECTS OF TISSUE AND MEDIA AMINO ACID POOLS ON TRANSPORT OF AMINO ACIDS BY RAT KIDNEY CORTEX SLICES

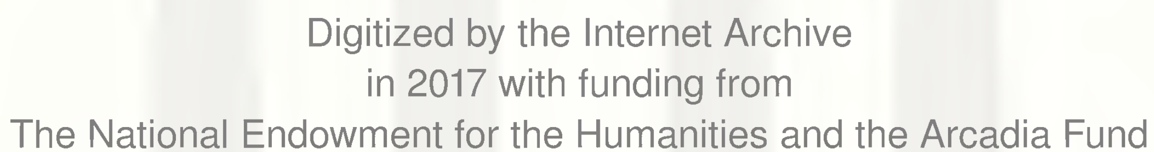
WILLIAM GAYLE KOEHNE

1970

MUDD
LIBRARY
Medical

YALE

MEDICAL LIBRARY


Digitized by the Internet Archive
in 2017 with funding from
The National Endowment for the Humanities and the Arcadia Fund

https://archive.org/details/effectsoftissuem00koeh

[illegible]

A Thesis

Presented to

the Faculty of the School of Medicine

Yale University

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Medicine

by

William [illegible]

April, [illegible]

EFFECTS OF TISSUE AND MEDIA AMINO ACID POOLS ON TRANSPORT OF AMINO ACIDS BY RAT KIDNEY CORTEX SLICES

A Thesis

Presented to

the Faculty of the School of Medicine

Yale University

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Medicine

by

William G. Koehne

April, 1970

YALE MEDICAL
JUN 1970
LIBRARY

Permission for microfilming of "Effects of Tissue and Media Amino Acid Pools on Transport of Amino Acids by Rat Kidney Cortex Slices" is hereby granted by the author.

ACKNOWLEDGMENTS

Any endeavor such as a thesis can rarely be attributed to one person. This study reflects the influences and concerns of many individuals. Most deeply and most directly, I am indebted to the following people:

Dr. Leon Rosenberg, whose numerous suggestions, wise counsel, and stimulus to scholarship have contributed so much to this study, and to my own personal and professional growth.

Joseph Durant and Isadora Albrecht, two colleagues and friends who by their example and willingness to share taught me that much of the success of laboratory research is dependent upon breadth of knowledge as well as rigorous performance of the task.

Dr. Louis Elsas, who offered thoughtful encouragement and constructive criticism throughout this study.

Dr. Jerry Mahoney, who contributed unselfishly of his time and expertise in assisting me to collect and interpret much of the data in this study.

My wife, Nancy, and daughter, Shannon, who shared the joys and frustrations of the past four years and provided, by their devotion, understanding, and support, a real reason to strive for the best I could offer.

W.G.K.

ABSTRACT

This study investigated changes in tissue and media amino acid pools during the course of incubation of rat kidney cortex slices in Krebs-Ringer bicarbonate buffer and in Krebs-Ringer buffer supplemented with plasma concentrations of amino acids, focusing on influences which these pools exert on transport of amino acids by the tissue. The tissue amino acid pool was found to be maintained at a more physiologic level when kidney slices were incubated in "amino acid buffer," although incubation of tissues in either this buffer or Krebs-Ringer buffer resulted in a substantial loss of amino acids from tissue to medium during the course of incubation. The predominant amino acids lost from the tissue pool during incubation were glutamic acid and glycine, the two amino acids present in highest concentrations in tissues prior to incubation. Transport of α-aminoisobutyric acid (AIB), glycine, diaminobutyric acid (DAB), and lysine by kidney slices was significantly reduced when the uptake studies were carried out in amino acid buffer. The effect was shown for AIB to be due to competitive inhibition, confirming previous observations that amino acids composing the media pool may influence transport phenomena by competing with similar amino acids for transport. Reduced AIB uptake following prolonged preincubation of tissues either in amino acid buffer or in the presence of high concentrations of AIB suggested that the tissue pool may also influence amino acid transport, perhaps by means of feedback regulation as previously postulated in a bacterial system but heretofore undescribed in a mammalian tissue.

TABLE OF CONTENTS

Page

INTRODUCTION.. 1

METHODS AND MATERIALS............................... 12

RESULTS... 19

Amino Acid Composition of Rat Plasma.............................. 20

Changes in Tissue and Media α-Amino Nitrogen Pools during Incubation of Rat Kidney Slices.............................. 20

Changes in Concentrations of Individual Amino Acids Composing the Tissue Pool During the Course of Incubation................ 22

Transport of AIB, Glycine, DAB, and Lysine in the Two Buffers...................... 23

Michaelis-Menten Kinetics of AIB and DAB Transport in the Two Buffers............... 25

AIB Transport Following Prolonged Preincubation of Tissues in Amino Acid Buffer or in the Presence of 10 mM AIB......... 26

DISCUSSION.. 30

TABLES.. 40

I. Amino Acid Concentrations in Rat Plasma and in Amino Acid Buffer.......... 41

II. Composition of the Tissue Amino Acid Pool at Various Incubation Times.................................. 42

III. Effect of Preincubation in Amino Acid Buffer on AIB Transport............. 43

IV. Effect of Prolonged Preincubation in Amino Acid Buffer on AIB Transport.............................. 44

V. Effect of Prolonged Preincubation in 10 mM AIB on AIB Transport............. 44

VI. Effect of Duration of Preincubation in 10 mM AIB on AIB Transport............. 45

VII. Changes in Tissue and Media α-Amino Nitrogen Pools Following Transfer of Tissues From Preincubation Buffer to Incubation Buffer...................... 46

VIII. AIB Transport in Differing Media.......... 47

IX. Ratios of Individual Amino Acids in Tissue and Plasma...................... 48

FIGURES.. 49

1. Tissue α-Amino Nitrogen Pool During the Course of Incubation of Rat Kidney Cortex Slices in Krebs-Ringer Buffer or Amino Acid Buffer............... 50

2. Media α-Amino Nitrogen Pool During the Course of Incubation of Rat Kidney Cortex Slices in Krebs-Ringer Buffer or Amino Acid Buffer............... 52

3. Time Course of AIB Uptake by Rat Kidney Cortex Slices Incubated in Krebs-Ringer Buffer or Amino Acid Buffer................................ 54

4. Time Course of Glycine Uptake by Rat Kidney Cortex Slices Incubated in Krebs-Ringer Buffer or Amino Acid Buffer................................ 56

5. Time Course of DAB Uptake by Rat Kidney Cortex Slices Incubated in Krebs-Ringer Buffer or Amino Acid Buffer............... 58

6. Time Course of Lysine Uptake by Rat Kidney Cortex Slices Incubated in Krebs-Ringer Buffer or Amino Acid Buffer................................ 60

7. Michaelis-Menten Kinetics of AIB Transport by Rat Kidney Cortex Slices Incubated in Krebs-Ringer Buffer or Amino Acid Buffer........................ 62

BIBLIOGRAPHY.. 64

INTRODUCTION

The cell membrane is known to be more than an inactive barrier separating vital intracellular components from the surrounding extracellular fluid. It is now recognized as a highly developed structure having the unique ability to mediate, in a selective fashion, the movement of organic and inorganic substances between the cell exterior and the cell interior. Knowledge about the mechanism of this mediation has been accumulating rapidly during the past few years, but interest in the problem dates back at least a century.

Tissue analyses carried out in the mid-nineteenth century revealed that sodium and calcium ions were largely extracellular in location, whereas potassium and magnesium were the principal intracellular cations. Liebig[1] noted in 1847 that muscle ash was rich in potassium and poor in sodium while the reverse was true in the blood. At about the same time Enderlin[2] reported that alkali phosphate exceeded alkali chloride in muscle. By 1875 Forster[3] had grouped tissue salts into either the "Gewebesalze" (potassium, magnesium, and phosphate) or the "Saftesalze" (sodium, chloride, and calcium).

A static placement or "fossilization" of the ions in one of the two compartments was assumed to explain these observations until labeled ions were first used to investigate the problem

thirty years ago. Cohn and Cohn[4] showed in 1939 that Na^{24} rapidly entered the erythrocytes of the intact dog, and two years later Harris[5] demonstrated a net exchange of sodium ions for potassium ions when erythrocytes were cooled or soon after they were depleted of glucose. These findings prompted Harris to comment:

> Plainly, the view that the erythrocyte membrane is impermeable to cations, the distribution of these ions being a static phenomenon, must be abandoned. In its place must be substituted a concept of normal membrane permeability to sodium and potassium, their concentrations in the cells being maintained by one or more metabolic functions of the cells.[5]

The manner in which amino acids distribute themselves between the cell interior and the surrounding environment is a more recent interest of biologists. Van Slyke was the first to demonstrate, in 1913, that animal tissues have a much higher concentration of free amino acids than does plasma.[6] Christensen *et al.*[7,8] reported in 1952 that all twenty-one amino acids they studied were concentrated by the Ehrlich mouse ascites tumor cell and that uptake of certain amino acids was inhibited by other amino acids of the same general class (neutral, cationic, or anionic). This prompted Christensen to postulate the presence of a trans-membrane "carrier" for amino acids:

> The evidence now seems very strong that the amino acids really are in a free form within the cell, or at least in a form with full osmotic activity and electric charge. Therefore, it seems necessary to assume that they exist in a modified form during transfer across the boundary. Presumably

> the amino acid reacts with a carrier to produce this modification. . . The inhibitory effects observed here are probably explained as competitions among the amino acids for the carrier, rather than as competitions between carrier and inhibitor for the amino acid to be transferred.[8]

Amino acid transport has now been studied in a number of *in vitro* systems including tumor cells[8,9], erythrocytes[10], various microorganisms[11], everted intestinal sac[12], isolated diaphragm muscle[13,14], brain slices[15], and kidney slices[16]. In general, the movement of amino acids across cell membranes in these various preparations has been found to be a mediated process, i.e. involving a reaction between the amino acid and a chemical structure or site present in a limited amount in the membrane[17]. Many aspects of mediated transport are similar to those found in enzyme-catalyzed reactions -- substrate specificity, saturation kinetics, responsiveness to competitive and noncompetitive inhibitors, etc. When the amino acid is transported against a chemical concentration gradient, the term "active transport" may be invoked, inasmuch as some force other than diffusion is required to maintain an asymmetric system[18]. This active process is energy-requiring and is presumably coupled with cellular metabolic processes.

This study will utilize a kidney slice preparation to investigate various features of amino acid transport. A brief

review of previous work in this system therefore seems in order. Kidney slices from the rabbit were introduced as a means of studying p-amino-hippurate transport by Cross and Taggart in 1950.[19] Rabbit kidney slices were subsequently employed by other investigators to study renal transport mechanisms for potassium[20] and galactose[21]; slices from guinea pig kidney were used to investigate both sodium and potassium transport[22]; and slices from a number of animals were used to study renal handling of uric acid.[23]

The system used in the present study, rat kidney cortex slices, was introduced by Rosenberg et al.[16] in 1961. Amino acid transport mechanisms in this tissue have been well characterized. In addition to many of the naturally occurring amino acids, at least two synthetic amino acids have been used extensively for kidney transport studies -- α-aminoisobutyric acid (AIB) and L-diaminobutyric acid (DAB). The former is a non-utilizable amino acid, first employed by Christensen, Noall, et al.[24], which is transported by kidney slices in much the same fashion as neutral amino acids but is neither metabolized nor incorporated into protein once within the cell. AIB is thus a valuable tool to dissociate transport phenomena from intracellular metabolic events. DAB serves somewhat the same function for the dibasic amino acids, being transported much like lysine. It is apparently not as metabolically inert as AIB, however, being oxidized significantly to carbon dioxide

although showing minimal incorporation into protein[25].

Amino acid transport in the rat kidney slice has been shown to be dependent on aerobic metabolism, temperature, and oxidative phosphorylation[16]. In addition, it exhibits the usual transport properties of substrate specificity[26,27], saturation kinetics at high substrate concentrations[16,26,27], and competitive inhibition among distinct groups of amino acids[26,27]. It is dependent on an adequate Na^+ concentration in the medium, with the transport of many amino acids being abolished in Na^+-free medium[28]. An interesting mathematical model has been designed by Rosenberg _et al._[29] to explain the kinetic data obtained from transport studies of AIB, glycine, and L-lysine in kidney slices. The model has three "parallel" compartments representing the medium, extracellular space, and intracellular space. It presumes that substrate molecules can enter the extra- and intra-cellular spaces simultaneously from the medium and need not traverse the extracellular space before entering the cellular compartment. Also, the exogenous amino acids apparently do not have to equilibrate with the intracellular pool before being incorporated into protein, but the oxidation of amino acids to carbon dioxide does seem to reflect their buildup in the intracellular pool.

Interest in the intracellular and extracellular amino

acid pools has been growing in recent years. One aspect of this interest has centered around the role which the pools might have in membrane transport of amino acids. The intracellular pool is basically determined by the equilibrium existing between amino acid influx and efflux on one hand and intracellular utilization of amino acids on the other. The former may be represented by passive diffusion, carrier mediated but energy independent transfer, and active transport. The latter consists of incorporation of amino acids into protein and metabolic degradation of amino acids. It now seems apparent that the intracellular pool is not homogeneous but is rather either structurally or functionally compartmentalized. It has been shown in single-cell systems[30,31] and in mammalian tissues including the kidney slice[29,32] that the pool is heterogeneous with respect to at least one cellular process, protein synthesis. Whether the protein synthesizing pool is structurally restricted to sites such as microsomes, nuclei, or mitochondria or whether it is only chemically differentiated by such mechanisms as selective activation or incorporation of amino acids into protein at the cell membrane is still undetermined.

Little quantitative information is available on the composition of the intracellular amino acid pool. Malathi

et al.[33] used a semi-quantitative paper chromatographic method to estimate the concentrations of amino acids in several rat tissues including kidney. It has been found that, in general, the intracellular patterns do not reflect the amino acid composition of plasma[34] and that the makeup of the intracellular pool is remarkably constant when changes take place in the external environment of the animal[35]. This is in contrast to the considerable variability found in the composition of the *plasma* amino acid pool in animals and man under a variety of conditions[36,37].

Influences of the intra- and extracellular pools on amino acid transport phenomena have thus far been investigated only in unicellular systems, with the exception of well defined competitive inhibitory effects observed between various amino acids when tissues are exposed to a medium containing both. That a transport carrier could be part of an inducible system was initially proposed in the remarkable work of Monod *et al.*[38] on the B-galactosidase system in *E. coli*. They theorized that this carrier, termed a "permease", was coded for by a distinct genetic locus in the lac operon and that the presence of galactose in the medium could "turn on" the permease locus so that it would begin coding for the carrier necessary to transport galactose into the cell. Several other inducible permease systems in a variety of

microorganisms have since been described[39-41]. Conversely, a sulfate transporting system has been characterized in *Salmonella typhimurium* which is sensitive to a *repressive* type of regulation by its end product, cystine[42]. In the realm of amino acid transport, Heinz has recently described a negative feedback control exerted by intracellular AIB on the AIB transport system in *Streptomyces hydrogenans*[43,44]. Heinz postulates that the accumulated substrate, AIB, can inhibit one of the first reactions in the transport process, perhaps the energy-requiring conversion of inactive to active carrier, thus exerting a regulatory influence at the level of the transport carrier rather than at the gene level. He notes that the numerous analogies between enzymatic reactions and transport processes seem to justify such a hypothesis[43].

It would seem important to extend to mammalian tissues these studies on the relationship between intra- and extra-cellular amino acid pools and membrane transport phenomena. The work reported in this paper is an attempt to do so in one specific system, the rat kidney cortex slice. An appealing feature of the kidney slice in this regard is that, while prepared by direct slicing of a mammalian organ, the tissue apparently consists almost entirely of renal tubules and has only minimal extraneous interstitial tissue[16]. It can therefore

be considered a tissue in which very reproducible data can be collected for transport by tubular cells, and yet the transport observations in this system can apparently be considered indicative of the manner in which these processes occur in the intact animal.

All previous studies of amino acid transport in rat kidney cortex slices have been carried out using a buffer which contains only one or two selected amino acids and appropriate concentrations of physiologic ions. To date no reports have appeared in which amino acid transport in kidney slices has been studied in a medium containing all the amino acids normally present in physiologic fluids. In addition, there has been no investigation of possible fluctuations in the tissue and media pools of amino acids during the time course of the transport studies or of the influences these pools may exert on transport processes. The present study was therefore designed to provide information on the following:

1) To characterize the transport of several amino acids by rat kidney cortex slices incubated in Krebs-Ringer bicarbonate buffer supplemented with plasma levels of amino acids.
2) To compare the changes in tissue and media amino acid pools during incubation of kidney slices in this buffer and in normal Krebs-Ringer buffer.

3) To study direct and indirect influences of the tissue and media amino acid pools on amino acid transport processes.

METHODS AND MATERIALS

Tissues obtained from male Sprague-Dawley rats were used for all phases of this study. Rats weighing 120-200 grams were fed water and Purina rat chow *ad libitum* until sacrificed by stunning and decapitation immediately prior to removal of the kidneys or collection of blood samples.

Preparation of an incubation buffer containing amino acids in concentrations comparable to those in rat plasma was the initial stage of this study. A pooled sample of blood from three rats was collected in a heparinized tube at the time of decapitation. The sample was centrifuged and the plasma deproteinized with 10% sulfosalicylic acid prior to quantitative analysis for amino acids on a Beckman Model 120C Amino Acid Analyzer using the method of Moore, Spackman, and Stein[45]. To ensure the accuracy of the amino acid values obtained from a single pooled plasma sample, a second pooled sample was obtained from two rats during the course of another experiment and analyzed in an identical manner. All amino acid concentrations from this sample were in close agreement with those previously determined. Using the quantitative plasma amino acid values obtained from the initial pooled sample, an aqueous solution of twenty amino acids was prepared. The amino acid concentrations in this solution were calculated such that when the solution was used in preparing Krebs-Ringer bicarbonate buffer a medium resulted which was identical with Krebs-Ringer except that it contained amino acids in conentrations comparable

to those found in rat plasma (Table I). This buffer was subsequently referred to as the "amino acid buffer".

The aqueous amino acid solution was freshly prepared at two-week intervals by quantitatively weighing crystalline preparations of the individual amino acids on a Mettler analytic balance and dissolving them in distilled water. The solution was then frozen until used.

For studies of amino acid pools and transport, both kidneys were quickly removed from the decapitated rat and placed in either cold Krebs-Ringer bicarbonate buffer or in the specially prepared Krebs-Ringer buffer containing plasma concentrations of amino acids. The kidneys were first bisected transversely, and then thin cortical slices (about 0.4 mm thick) were prepared with a Stadie-Riggs microtome. The initial (polar) slice from each hemi-kidney was discarded, and two or three slices containing only cortical tissue were obtained from each hemi-kidney. Slices were prepared from three animals in rapid succession. Groups of three slices (one from each animal) were transferred to a 25-ml Erlenmeyer flask containing 2.0 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, or to a comparable flask containing 2.0 ml of amino acid buffer, pH 7.4. For transport studies a tracer quantity of labeled substrate was present in the incubation flask. The flasks were gassed

with 95% O_2, 5% CO_2 for 20 seconds, sealed, and incubated for specific lengths of time in a Dubnoff metabolic shaker at 37.5°C.

Following incubation, the tissue slices were removed from the flask, dipped twice in isotonic saline to remove surface radioactivity, blotted, weighed on a torsion balance, and placed in 2.0 ml of distilled water in conical centrifuge tubes. The tubes were placed in beakers of boiling water for six minutes to allow complete equilibration of the tissue water with the suspending solution and to end the reaction. The tubes were then cooled and centrifuged.

Total tissue and media pools of free amino acids were estimated by measuring the concentration of α-amino nitrogen present in either an aliquot of the aqueous tissue supernate or an aliquot of the final medium. The α-amino nitrogen determinations provided an index of total free amino acids in the respective pools but did not distinguish individual amino acid forms. The method of Fisher, Bunting, and Rosenberg[46] was used for α-amino nitrogen determinations. The tissue α-amino nitrogen pool was expressed in mmoles per liter of total tissue water. To ensure that only the free α-amino nitrogen pool was being measured, certain tissues were homogenized in trichloroacetic acid and α-amino nitrogen determinations made on these tissue homogenates. Values obtained by

this method were in close agreement with those obtained by boiling the tissues.

In the transport studies, radioactivity was assessed by pipetting 0.2 ml of the aqueous tissue supernate or the remaining incubation medium into glass counting vials, followed by 2.8 ml of absolute ethyl alcohol and 7.0 ml of DPO-POPOP phosphor (0.457% 2,5 diphenyloxazole and 0.010% 1,4-bis-2-phenyloxazolyl-benzene in toluene). The vials were counted in a Packard Tricarb Liquid Scintillation Spectrometer.

Values for total tissue water (80.0% of the wet tissue weight) and extracellular space (25.7% of the wet weight) were taken from previous studies using the same tissue[47]. Uptake of labeled substrate was defined as the distribution ratio of cpm per ml of intracellular fluid to cpm per ml of incubation medium. The following formula was used to calculate the net cpm per ml of intracellular fluid, based on the fact that labeled substrate in the medium equilibrates rapidly with the extracellular fluid:

$$\text{Net counts/min/ml ICF} = \frac{(\text{net counts/min}) - (\text{counts/min/ml medium})(\text{ml ECF})}{(\text{ml water}) - (\text{ml ECF})}$$

Composition of the tissue amino acid pool was assessed first by semi-quantitative electrophoretic techniques and then more quantitatively by means of a Beckman Model 120C Amino Acid Analyzer. An aliquot of the aqueous supernate following boiling

and centrifuging of the tissue was used in both cases. This provided information on the composition of the "tissue pool" but did not differentiate intracellular from extracellular components. For semi-quantitative analysis a 30 µl aliquot of the supernate was spotted on Whatman #3MM paper prior to immersion in a Gilson Model D high voltage electrophorator containing 6.8% formic acid buffer for a two-hour unidimensional run at 2500 volts. The paper was then dried, sprayed with a 0.2% (w/v) solution of ninhydrin in acetone, heated, and compared visually with the amino acid pattern of a control solution run on the same paper. Following this initial screening, a 0.5 ml aliquot of the tissue supernate was analyzed on the Beckman Amino Acid Analyzer to obtain more precise quantitative information on the concentrations of various amino acids in the tissue pool. Concentrations of glutamine and asparagine could not be derived by this method since the two amino acids are inseparable on the Beckman Analyzer column with the sodium citrate buffer system used.

Chemicals used in this study included the following: α-Aminoisobutyric acid-1-C^{14} (specific activity 3.97 mC per mmole), glycine-2-C^{14} (specific activity 2.5 mC per mmole), and L-lysine-U-C^{14} (specific activity 126 mC per mmole) were obtained from New England Nuclear Corporation. Uniformly labeled L-2,4-diaminobutyric acid-U-C^{14} (specific activity

1.9 mC per mmole), unlabeled L-2,4-diaminobutyric acid, and unlabeled α-aminoisobutyric acid were purchased from Calbiochem. Samples of the unlabeled naturally occurring amino acids used in this study were obtained from Nutritional Biochemicals Company.

RESULTS

Amino Acid Composition of Rat Plasma

Concentrations of the free amino acids in rat plasma are shown in Table I. These values are similar to those reported by Scharff and Wool[48] in 400 gram Sprague-Dawley rats and by Schimassek and Gerok[49] in Wistar rats. A striking observation is the apparent absence of cystine in the plasma of young rats. Schimassek and Gerok similarly reported that cystine was "not measurable" in the plasma of young Wistar rats. Scharff and Wool, however, found a 0.061 mM concentration of cystine in plasma from older Sprague-Dawley animals.

Based on the data recorded in Table I, the amino acid buffer was prepared in such a way that it contained amino acids in the exact concentrations found in rat plasma. The concentrations of glutamine and asparagine, two amino acids which could not be measured by the technique used, were derived from composite data available on the concentrations of these amino acids in human plasma[50].

Changes in Tissue and Media α-Amino Nitrogen Pools during Incubation of Rat Kidney Slices

Tissues were incubated for varying lengths of time in either Krebs-Ringer buffer or amino acid buffer to provide preliminary information on changes in tissue and media amino

acid pools during the course of such an incubation. The pools were expressed in terms of their α-amino nitrogen content, an index of the total pool of free amino acids.

Changes in the tissue α-amino nitrogen pool during a 360-minute incubation are illustrated in Figure 1. When the slices were incubated in Krebs-Ringer buffer, the tissue α-amino nitrogen pool decreased markedly during the initial 60 minutes of incubation and continued to fall at a slower rate thereafter. Incubation in amino acid buffer resulted in a similar decrease in the tissue pool, but the pool was maintained at a concentration 6-8 mM greater in this buffer. An interesting observation was the significant ($p<0.01$) rise in the tissue pool between 15 and 30 minutes incubation for tissues incubated in Krebs-Ringer buffer, followed by an equally significant ($p<0.01$) fall by 60 minutes. Whether this effect represents re-uptake of amino acids initially lost from the tissue, a brief increase in protein catabolism to maintain the tissue amino acid pool, or some other phenomenon was not investigated further.

The media concentration of α-amino nitrogen increased almost linearly in the two buffers throughout the 360-minute incubation (Figure 2). There was no evidence of a leveling off in the size of the media pool at longer incubation times.

Changes in Concentrations of Individual Amino Acids Composing the Tissue Pool during the Course of Incubation

More extensive information on the composition of the tissue amino acid pool seemed desirable in order to compare it with the previously determine plasma pool and to evaluate fluxes in individual amino acids during the course of incubation of the tissue. As an initial screening procedure, the amino acid patterns of several tissue supernates prepared from kidney slices without incubation were examined by means of high voltage electrophoresis. Detectable amounts of glutamine, glycine, serine, and alanine were observed, together with traces of lysine and the branched-chain amino acids. The glutamine spot was significantly more prominent than the others.

Aqueous supernates from tissues incubated in Krebs-Ringer buffer for varying lengths of time were then analyzed quantitatively on the Beckman Amino Acid Analyzer. Certain tissues were quickly prepared, weighed, and placed in boiling water without exposure to buffer. These were labeled "direct from animal". Others were placed in cold Krebs-Ringer buffer for 8-10 minutes prior to weighing and boiling. These were labeled "0 minutes incubation". The remaining tissues were incubated for either 180 or 360 minutes. As shown in Table II, glutamic acid (15.90 mM) and glycine (11.15 mM) were found to be present

in the highest concentrations in the tissue pool. All other amino acids were present in less than 2.25 mM concentrations in tissues direct from the animal. Once again concentrations of glutamine and asparagine could not be obtained by the method used. It will be noted that the tissue concentrations of glutamic acid and glycine decreased dramatically during the course of 360 minutes incubation, while the concentrations of most other amino acids declined by 50% or less. Hydroxyproline, proline, and methionine showed marked fractional decreases in concentration but were initially present in rather small amounts. Tissue concentrations of ornithine, lysine, and histidine increased during the course of the incubation. An intriguing finding is the presence of measurable cystine in the tissue pool despite its apparent absence in plasma.

Transport of AIB, Glycine, DAB, and Lysine in the Two Buffers

Characteristics of the transport of four amino acids by rat kidney cortex slices in Krebs-Ringer or amino acid buffer was next investigated. Two neutral amino acids, α-aminoisobutyric acid (AIB) and glycine, as well as two dibasic amino acids, diaminobutyric acid (DAB) and lysine, were selected for these studies. Whereas the naturally occurring amino acids, glycine and lysine, are known to be metabolized after transport into the cell, AIB and DAB have been shown to be utilized only

minimally by the cell[24,25]. Tissues were incubated with the labeled substrate for varying lengths of time in either Krebs-Ringer or amino acid buffer and the resulting distribution ratios determined.

Time curves for the uptake of AIB, glycine, DAB, and lysine revealed reduced distribution ratios for each amino acid when incubation was carried out in amino acid buffer (Figures 3-6). The effect was seen within 10-15 minutes incubation for AIB and DAB and by 30 minutes for all four amino acids, suggesting that reduced influx rather than increased efflux was the phenomenon being observed. By 60 minutes incubation distribution ratios were reduced by 45% for AIB, 20% for glycine, 40% for DAB, and 10% for lysine.

To ascertain whether the reduced uptake was related to the presence of amino acids in the incubation buffer rather than to maintenance of higher tissue amino acid pools in those slices incubated in amino acid buffer, tissues were preincubated for 60 minutes in either Krebs-Ringer or amino acid buffer and then transferred to fresh buffer containing labeled substrate for a 15-minute incubation. The results, shown in Table III, reveal that the initial size of the tissue amino acid pool had no apparent effect on AIB transport. The effect seemed instead related to the presence of amino acids in the incubation buffer.

Michaelis-Menten Kinetics of AIB and DAB Transport in the Two Buffers

Kinetic studies were undertaken to further elucidate the characteristics of AIB and DAB transport in tissues exposed to a medium containing amino acids. Kidney slices were incubated in either Krebs-Ringer or amino acid buffer for 15 minutes in the presence of increasing concentrations of AIB or DAB. A 300-fold range of AIB concentrations and a 200-fold range of DAB concentrations were used. The final intracellular concentration of each amino acid was corrected for diffusion to obtain the final intracellular concentration attributable to mediated transport alone[14,16]. Uptake data were then plotted by the double reciprocal method of Lineweaver and Burk[51] to provide a means of determining Michaelis constants for the substrates under the two incubation conditions.

Curves for AIB uptake in the two buffers (Figure 7) had differing slopes but an identical ordinate intercept. The extrapolated abscissa intercepts, different for the two buffers, gave K_m values of 3.4 mM for Krebs-Ringer buffer and 12.5 mM for amino acid buffer. The former value agrees with that reported previously by other investigators[52,53]. No evidence was seen of the "high K_m system" for AIB transport in the kidney slice detected by Scriver and Mohyuddin[53]. Maximal velocity of AIB transport (V_{max}) in both buffers was calculated to be 3.3 mmoles

per liter per 15 minutes. The greater K_m for AIB transport in amino acid buffer with no difference in V_{max} implies that AIB transport is competitively inhibited when tissues are exposed to the spectrum of amino acids in this buffer.

DAB uptake in the two buffers showed no evidence of saturability even at 20 mM substrate concentrations, although distribution ratios were lower in amino acid buffer throughout the range of substrate concentrations used. It therefore appears that DAB transport in the kidney slice is handled by a system (or systems) of very high capacity. Previous studies of DAB transport in kidney slices have not included similar kinetic analysis, but it has been shown for a number of amino acids, including the naturally occurring dibasics, that steady state distribution ratios exceeding 1.0 are attained even at high substrate concentrations (15 mM)[25]. It was of course impossible to calculate Michaelis constants for DAB transport in the two buffers since a saturable transport component could not be demonstrated.

AIB Transport Following Prolonged Preincubation of Tissues in Amino Acid Buffer or in the Presence of 10 mM AIB

Following the above studies on influences which the media amino acid pool has on transport processes, it was deemed important to investigate the more subtle matter of effects

exerted by the tissue amino acid pool on uptake of amino acids by the tissue. In particular, the possibility of eliciting evidence of feedback regulation of membrane transport by intracellular amino acids, an effect previously observed in bacterial systems, seemed worthy of investigation in a mammalian tissue. The amino acid buffer proved to be a valuable tool in these studies since the tissue pool of free amino acids had previously been shown to be maintained at a higher level in tissues incubated in this buffer. The tissue pool could also be altered by preloading the kidney slice with one particular amino acid. Presumably, changes in the composition of the tissue amino acid pool might promote alterations in uptake of amino acids by the tissue if the pool did indeed exert regulatory effects on amino acid transport.

It was decided to study the 10-minute uptake of labeled AIB by tissues which had been preincubated for 180 minutes either in amino acid buffer or in Krebs-Ringer containing 10 mM AIB, using tissues preincubated in plain Krebs-Ringer as controls. A 180-minute duration for the preincubation was selected in view of data reported by Elsas and Rosenberg[52] which suggests that the catabolic "half-life" of the protein(s) postulated to be responsible for mediated transport of AIB in the rat kidney slice is about 3½ hours. Presumably, a repressive effect exerted by the tissue pool on the synthesis of this

protein would be more clearly revealed following a preincubation of at least 180 minutes. Preincubation in amino acid buffer or in the presence of 10 mM AIB led to reduced AIB uptake compared with control tissues (Tables IV-V). The effect was highly significant ($p < 0.01$) in both cases. To determine whether a similar effect could be observed following a briefer preincubation, certain tissues were preincubated in 10 mM AIB or in Krebs-Ringer for only 60 minutes while the remainder were preincubated for 180 minutes. Again, significantly reduced AIB uptake was noted in tissues exposed to high media concentrations of AIB during the 60-minute preincubation ($p < 0.01$), but the effect was not as marked as that observed following 180 minutes preincubation (Table VI).

To determine the extent to which the above findings might reflect differences in media amino acid pools in the incubation flasks as a result of rapid efflux of amino acids from tissues preincubated in amino acid buffer or 10 mM AIB, tissue and media α-amino nitrogen pools were assessed during the 10-minute incubation. As shown in Table VII, the concentration of amino acids in the tissue pool decreased by approximately 5 mM during the 10-minute period. Concentration of the media pool increased by 0.25 mM during the incubation if the tissues had been preincubated in Krebs-Ringer buffer and by 0.50 mM if the tissues had been exposed to amino acid buffer during the preincubation. It was deemed essential to demonstrate

conclusively whether the 0.25 mM difference in the media pools could account for the reduced AIB uptake noted in tissues preincubated in amino acid buffer. Ten-minute AIB uptake studies without preincubation were therefore performed in plain Krebs-Ringer buffer or in Krebs-Ringer containing amino acids in 1/10 or 1/20 the concentrations found in amino acid buffer (approximately 0.50 mM and 0.25 mM respectively). In addition, certain tissues were incubated in the presence of 0.32 mM AIB, the concentration of AIB expected in the incubation medium had the entire AIB content of the tissue pool (at 10 mM concentration) shifted from tissue to media following transfer of tissues from preincubation to incubation flasks. There was no difference in AIB uptake under any of these incubation conditions (Table VIII). Evidence, then, seems to indicate that the factors responsible for reduced amino acid transport are not present in the incubation medium but are instead related to a property of the tissue. The greater tissue amino acid pool known to exist in those slices which transport less substrate implies that the relative size of the tissue pool may be a regulatory influence on the amount of substrate transported by the tissue.

DISCUSSION

Transport of amino acids by rat kidney cortex slices has been considered indicative of the manner in which amino acids are transported in the intact kidney. The medium commonly used in transport experiments has, however, contained only one or two amino acids rather than the full spectrum of amino acids normally filtered by the glomeruli and reabsorbed in the renal tubules. Incubation of kidney slices in a medium containing plasma conentrations of amino acids would seem to more closely approximate physiologic conditions, and use of this medium for transport studies might provide a more accurate indication of transport processes as they occur in the kidney *in vivo*. Preparation and utilization of such a buffer to investigate influences of tissue and media amino acid pools on transport processes were the goals of this study.

The concentrations of various amino acids in rat plasma as determined in this study are in close agreement with values found by other investigators in rats of different age or strain[48,49]. Discrepancies are perhaps related not only to age or strain differences but also to differences in the time of blood sampling in view of a recent report noting significant diurnal variations in the plasma concentrations of most amino acids in man[37]. The absence of measurable cystine in rat plasma, while agreeing with a previous report[49], is somewhat

surprising when contrasted with the small but quantifiable amount of cystine detected in an analysis of the tissue amino acid pool. It is probably worth noting in this regard that only ornithine was present in the tissue pool in a concentration smaller than that of cystine. Perhaps the minute concentration of plasma cystine required to maintain renal tissue pools in the range of 0.36 mM is undetectable even by the sensitive analytic technique used in this study.

Heterogeneity of the "tissue amino acid pool" complicates interpretation of the quantitative data on concentrations of amino acids in the tissue. Not only does such a pool consist of those amino acids free in the extracellular fluid and in the intracellular fluid but also may encompass several compartmentalized pools within the intracellular space. It may be useful for analytic purposes to assume that the extracellular amino acid pool reflects quite closely the relative amino acid pattern found in plasma. Coupling this supposition with the documented fact that the ICF includes 75% of the tissue wet weight, it is apparent that the "tissue pool" reflects predominantly the pattern of amino acids found intracellularly. A striking observation, then, is the variability between the relative concentrations of amino acids in plasma and in the tissue pool (Table IX). Whereas a significant

gradient exists for certain amino acids such as glutamic acid (0.18 mM in plasma, 15.9 mM in the tissue pool), other amino acids such as arginine show only a small gradient (0.22 mM in plasma, 0.27 mM in the tissue pool). In general, the concentrations of glutamic acid, aspartic acid, glycine, and methionine are vastly greater in the tissue pool than in plasma, while the tissue/plasma ratios of most other amino acids range only from about 3/1 to 6/1. Scharff and Wool[48] reported a similar wide variability in the relative tissue/plasma concentrations of individual amino acids in rat diaphragm and heart muscle. They also noted the gradient to be greatest for aspartic acid and glutamic acid.

It is interesting to compare the ratio of tissue/plasma concentrations of amino acids with ICF/ECF distribution ratios obtained for the same amino acids in transport studies using labeled substrate. Glycine, for example, attains a steady state distribution ratio of only 6.0 to 7.0 in uptake studies in the kidney slice[16], yet this amino acid was shown to have a tissue/plasma gradient of 19.6 in tissues direct from the animal (Table IX). After 180 minutes incubation the concentration of glycine in the tissue pool had decreased to the extent that the tissue/plasma ratio was only about 4.1. It may be more appropriate to use the 180 minute tissue/plasma ratio for

comparison since glycine is known to attain a steady state distribution ratio rather slowly. A glycine tissue/plasma ratio of 4 or 5 would, then, agree rather well with the ICF/ECF distribution ratio of labeled substrate reported for this amino acid. Similar reasoning reveals that tissue/plasma ratios for lysine (3.1) and proline (1.6) at 180 minutes incubation closely approximate their reported ICF/ECF steady state distribution ratios (3.0 and 2.3 respectively[25,54]). In general, the distribution ratios of those amino acids whose transport has been studied in the rat kidney slice do not differ markedly from their respective tissue/plasma ratios, as reported in the present investigation.

The finding that the tissue pool of free amino acids is maintained at a slightly higher concentration when tissues are incubated in amino acid buffer is encouraging evidence that, in at least one respect, use of this buffer provides a more physiologic environment for the tissue. A substantial loss of tissue amino acids occurs even in this buffer, however, presumably as an inevitable consequence of rupturing and traumatizing renal cells in the slicing process.

Quantitative data on changes in the composition of the tissue pool during prolonged incubation in Krebs-Ringer

buffer revealed that those amino acids present in the highest concentrations in tissues taken directly from the animal showed the greatest decrease in concentration during the course of incubation. The concentration of glutamic acid, originally 15.90 mM in the tissue pool, fell to 1.96 mM after 180 minutes incubation and remained relatively constant thereafter. The concentration of glycine, 11.15 mM in tissues direct from the animal, decreased to 2.32 mM at 180 minutes and also stabilized at that point. It is perhaps important to note that glutamic acid and glycine are two of the amino acids whose concentrations in the tissue pool far exceed their plasma concentrations. Disruptions in normally functioning transport processes caused by slicing and traumatizing the renal tissue might therefore be reflected rapidly and dramatically as a decrease in the steep tissue/plasma gradients of these two amino acids. In addition, glutamic acid and glycine are among the amino acids most actively involved in intracellular metabolic processes. Such processes might, over the course of 180 minutes, promote a more striking decrease in the intracellular concentrations of glutamic acid and glycine than in the concentrations of most other amino acids.

The marked decrease in the tissue amino acid pool observed over the course of 180 minutes incubation was followed by little subsequent decline from 180 to 360 minutes. This

relative stability of the tissue pool after 180 minutes exposure of the tissue to a buffered medium suggests that advantages might be derived by conducting amino acid transport studies after such a steady state has been attained. The flux of amino acids from tissue to medium prior to that time undoubtedly has some unassessed impact on uptake of amino acids by the tissue. Transport of glutamic acid and glycine would seem most likely affected by such spontaneous changes in the composition of the tissue pool since these are the amino acids subject to the greatest and most rapid decrease in tissue concentration.

Transport of two neutral amino acids, glycine and AIB, and of two dibasic amino acids, lysine and DAB, has been shown to be greatly influenced by the size of the media amino acid pool. Incubation of slices in a medium whose amino acid composition simulates that of plasma was shown to lead to a significant decrease in glycine, AIB, lysine, and DAB uptake by the tissue. This is perhaps not surprising in view of the well documented competition for transport sites among various groups of amino acids.[26,27] Indeed, it was clearly demonstrated in this study that AIB uptake was competitively inhibited when tissues were incubated in amino acid buffer. An attempt was made to illustrate the same effect for DAB, but failure to elicit saturability of the DAB transport system even at 20 mM substrate

concentrations prevented kinetic analysis of the uptake data. It seems reasonable to assume, however, that competitive inhibition accounted for the decreased transport of all four substrates in amino acid buffer. The degree of inhibition of transport for a given amino acid may be related to the relative concentrations in amino acid buffer of those amino acids sharing transport sites with the labeled substrate and which therefore actively compete with it for transport.

This study presents the first evidence that the tissue amino acid pool may exert effects on amino acid transport more subtle than those exerted by the media pool. It is postulated that one such effect may involve a feedback control mechanism, similar to that suggested in a bacterial system[43,44] but heretofore undescribed in a mammalian tissue. Markedly reduced AIB uptake was observed in tissues which had been preincubated for 180 minutes either in amino acid buffer or in the presence of a high concentration of AIB, two conditions which are known to alter the composition of the tissue amino acid pool. Furthermore, it was found that the reduction in AIB uptake under the latter condition appeared related to the duration of preincubation in AIB, the effect being more striking following 180 minutes preincubation than after 60 minutes preincubation. Whether such reduced uptake is due to control exerted by a

greater tissue amino acid pool cannot be answered conclusively from data obtained in this study. It does seem apparent, however, that the regulatory influence resides within the tissue since no factor was detected in the media to account for the findings. The effect being observed differs significantly from that of exchange diffusion as reported for dibasic amino acid transport in the kidney slice by Schwartzman, Blair, and Segal[55] since preloading the tissue with an amino acid involved in exchange diffusion leads to an increase rather than a decrease in initial uptake of that or a similar amino acid. The possibility of generalized tissue damage or metabolic derangement following prolonged exposure to amino acid buffer or high concentrations of AIB, effects which may lead to lowered amino acid transport, has not been ruled out by this study. With this in mind, it would seem important to investigate the transport of amino acids other than AIB following prolonged preincubation to assess the specificity of the observed effect. Further, if a regulatory influence of the tissue pool is indeed responsible for the alteration in amino acid uptake, the site of such regulation should be sought. In view of the apparent dependence of the magnitude of reduction in AIB uptake on the duration of pre-incubation, with the effect being more striking following longer exposure of the tissue to a high concentration of AIB, repression of synthesis of a protein(s) responsible for AIB

transport should perhaps be considered a potential site for such regulation.

Tissue and media amino acid pools have been shown in this study to be a major influence on amino acid transport in the rat kidney cortex slice. The two pools were demonstrated to change in composition and relative size during the course of incubation, with the magnitude of such changes being governed at least partially by the nature of the incubation buffer. More important, evidence was presented indicating direct and indirect effects which amino acids in the pools exert on the amount of substrate taken up by the tissue. An all too common practice has been to view amino acid transport as a rather isolated event involving mediated movement of an amino acid across a cell membrane. This study will have contributed significantly to an understanding of transport phenomena if it merely creates an increased awareness of the importance of the pools of amino acids residing on either side of the cell membrane.

TABLES

TABLE I

Amino Acid Concentrations in Rat Plasma and in Amino Acid Buffer

Amino Acid	Rat Plasma mg/100ml	Amino Acid Buffer mg/100ml	mM
Hydroxyproline	1.4	1.4	0.11
Aspartic acid	0.6	0.6	0.05
Threonine	4.7	4.7	0.39
Serine	3.3	3.3	0.31
Glutamine	9.7	9.7	0.66
Proline	3.9	3.9	0.34
Glutamic acid	2.7	2.7	0.18
Glycine	4.3	4.3	0.57
Alanine	5.3	5.3	0.59
Valine	3.0	3.0	0.26
Cystine	0.0	0.0	0.00
Methionine	0.9	0.9	0.06
Isoleucine	1.2	1.2	0.09
Leucine	2.3	2.3	0.18
Tyrosine	1.9	1.9	0.10
Phenylalanine	1.1	1.1	0.07
Ornithine	1.0	1.0	0.08
Lysine	8.0	8.0	0.55
Histidine	1.1	1.1	0.07
Arginine	3.9	3.9	0.22
Asparagine	0.6	0.6	0.05
			4.93 mM

Concentrations of amino acids in rat plasma were assessed by analysis of an aliquot of deproteinized plasma pooled from three animals. A Beckman Model 120C Amino Acid Analyzer was used for the determinations. Amino acid buffer was then prepared so that it contained concentrations of individual amino acids exactly the same as those found in the pooled plasma sample.

TABLE II

Composition of the Tissue Amino Acid Pool at Various Incubation Times

Amino Acid	Direct from Animal	0 min.	180 min.	360 min.
Hydroxyproline	0.46 mM	0.42 mM	0.00 mM	0.00 mM
Aspartic acid	2.00	1.18	1.67	1.32
Threonine	1.07	0.79	0.62	0.61
Serine	1.72	1.13	1.04	1.04
Proline	1.32	0.64	0.54	0.31
Glutamic acid	15.90	10.62	1.96	2.00
Glycine	11.15	6.88	2.32	2.91
Alanine	2.25	2.29	1.16	1.12
Valine	0.72	0.57	0.54	0.58
Half cystine	0.36	0.25	Trace	0.15
Methionine	0.97	0.64	0.27	0.07
Isoleucine	0.39	0.39	0.36	0.39
Leucine	1.14	0.79	0.74	0.73
Tyrosine	0.57	0.39	0.42	0.34
Phenylalanine	0.43	0.27	0.39	0.32
Ornithine	0.00	0.00	Not analyzed	0.06
Lysine	1.03	1.19		1.73
Arginine	0.27	0.35		0.16
Histidine	0.31	0.35		0.48

Rat kidney cortex slices were incubated in Krebs-Ringer bicarbonate buffer for the designated length of time. An aliquot of aqueous tissue extract was then analyzed on a Beckman Model 120C Amino Acid Analyzer. Concentrations of individual amino acids are expressed as mmoles per liter of total tissue water. One tissue was analyzed at each incubation time. The terms "direct from animal" and "0 minutes incubation" are explained in the text.

TABLE III

Effect of Preincubation in Amino Acid Buffer on AIB Transport

Preincubation Buffer	Incubation Buffer	Distribution Ratio
Krebs-Ringer	Krebs-Ringer	4.45
Krebs-Ringer	Amino Acid	2.74
Amino Acid	Krebs-Ringer	4.85
Amino Acid	Amino Acid	2.55

Tissues were preincubated in the designated buffer for 60 minutes and then transferred to different flasks containing fresh buffer and AIB-C^{14} (0.06 mM) for a 45-minute uptake study. Each distribution ratio represents the mean of 4 observations. Distribution ratio is defined as the ratio of cpm per ml of intracellular fluid to cpm per ml of incubation medium.

TABLE IV

Effect of Prolonged Preincubation in Amino Acid Buffer on AIB Transport

Preincubation Condition	Distribution Ratio
Krebs-Ringer	2.25
Amino Acid Buffer	1.73

Tissues were preincubated for 180 minutes in either Krebs-Ringer or amino acid buffer and then transferred to different flasks containing fresh Krebs-Ringer buffer and 0.1 mM AIB-C^{14} for a 10-minute uptake study. Each distribution ratio represents the mean of seven observations. The "p" value for the difference in distribution ratios is <0.01 (Student's "t" test).

TABLE V

Effect of Prolonged Preincubation in 10 mM AIB on AIB Transport

Preincubation Condition	Distribution Ratio
Krebs-Ringer	2.84
10 mM AIB	1.44

Tissues were preincubated for 180 minutes in Krebs-Ringer buffer with or without 10mM AIB and then transferred to different flasks containing fresh buffer with 0.1 mM AIB-C^{14} for a 10-minute uptake study. Each distribution ratio represents the mean of four observations. The "p" value for the difference in distribution ratios is < 0.01 (Student's "t" test).

TABLE VI

Effect of Duration of Preincubation
in 10 mM AIB on AIB Transport

Duration of Preincubation	Preincubation Condition	Distribution Ratio
180 Minutes	Krebs-Ringer	2.09
	10 mM AIB	1.05
60 Minutes	Krebs-Ringer	2.00
	10 mM AIB	1.42

Tissues were preincubated for either 60 minutes or 180 minutes in Krebs-Ringer buffer with or without 10 mM AIB and then transferred to different flasks containing fresh Krebs-Ringer buffer with 0.1 mM AIB-C^{14} for a 10-minute uptake study. Each distribution ratio represents the mean of three or four observations. The "p" values for differences in distribution ratios is < 0.01 (Student's "t" test) for both preincubation times.

TABLE VII

Changes in Tissue and Media ∝-Amino Nitrogen Pools Following Transfer of Tissues From Preincubation Buffer to Incubation Buffer

Tissue Pool -

Preincubation Condition	Following 180-Min. Preincub.	Following 180-Min. Preincubation + 10-Min. Incubation
Krebs-Ringer	29.0 mM	25.5 mM
Amino Acid Buffer	33.6 mM	28.6 mM

Media Pool (Incubation Flasks) -

Preincubation Condition	Following 180-Min. Preincub.	Following 180-Min. Preincubation + 10-Min. Incubation
Krebs-Ringer	0.00 mM	0.25 mM
Amino Acid Buffer	0.00 mM	0.55 mM

Tissues were preincubated for 180 minutes in either Krebs-Ringer or amino acid buffer and then transferred to different flasks containing fresh Krebs-Ringer buffer for a 10-minute incubation without substrate. Tissue ∝-amino nitrogen concentrations are expressed as mmoles per liter of total tissue water. Media concentrations are expressed as mmoles per liter of the appropriate media. Each value represents the mean of three observations.

TABLE VIII

AIB Transport in Differing Media

Incubation Condition	Distribution Ratio
Krebs-Ringer	1.03 ± 0.07
0.32 mM AIB	0.92 ± 0.09
Amino Acids (0.50 mM)	0.95 ± 0.10
Amino Acids (0.25 mM)	1.09 ± 0.07

Tissues were incubated for 10 minutes in Krebs-Ringer buffer or in Krebs-Ringer buffer containing the designated concentration of amino acid. Substrate was AIB-C^{14} 0.1 mM. Each distribution ratio represents the mean of four observations ± S.E.

TABLE IX

Ratios of Individual Amino Acids in Tissue and Plasma

Amino Acid	Tissue/Plasma Ratio
Glutamic acid	15.90/0.18 = 88.3
Aspartic acid	2.00/0.05 = 40.0
Glycine	11.15/0.57 = 19.6
Methionine	0.97/0.06 = 16.1
Leucine	1.14/0.18 = 6.3
Phenylalanine	0.43/0.07 = 6.1
Tyrosine	0.57/0.10 = 5.7
Serine	1.72/0.31 = 5.5
Histidine	0.31/0.07 = 4.4
Isoleucine	0.39/0.09 = 4.3
Hydroxyproline	0.46/0.11 = 4.2
Proline	1.32/0.34 = 3.9
Alanine	2.25/0.59 = 3.8
Valine	0.72/0.26 = 2.8
Threonine	1.07/0.39 = 2.7
Lysine	1.03/0.55 = 1.9
Arginine	0.27/0.22 = 1.2
Ornithine	0.00/0.08 = 0.0
Half cystine	0.36/0.00 = --

Tissue amino acid concentrations are expressed as mmoles per liter of total tissue water (see Table II). Plasma concentrations are in mmoles per liter of rat plasma (Table I).

FIGURES

Figure 1 --- Changes in the tissue α-amino nitrogen pool during the course of 360 minutes incubation of rat kidney cortex slices in either Krebs-Ringer or amino acid buffer. Tissue α-amino nitrogen content, expressed in mmoles per liter of total tissue water, was used as an index of the concentration of free amino acids in the tissue pool. Each point from 0 to 180 minutes incubation represents the mean of 7 or 10 observations ± S.E., while points at 270 and 360 minutes incubation represent the mean of 4 observations ± S.E. The value at 30 minutes incubation in Krebs-Ringer buffer is significantly greater ($p < 0.01$) than values at either 15 or 60 minutes.

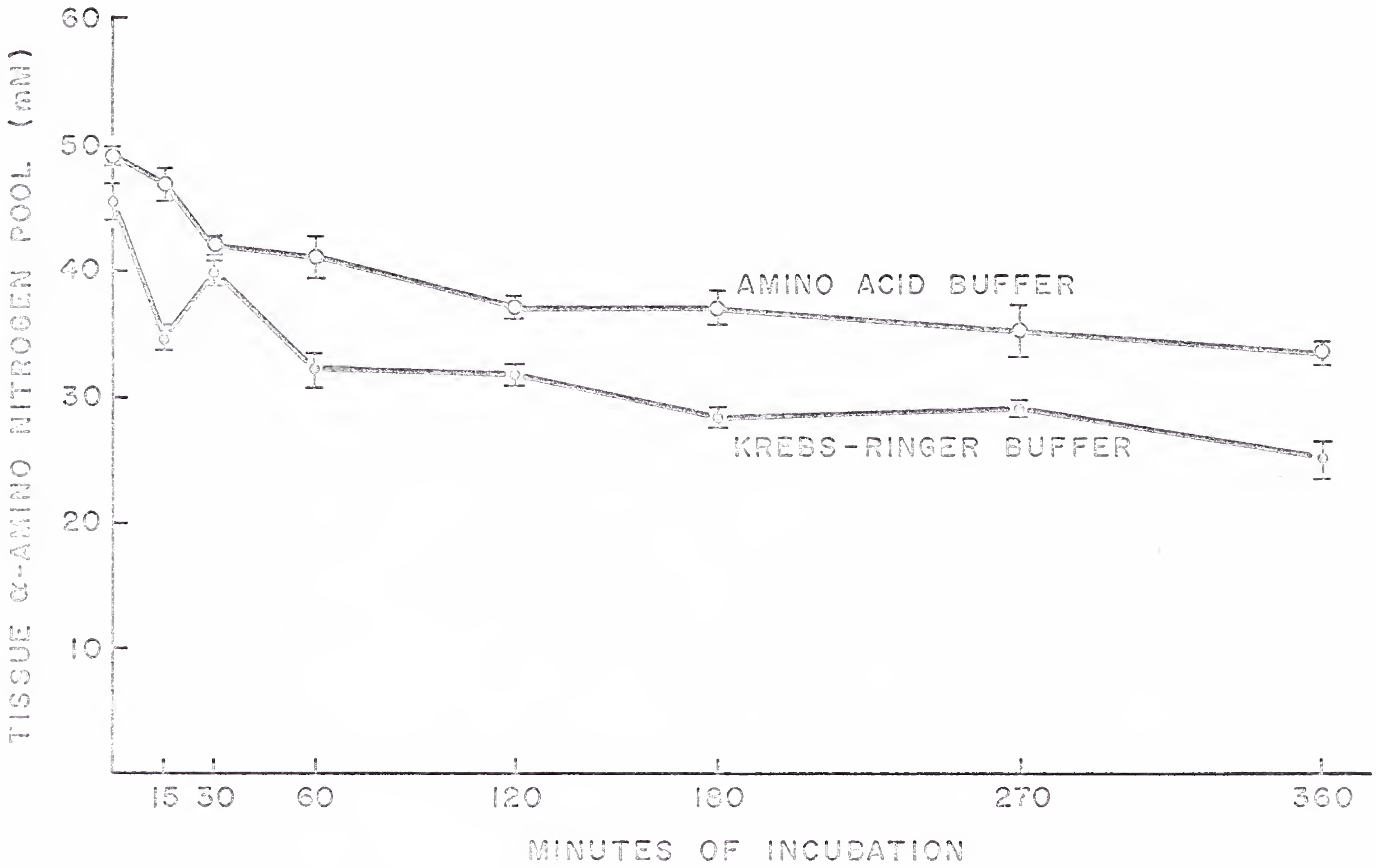

TISSUE α-AMINO NITROGEN POOL (mM)
60
50
40
30
20
10
AMINO ACID BUFFER
KREBS-RINGER BUFFER
15
30
60
120
180
270
360
MINUTES OF INCUBATION

Figure 2 -- Changes in the media α-amino nitrogen pool during the course of 360 minutes incubation of rat kidney cortex slices in either Krebs-Ringer or amino acid buffer. Media α-amino nitrogen concentration, expressed in mmoles per liter of final medium, was used an an index of the total concentration of free amino acids present in the media at various incubation times. Each point from 15 to 180 minutes incubation represents the mean of 7 or 10 observations ± S.E., while points at 270 and 360 minutes incubation represent the mean of 4 observations ± S.E. α-amino nitrogen concentration of the initial amino acid buffer, representing the mean of 4 observations ± S.E., is plotted at 0 minutes incubation.

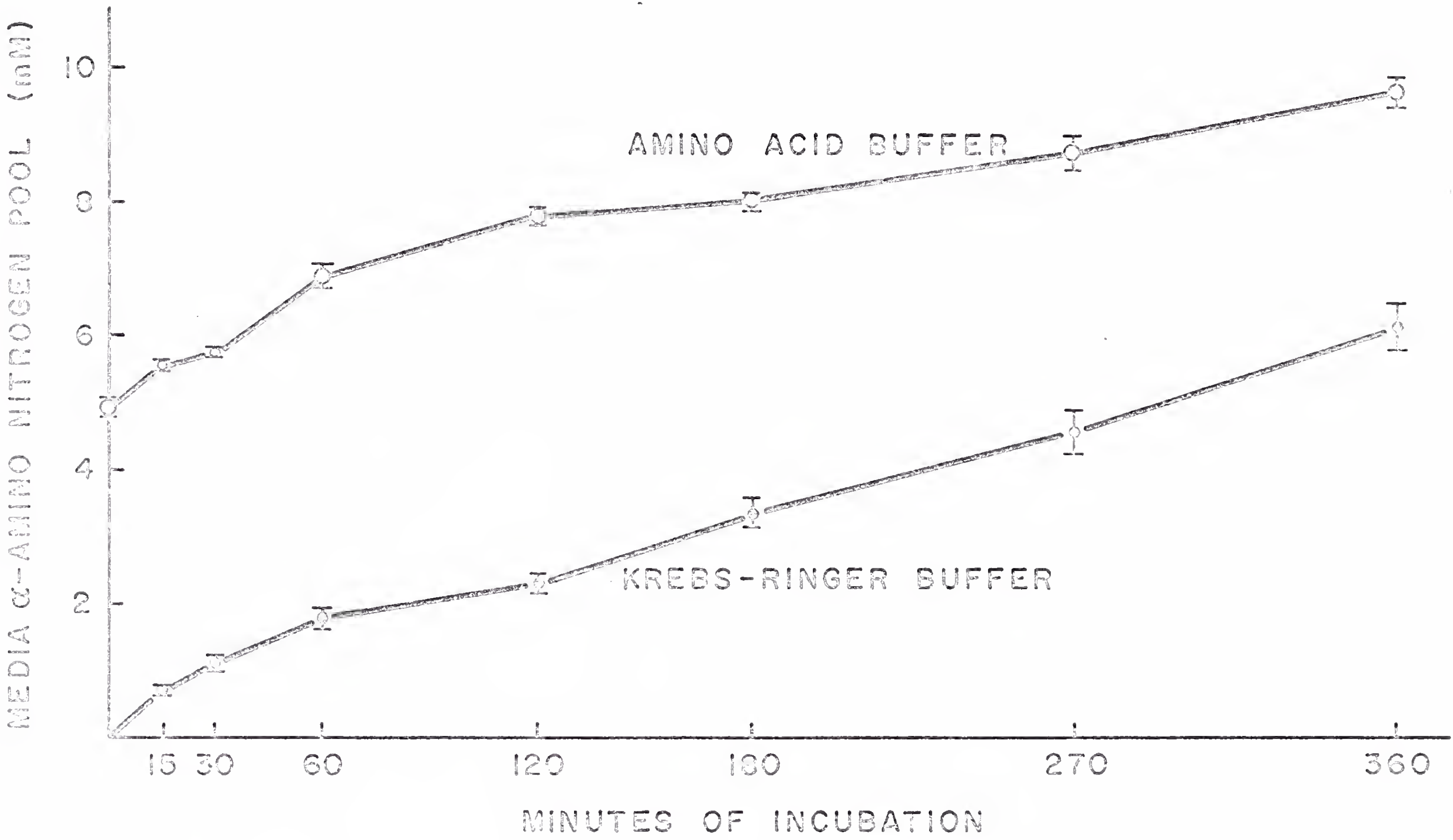

MEDIA α-AMINO NITROGEN POOL (mM)
10
8
6
4
2
AMINO ACID BUFFER
KREBS-RINGER BUFFER
15
30
60
120
180
270
360
MINUTES OF INCUBATION

Figure 3 -- Time course of AIB uptake by rat kidney cortex slices incubated in either Krebs-Ringer bicarbonate buffer or amino acid buffer. Initial medium concentration of AIB was 0.06 mM. Rat kidney cortex slices were incubated aerobically (95% O_2, 5% CO_2) in 2.0 ml Krebs-Ringer buffer or amino acid buffer (pH 7.4) at 37°C. Each point represents the mean of 3 observations. Uptake was defined as the distribution ratio of cpm per ml of intracellular fluid to cpm per ml of incubation medium.

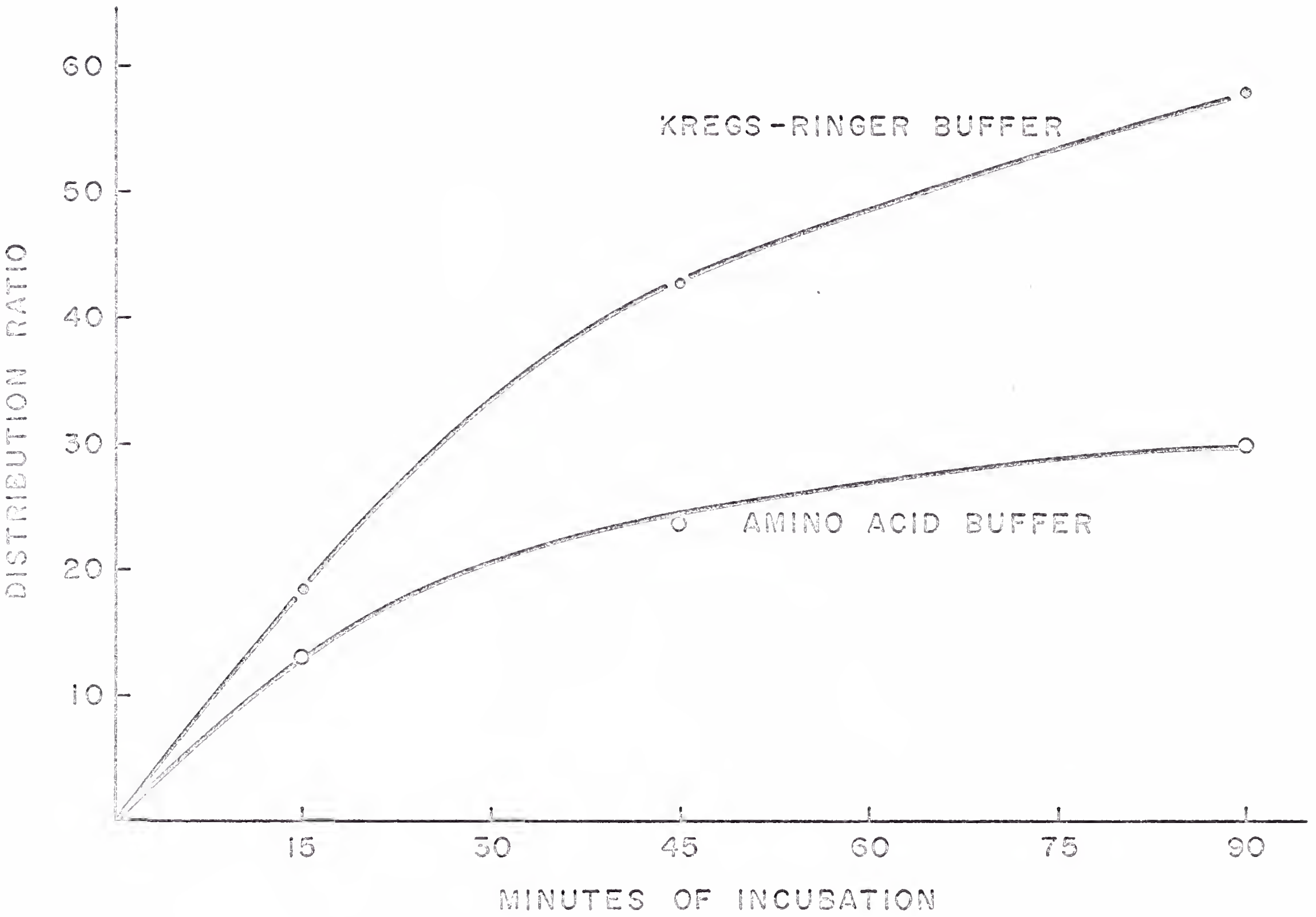

KREGS-RINGER BUFFER
AMINO ACID BUFFER
DISTRIBUTION RATIO
60
50
40
30
20
10
15
30
45
60
75
90
MINUTES OF INCUBATION

Figure 4 -- Time course of glycine uptake by rat kidney cortex slices incubated in either Krebs-Ringer bicarbonate buffer or amino acid buffer. Initial medium concentration of glycine was 0.57 mM. Rat kidney cortex slices were incubated aerobically (95% O_2, 5% CO_2) in 2.0 ml Krebs-Ringer buffer or amino acid buffer (pH 7.4) at 37°C. Each point represents the mean of 3 observations. Uptake was defined as the distribution ratio of cpm per ml of intracellular fluid to cpm per ml of incubation medium.

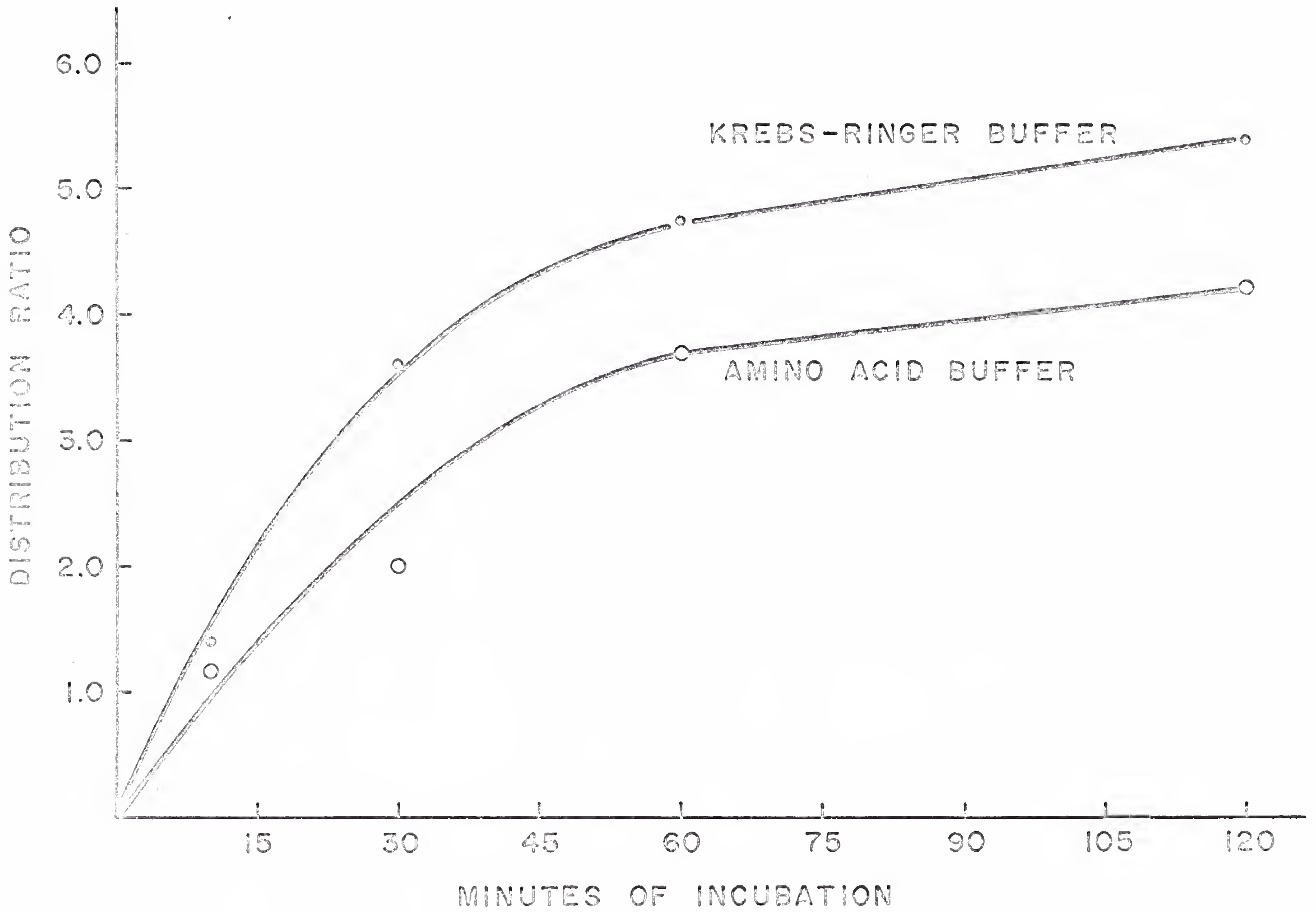
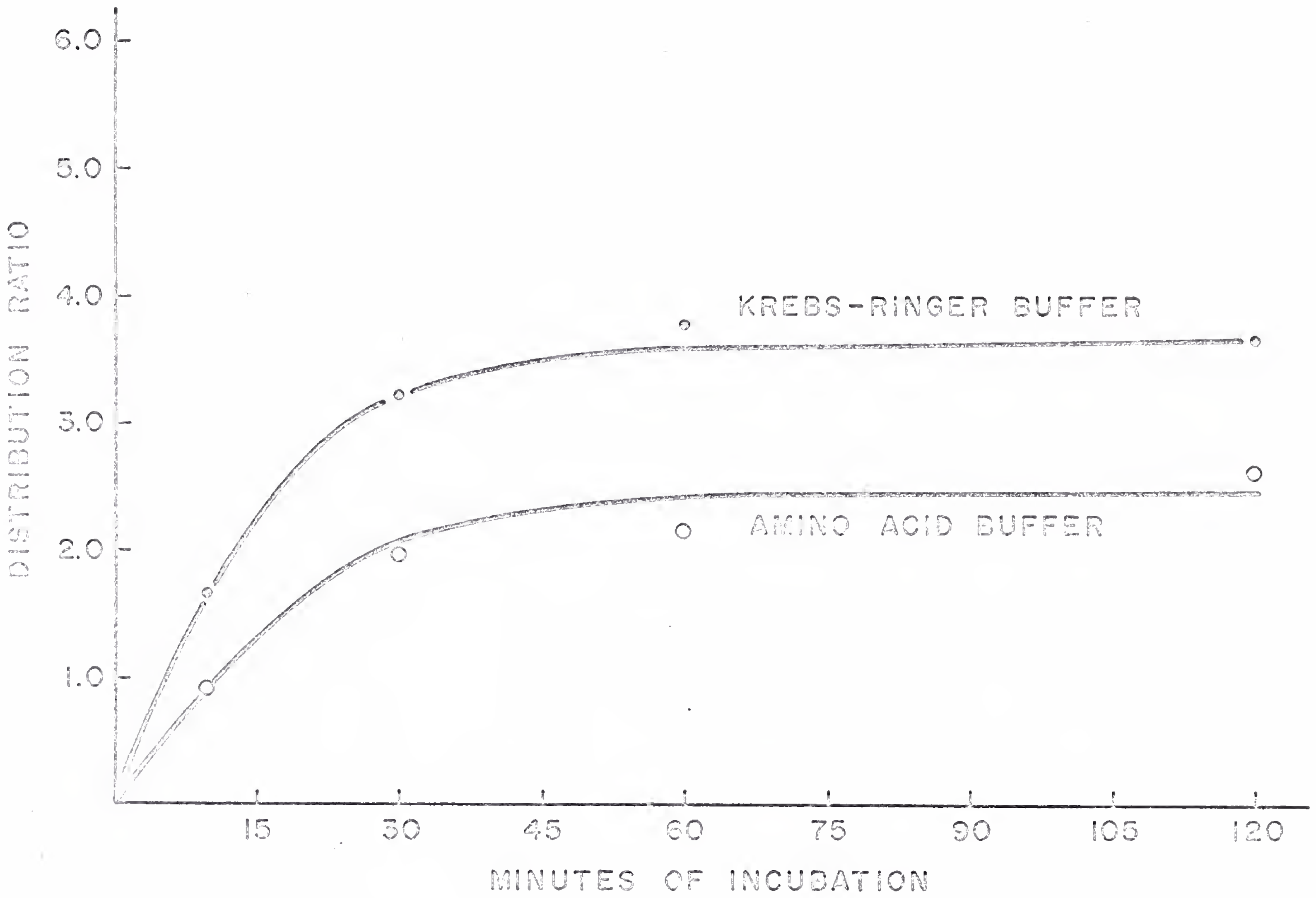
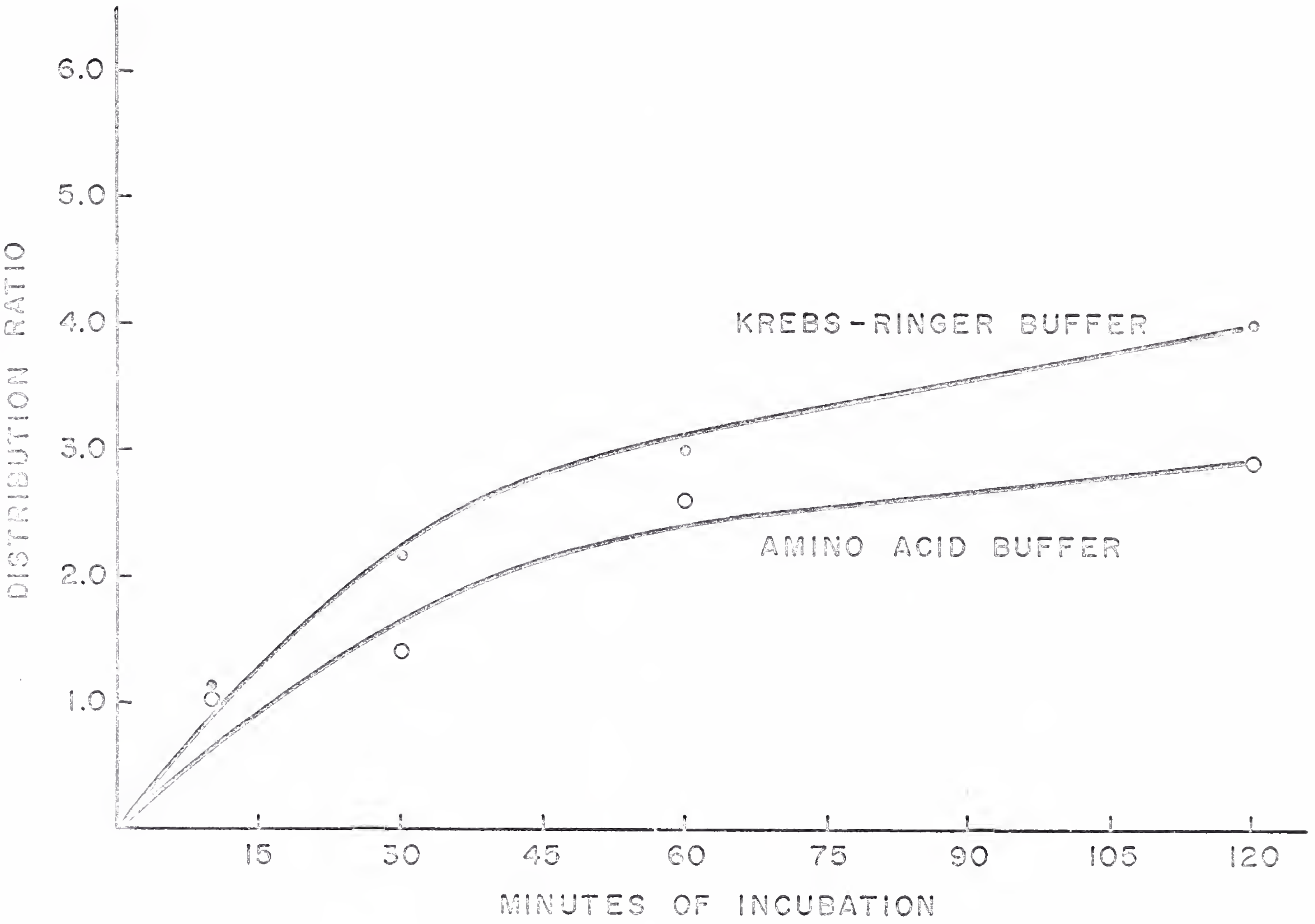

KREBS-RINGER BUFFER
AMINO ACID BUFFER
DISTRIBUTION RATIO
6.0
5.0
4.0
3.0
2.0
1.0
15
30
45
60
75
90
105
120
MINUTES OF INCUBATION

Figure 5 -- Time course of DAB uptake by rat kidney cortex slices incubated in either Krebs-Ringer bicarbonate buffer or amino acid buffer. Initial medium concentration of DAB was 0.1 mM. Rat kidney cortex slices were incubated aerobically (95% O_2, 5% CO_2) in 2.0 ml Krebs-Ringer buffer or amino acid buffer (pH 7.4) at 37°C. Each point represents the mean of 6 observations. Uptake was defined as the distribution ratio of cpm per ml of intracellular fluid to cpm per ml of incubation medium.



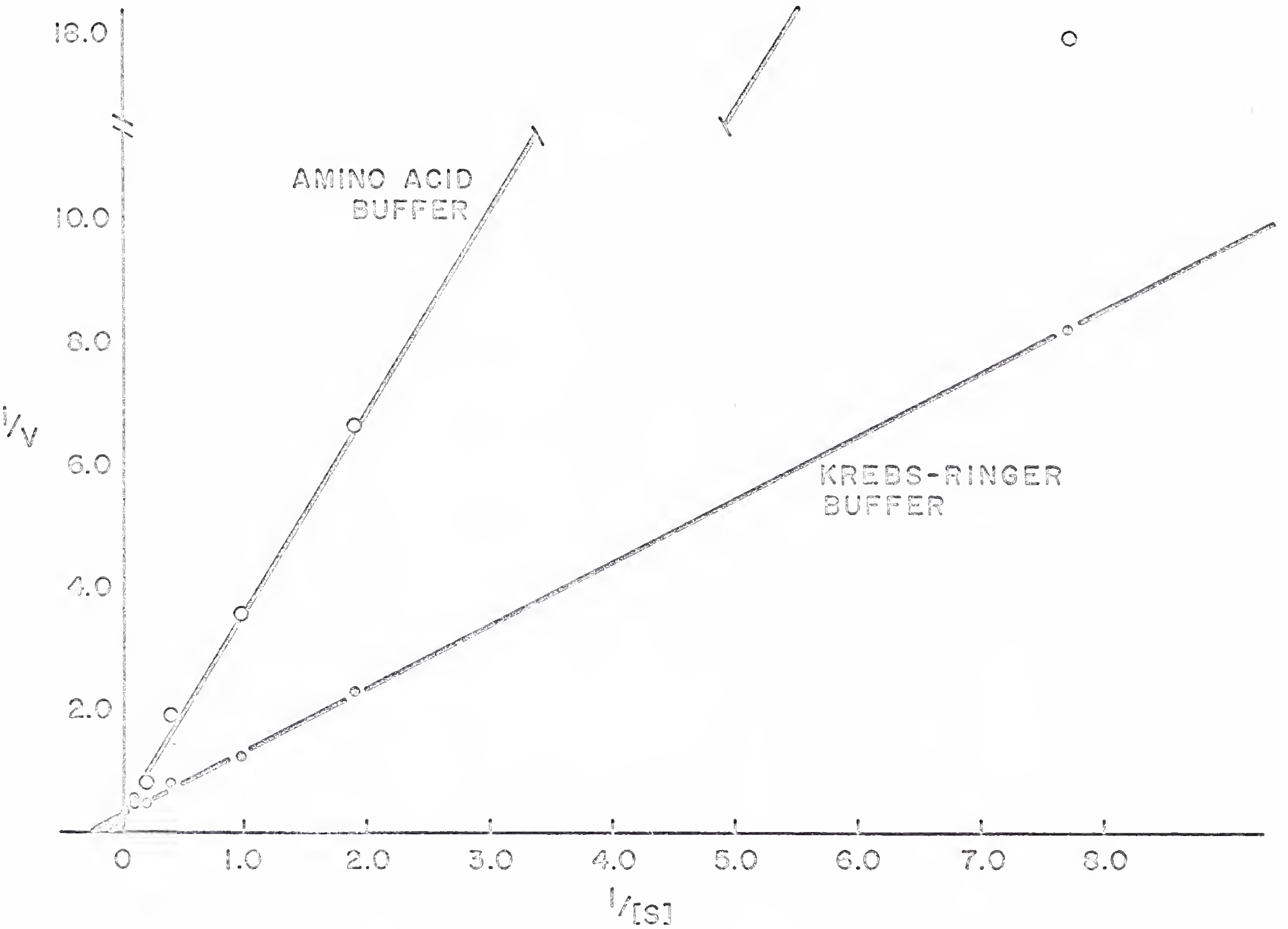
6.0
5.0
4.0
3.0
2.0
1.0
DISTRIBUTION RATIO
KREBS-RINGER BUFFER
AMINO ACID BUFFER
15
30
45
60
75
90
105
120
MINUTES OF INCUBATION

Figure 6 -- Time course of lysine uptake by rat kidney cortex slices incubated in either Krebs-Ringer bicarbonate buffer or amino acid buffer. Initial medium concentration of lysine was 0.55 mM. Rat kidney cortex slices were incubated aerobically (95% O_2, 5% CO_2) in 2.0 ml Krebs-Ringer buffer or amino acid buffer (pH 7.4) at 37°C. Each point represents the mean of 3 observations. Uptake was defined as the distribution ratio of cpm per ml of intracellular fluid to cpm per ml of incubation medium.



KREBS-RINGER BUFFER
AMINO ACID BUFFER
DISTRIBUTION RATIO
6.0
5.0
4.0
3.0
2.0
1.0
15
30
45
60
75
90
105
120
MINUTES OF INCUBATION

Figure 7 -- Lineweaver-Burk plot of AIB uptake by rat kidney cortex slices incubated in either Krebs-Ringer buffer or amino acid buffer. Initial medium concentrations of AIB ranged from 0.03 mM to 10.0 mM. Tissues were incubated for 15 minutes. Each point represents the mean of 6 observations. V is the velocity of mediated uptake in mmoles per liter per 15 minutes as determined by subtracting the uptake component due to passive diffusion[14]. S represents the initial substrate concentration in mmoles per liter. Appropriate K_m values for AIB uptake in each buffer were calculated from the respective abscissa intercepts: K_m(Krebs-Ringer)=3.4 mM, K_m(amino acid)= 12.5 mM. V_{max}, as calculated from the ordinate intercept, was 3.3 mmoles per liter per 15 minutes for both buffers.



AMINO ACID BUFFER
KREBS-RINGER BUFFER
1/V
1/[S]
18.0
10.0
8.0
6.0
4.0
2.0
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0

BIBLIOGRAPHY

1. Liebig, J., Ann. d. Chem. u. Pharm. 62: 257, 1847.

2. Enderlin, C., Ann. d. Chem. u. Pharm. 50: 53, 1844.

3. Forster, J., Ztschr. f. Biol. 9: 297, 1873.

4. Cohn, W.E., and Cohn, E.T., "Permeability of Red Corpuscles of the Dog to Sodium Ion," Proc. Soc. Exptl. Biol. Med. 41: 445, 1939.

5. Harris, J.E., "The Influence of the Metabolism of Human Erythrocytes on their Potassium Content," J. Biol. Chem. 141: 579, 1941.

6. Van Slyke, D.D., and Meyer, G.M., "The Fate of Protein Digestion Products in the Body. III. The Absorption of Amino-Acids From the Blood by the Tissues," J. Biol. Chem. 16: 197, 1913.

7. Christensen, H.N., and Riggs, T.R., "Concentrative Uptake of Amino Acids by the Ehrlich Mouse Ascites Carcinoma Cell," J. Biol. Chem. 194: 57, 1952.

8. Christensen, H.N., Riggs, T.R., Fischer, H., and Palatine, I.M., "Amino Acid Concentration by a Free Cell Neoplasm: Relations Among Amino Acids," J. Biol. Chem. 198: 1, 1952.

9. Johnstone, R.M., and Scholefield, P.G., "Amino Acid Transport in Tumor Cells," Advan. in Cancer Res. 9: 143, 1965.

10. Riggs, T.R., Christensen, H.N., and Palatine, I.M., "Concentrating Activity of Reticulocytes for Glycine," J. Biol. Chem. 194: 53, 1952.

11. Holden, J.T., "Transport and Accumulation of Amino Acids by Microorganisms," in Holden, J.T., Amino Acid Pools, New York, Elsevier Press: 566, 1962.

12. Wilson, T.H., Intestinal Absorption, Philadelphia, W.B. Saunders Company, 1962.

13. Akedo, H., and Christensen, H.N., "Transfer of Amino Acids across the Intestine: a New Model Amino Acid," J. Biol. Chem. 237: 113, 1962.

14. Akedo, H., and Christensen, H.N., "Nature of Insulin Action on Amino Acid Uptake by the Isolated Diaphragm," J. Biol. Chem. 237: 118, 1962.

15. Stern, J.R., Eggleston, L.V., Hems, R., and Krebs, H.A., "Accumulation of Glutamic Acid in Isolated Brain Tissue," Biochem. J. 44: 410, 1949.

16. Rosenberg, L.E., Blair, A., and Segal, S., "Transport of Amino Acids by Slices of Rat Kidney Cortex," Biochim. et Biophys. Acta 54: 479, 1961.

17. Christensen, H.N., Biological Transport, New York, W.A. Benjamin, Inc.: 16, 1962.

18. Ussing, H.H., "Transport of Ions Across Cellular Membranes," Physiological Reviews 29: 127, 1949.

19. Cross, J.C., and Taggart, J.V., "Renal Tubular Transport: Accumulation of p-Aminohippurate by Rabbit Kidney Slices," Am. J. Physiol. 161: 181, 1950.

20. Mudge, G.H., "Studies on Potassium Accumulation by Rabbit Kidney Slices: Effect of Metabolic Activity," Am. J. Physiol. 165: 113, 1951.

21. Krane, S.M., and Crane, R.K., "The Accumulation of D-Galactose Against a Concentration Gradient by Slices of Rabbit Kidney Cortex," J. Biol. Chem. 234: 211, 1959.

22. Whittam, R., and Davies, R.E., "Active Transport of Water, Sodium, Potassium, and α-Oxoglutarate by Kidney Cortex Slices," Biochem. J. 55: 880, 1953.

23. Platts, M.M., and Mudge, G.H., "Accumulation of Uric Acid by Slices of Kidney Cortex," Am. J. Physiol. 200: 387, 1961.

24. Noall, M.W., Riggs, T.R., Walker, L.M., and Christensen, H.N., "Endocrine Control of Amino Acid Transfer: Distribution of an Unmetabolizable Amino Acid," Science 126: 1002, 1957.

25. Segal, S., Schwartzman, L. Blair, A., and Bertoli, D., "Dibasic Amino Acid Transport in Rat Kidney Cortex Slices," Biochim. Biophys. Acta 135: 127, 1967.

26. Rosenberg, L.E., Downing, S.J., and Segal, S., "Competitive Inhibition of Dibasic Amino Acid Transport in Rat Kidney," J. Biol. Chem. 237: 2265, 1962.

27. Wilson, O.H., and Scriver, C.R., "Specificity of Transport of Neutral and Basic Amino Acids in Rat Kidney," Am. J. Physiol. 213: 185, 1967.

28. Fox, M. Their, S., Rosenberg, L., and Segal, S., "Ionic Requirements for Amino Acid Transport in the Rat Kidney Cortex Slice," Biochim. Biophys. Acta 79: 167, 1964.

29. Rosenberg, L.E., Berman, M., and Segal, S., "Studies of the Kinetics of Amino Acid Transport, Incorporation into Protein, and Oxidation in Kidney Cortex Slices," Biochim. Biophys. Acta 71: 664, 1963.

30. Cowie, D.B., and McClure, F.T., "Metabolic Pools and the Synthesis of Macromolecules," Biochim. Biophys. Acta 31: 236, 1959.

31. Halvorson, H.O., Cohen, G.N., "Incorporation des Amino Acides Endogenes et Exogenes Dans Les Proteines de la Levure," Ann. Inst. Pasteur 95: 73, 1958.

32. Kipnis, D.M., Reiss, E., and Helmreich, E., "Functional Heterogeneity of the Intracellular Amino Acid Pool in Mammalian Cells," Biochim. Biophys. Acta 51: 519, 1961.

33. Malathi, P., Sastry, P.S. and Ganguly, J., "Amino Acid Patterns in Vitamin A-Deficient Rats," Nature 189: 660, 1961.

34. Roberts, E., and Simonsen, D.G., "Free Amino Acids in Animal Tissue," in Amino Acid Pools, ed. J.T. Holden, Amsterdam, Elsevier Pub. Co.: 286, 1962.

35. Ibid, p. 284.

36. Rouser, G., Jelinek, B., Samuels, A.J., and Kinugasa, K., "Free Amino Acids in the Blood of Man and Animals. I. Method of Study and the Effects of Venipuncture and Food Intake on Blood Free Amino Acids," in Amino Acid Pools, ed. J.T. Holden, Amsterdam, Elsevier Pub. Co.: 350, 1962.

37. Wurtman, R.J., Rose, C.M., Chou, C., and Larin, F.F., "Daily Rhythms in the Concentrations of Amino Acids in Human Plasma," New England J. Med. 279: 171, 1968.

38. Rickenberg, H.V., Cohen, G.N., Buttin, G., and Monod, J., "La Galactodise-Permease d'Escherichia Coli," Ann. Inst. Pasteur 91: 829, 1956.

39. Bibb, W.R., and Straughn, W.R., "An Inducible Transport System for Citrulline in Streptococcus Faecalis," J. Bacteriology 87: 815, 1964.

40. Prestige, L.S., and Pardee, A.B., "A Second Permease for Methyl-Thio-β-D-Galactoside in Escherichia Coli," Biochim. Biophys. Acta 100: 591, 1965.

41. Novotny, S.P., and Englesberg, E., "The L-Arabinose Permease System in Escherichia Coli B/r," Biochim. Biophys. Acta 117: 217, 1966.

42. Dreyfuss, J., "Characterization of a Sulfate and Thiosulfate Transporting System in Salmonella Typhimurium," J. Biol. Chem. 239: 2292, 1964.

43. Ring, K., and Heinz, E., "Active Amino Acid Transport in Streptomyces Hydrogenans. I. Kinetics of Uptake of α-Aminoisobutyric Acid," Biochem. Z. 344: 446, 1966.

44. Heinz, E., Ring, K., and Gross, W. "Feedback Control of Amino Acid Transport," Fed. Proc. 26: 393, 1967.

45. Moore, S., Spackman, D.H., and Stein, W.H., "Chromatography of Amino Acids on Sulfonated Polystyrene Resins," Analyt. Chem. 30: 1185, 1958.

46. Fischer, L.J., Bunting, S.L., and Rosenberg, L.E., "A Modified Ninhydrin Colorimetric Method for the Determination of Plasma Alpha-Amino Nitrogen," Clin. Chem. 9: 573, 1963.

47. Rosenberg, L.E., Downing, S.J., and Segal, S., "Extracellular Space Estimation in Rat Kidney Slices Using C^{14} Saccharides and Phlorizin," Am. J. Physiol. 202: 800, 1962.

48. Scharff, R., and Wool, I.G., "Concentrations of Amino Acids in Rat Muscle and Plasma," Nature 202: 603, 1964.

49. Schimassek, H., and Gerok, W., "Control of the Levels of Free Amino Acids in Plasma by the Liver," Biochem. Z. 343: 407, 1965.

50. Westall, R.G., "The Free Amino Acids of Body Fluids and Some Hereditary Disorders of Amino Acid Metabolism," in *Amino Acid Pools*, ed. J.T. Holden, Amsterdam, Elsevier Pub. Co.: 198, 1962.

51. Lineweaver, H., and Burk, D., "The Determination of Enzyme Dissociation Constants," *J. Am. Chem. Soc.* 56: 658, 1934.

52. Elsas, L.J., and Rosenberg, L.E., "Inhibition of Amino Acid Transport in Rat Kidney Cortex by Puromycin," *Proc. Nat. Acad. Sc.* 57: 371, 1967.

53. Scriver, C.R., and Mohyuddin, F., "Amino Acid Transport in Kidney: Heterogeneity of α-Aminoisobutyric Uptake," *J. Biol. Chem.* 243: 3207, 1968.

54. Scriver, C.R., and Wilson, O.H., "Possible Locations for a Common Gene Product in Membrane Transport of Imino-acids and Glycine," *Nature* 202: 92, 1964.

55. Schwartzman, L., Blair, A., and Segal, S., "Exchange Diffusion of Dibasic Amino Acids in Rat Kidney Cortex Slices," *Biochim. Biophys. Acta* 135: 120, 1967.

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS DATE