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AMINO ACID TRANSPORT IN THE RAT RENAL PAPILLA

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SUMMARY

The uptake of amino acids in slices of renal papilla of rats has been investigated *in vitro* under environmental conditions of high sodium and non-electrolyte concentrations and low O_2 tensions, and compared with uptake under similar conditions in slices of renal cortex. L-Lysine, cycloleucine and α-aminoisobutyric acid were actively concentrated in Krebs–Ringer-bicarbonate buffer up to 8 times the concentration of the media. The accumulation was 1.7 times greater in the papilla than in the cortex and behaved as a saturable transport system. The papilla actively accumulated the above amino acids in media of increasing sodium concentrations up to 439 mequiv/l and in media made hyperosmolal with sucrose, whereas active accumulation of the amino acids in the renal cortex was absent or greatly diminished under these same experimental conditions. Slices of renal papilla also concentrated α-aminoisobutyric acid and lysine in oxygen-deficient media. Aldosterone and vasopressin did not influence the amino acid uptake in the renal papilla or cortex. The results of this study indicate that the papilla is able to accumulate amino acids actively *in vitro* under conditions which simulate the environment of the papilla *in vivo* and which are detrimental to the accumulation of amino acids in the renal cortex.

INTRODUCTION

The renal papilla functions in a physiological environment unique to the body, an environment of high osmolality, high concentrations of sodium and other electrolytes, and low O_2 tension[1–5]. The concentration and osmotic gradients, which are maintained by a counter-current system created in the loops of Henle, are of prime importance in the production of a concentrated urine. In contrast, the renal cortex and most other tissues have a narrow concentration range of sodium, amino acids, and O_2 concentrations in the extracellular fluid. It is known that the accumulation of amino acids by slices of renal cortex is affected by the extracellular concentration of these substances[6,7]. However, little is known of the amino acid transport into the papilla. This paper explores the effect of this unique environment on amino acid transport in the papilla of the rat *in vitro*. α-Aminoisobutyric acid, L-lysine, and cycloleucine uptake into renal papillary slices was measured in media of various sodium and amino acid concentrations, various osmolalities, and under anaerobiosis. The

results were compared with similar experiments performed on renal cortical slices. The results of this study indicate that the papilla is able to actively accumulate amino acid *in vitro* under conditions which simulate its physiologic environment but which appear detrimental to the accumulation of amino acids in the renal cortex.

METHODS AND MATERIALS

Male Sprague Dawley rats, weighing 150–170 g, were used for the experiments. The rats were fed on a Purina chow diet and water until sacrifice by stunning and decapitation. The kidneys were removed, bisected, and sliced, 0.4 mm thick, with a Stadie–Riggs tissue slicer. Papillary slices were cut from the center of the kidney and were trimmed to include the pale portion of the inner medulla. Cortical slices were cut from the poles. The methods for incubation have been previously described[6]. One slice from the kidneys of each of 3 rats, weighing a total of 30 mg, was incubated at 37° under O_2–CO_2 (95:5, v/v) in 25-ml plastic flasks, with 2 ml of buffer, usually Krebs–Ringer-bicarbonate buffer and the appropriate ^{14}C-labeled amino acid. After various time intervals the tissues were removed and the free amino acid pools were extracted into hot water, and the radioactivity was counted in a liquid scintillation spectrometer. The amino acid uptake was expressed as a ratio of the concentration of the amino acid in the intracellular fluid to that in the medium. This ratio, termed the distribution ratio, was calculated as follows:

$$\text{Counts/min per ml intracellular fluid} = \frac{\text{net tissue counts/min} - [(\text{medium counts/min per ml})\ (\text{ml } [^{14}\text{C}]\text{inulin space})]}{\text{ml tissue water} - \text{ml } [^{14}\text{C}]\text{inulin space}}$$

$$\text{Distribution ratio} = \frac{\text{counts/min per ml intracellular fluid}}{\text{counts/min per ml medium}}$$

Extracellular spaces were calculated using the distribution of [^{14}C]inulin, a substance that does not enter the cells. Total tissue water was determined by the difference between tissue weight before and after drying in a vacuum oven at 105° for 24 h.

In experiments on the kinetics of amino acid uptake, slices of papilla were incubated for 20 min at 37° in media of increasing concentrations of α-aminoisobutyric acid or cycloleucine varying from 0.065 mM to 10 mM. The distribution ratios were determined and corrected for diffusion of the amino acids.

To study exchange diffusion, slices of renal papilla or cortex were placed in appropriate control media or the same media with 30 mM lysine added. The flasks were incubated at 37° in O_2–CO_2 (95:5, v/v) for 30 min, then incubated for 5 min at the same temperature in flasks containing the fresh control media *plus* 0.2 μC of [^{14}C]lysine at 65 μM. The initial uptake of 30 mM lysine was determined by incubating slices for 30 min in 30 mM lysine with 2 μC of [^{14}C]lysine added.

Solutions

L-Lysine, cycloleucine, and α-aminoisobutyric acid were obtained from Mann Research Laboratories. [^{14}C]Lysine, cycloleucine, and α-aminoisobutyric acid were obtained from New England Nuclear. Each radioactive amino acid ran as a single

chemical and radioactive spot on descending paper chromatograms with a solvent of butanol–acetic acid–water (4:1:5, v/v/v). The specific activity of each amino acid solution was adjusted so that 10 μl of each solution added to 2 ml of incubation media resulted in a final concn. of 0.2 μC and 65 mμmoles per ml. The water from extracts of the tissues after incubation moved as a single radioactive spot on descending paper chromatography, at the appropriate R_F of the specific amino acid, indicating that the label remained attached to the amino acid during the experiment. Buffers of varying NaCl concentrations were made using Krebs–Ringer-bicarbonate buffer without affecting the final concentrations of the other electrolytes or the pH of the buffer. Solutions of varying sucrose concentrations were also made in Krebs–Ringer-bicarbonate buffer without affecting the electrolyte concentrations of the buffer. Buffers with low sodium concentration were made isotonic with Tris. To produce anaerobiosis, the Krebs–Ringer-bicarbonate buffer and flasks were gassed with N_2–CO_2 (95:5, v/v). 8-Lysine-vasopressin was kindly supplied by Sandoz Pharmaceuticals, and aldosterone, by Dr. W. EBERLEIN.

RESULTS

Water spaces

The intracellular, extracellular, and total tissue water spaces varied with the incubation media used (Table I). After incubation for 60 min in Krebs–Ringer-bicarbonate buffer, the total tissue water space of the cortical slices was 78.8 $\pm$ 0.40 (S.E.) % and those of the papillary slice, 87.9 $\pm$ 0.67 %. The higher water space of the papilla consisted of an increased amount of extracellular fluid rather than intracellular fluid. At sodium concentrations of 438 mequiv/l, the intracellular fluid space diminished and the extracellular fluid space increased in both cortex and papilla. Following 60-min incubation periods in buffers of low sodium concentration or low O_2 tension, the intracellular and extracellular fluid spaces varied slightly in both cortex and papilla.

Concentrative uptake of amino acids

The accumulation of lysine, cycloleucine, and α-aminoisobutyric acid by cortex and papilla in Krebs–Ringer-bicarbonate buffer at 37° reached equilibrium within 60 min (Fig. 1). All the distribution ratios were greater than one, indicating active transport. The distribution ratios of each of the amino acids studied were similar for papilla and cortex at 30 min, but when equilibrium was reached, the distribution ratio was greater in the papilla by a factor of 1.75 for α-aminoisobutyric acid, 1.57 for cycloleucine, and 1.70 for lysine (Table II).

Saturability of amino acid transport

To determine if the kinetics of amino acid accumulation were similar to those of the cortex, slices of papilla were incubated in graded concentrations of α-aminoisobutyric acid or cycloleucine. It had previously been demonstrated that in the cortex, transport of amino acids behaves as a saturable system and can be expressed in terms of the equations for kinetics of catalytic reactions[6]. Lineweaver–Burk plots of the data shown in Fig. 2 indicate that saturable transport of the amino acids does occur in the papilla, similar to that occurring in the cortex.

TABLE I

FLUID SPACES OF RENAL PAPILLARY AND CORTICAL SLICES

Slices of renal papilla or cortex (30 mg) were incubated in the following buffers at pH 7.4, at 37° for 60 min with 0.2 μC of [^{14}C]inulin added. Krebs–Ringer-bicarbonate buffer with various Na^+ concentrations contained the same final concentrations of other electrolytes (except for Na^+ and Cl^-) as in Krebs–Ringer-bicarbonate buffer. Tris was added to Krebs–Ringer-bicarbonate buffer with 50 mequiv/l Na^+ to make it isotonic with Krebs–Ringer-bicarbonate buffer. N_2 buffer was Krebs–Ringer-bicarbonate buffer gassed with N_2–CO_2 (95:5, v/v) . At the end of the incubation period, tissue water space was determined by the difference in wet and dry tissue weight. Extracellular fluid space was represented by the [^{14}C]inulin space. The intracellular fluid space was calculated as the difference between the tissue water and extracellular fluid spaces. Each value is the mean value for 18 rats, pooled in groups of 3 and is expressed as % of wet tissue weight.

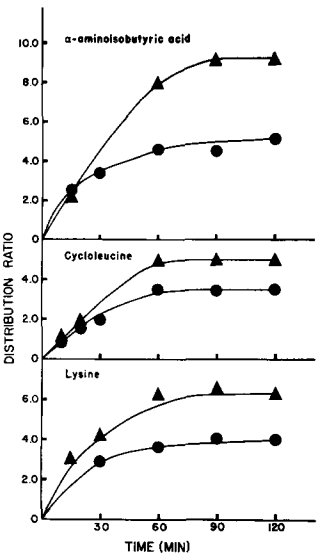
Media	*Cortex*			*Papilla*		
	Tissue water	*Intracellular fluid space*	*Extracellular fluid space*	*Tissue water*	*Intracellular fluid space*	*Extracellular fluid space*
Krebs–Ringer-bicarbonate buffer	78.8	52.8	26.0	87.9	50.0	37.9
Krebs–Ringer-bicarbonate buffer (439 mequiv Na^+/l)	79.6	33.5	46.1	85.3	33.5	52.0
Krebs–Ringer-bicarbonate buffer (50 mequiv Na^+/l)	80.27	54.3	26.0	87.4	54.8	32.6
N_2	83.0	47.3	35.7	89.5	47.0	42.5

TABLE II

EFFECT OF Na^+ CONCENTRATION ON AMINO ACID ACCUMULATION IN RENAL PAPILLARY AND CORTICAL SLICES

Slices of renal cortex or papilla (30 mg) were incubated for 60 min at 37° in 2 ml of Krebs–Ringer-bicarbonate (pH 7.4) with various concentrations of Na^+ and 0.2 μC of [^{14}C]amino acids, final concn. 65 μM. The concentration of other electrolytes in the buffer was constant. The accumulation of amino acids in the slices was measured and expressed as the distribution ratio (see text for explanation). Each mean is the average value of 9 to 18 rats, pooled into groups of 3.

Amino acid	*Distribution ratio*					
	50 mequiv Na^+/l		*142 mequiv Na^+/l*		*438 mequiv Na^+/l*	
	Papilla	*Cortex*	*Papilla*	*Cortex*	*Papilla*	*Cortex*
α-Aminoisobutyric acid	1.84 ± 0.15	2.99 ± 0.38	7.27 ± 0.49	4.15 ± 0.47	5.92 ± 1.1	1.73 ± 0.52
Cycloleucine	2.47 ± 0.17	2.16 ± 0.36	5.59 ± 0.33	3.57 ± 0.42	3.49 ± 0.17	1.21 ± 0.067
Lysine	3.17 ± 0.99	2.79 ± 0.31	6.38 ± 0.55	3.75 ± 0.72	4.73 ± 0.49	1.70 ± 0.12



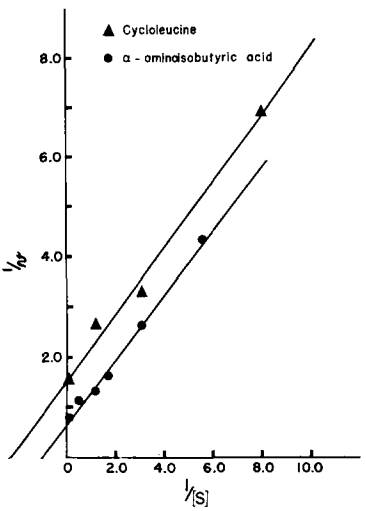


Fig. 1. The accumulation of α-aminoisobutyric acid, cycloleucine, and lysine in slices of renal cortex and papilla. Slices (30 mg) were incubated at 37° in 2 ml of Krebs–Ringer-bicarbonate buffer (pH 7.4) gassed with O_2–CO_2 (95 : 5, v/v), with 0.2 μC of [^{14}C]amino acid (final concn. 65 μM). Ordinate, ratio of the intracellular to the extracellular concn. of labelled amino acid. Abscissa, time of incubation. ▲, papilla; ●, cortex. Each point represents the average of the slices from 9 rats, grouped into pools of 3.

Fig. 2. Lineweaver–Burk plot of the reciprocal of velocity *vs.* the reciprocal of substrate concentration in renal papillary slices. Slices (30 mg) were incubated for 20 min at 37° in 2 ml of Krebs–Ringer-bicarbonate buffer containing various concentrations of α-aminoisobutyric acid or cycloleucine *plus* 0.2 μC of [^{14}C]amino acid. v = mmoles/l per 20 min; [S] = concentration of the amino acid in mM. Each point represents the average of the slices from 9 rats, grouped into pools of 3.

Effect of hyperosmolality

As the sodium concentration of the medium was increased, from 142 to 439 mequiv/l, the ability of the cortex to accumulate amino acids diminished (Table II). At the highest sodium concentrations, the distribution ratios were low. The papilla, however, increased in its ability to concentrate α-aminoisobutyric acid until an extracellular concentration of 202 mequiv/l of sodium was reached (Fig. 3). The distribution ratio at this concentration was 8.90. The distribution ratio then fell as the sodium concentration was further increased, but remained higher than the values for the cortex at all times.

Both the papilla and cortex accumulated α-aminoisobutyric acid less well at low sodium concentrations of 50 mequiv/l than at 142 mequiv/l. These observations were expected since the transport of α-aminoisobutyric acid is known to depend on the presence of Na^+ (ref. 2). Cycloleucine accumulation was also affected by the low sodium concentration. Lysine accumulation by the cortex was little affected by the decrease in sodium concentration but lysine accumulation in the papilla fell by 50 %.

The uptake α-aminoisobutyric acid and lysine was also determined in slices incubated for 60 min in a buffer which had the electrolyte content of Krebs–Ringer-bicarbonate buffer, and sucrose added to a final osmolality of 850 mosmoles/l. The distribution ratio for α-aminoisobutyric acid uptake in the cortex was 0, and for

lysine uptake 1.12 ± 0.46, indicating that only diffusion occurred under these conditions. In contrast, the distribution ratio in the papilla for α-aminoisobutyric acid was 2.36 and for lysine, 7.00 ± 1.13, indicating that concentration of the amino acids occurred.

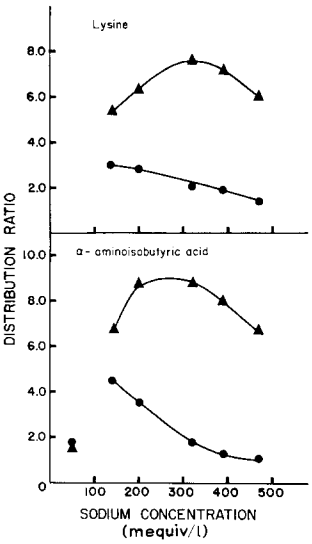


Fig. 3. The accumulation of lysine and α-aminoisobutyric acid in slices of renal papilla and cortex. Slices (30 mg) were incubated for 60 min at 37° in 2 ml of Krebs–Ringer-bicarbonate buffers of varying sodium concentrations, with 0.2 μC of [^{14}C]amino acid added to a final concn. of 65 μM. Ordinate, ratio of the intracellular to the extracellular concentration of labelled amino acid. Abscissa, sodium concn. (mequiv/l). ▲, papilla; ●, cortex. Each point represents the average of the slices from 9 rats, grouped into pools of 3.

Anaerobiosis

Slices of renal papilla concentrated α-aminoisobutyric acid without O_2 in buffers of high isotonic sodium concentrations (Table III). However, the distribution ratio of α-aminoisobutyric acid at high sodium concentrations fell from 10.06 ± 1.14 with O_2 to 4.10 ± 0.23 without O_2. The absence of O_2 did not affect the accumulation of lysine. On the other hand, the cortex could not accumulate α-aminoisobutyric acid without O_2, at either high or isotonic sodium concentrations. The accumulation of lysine was also diminished in the cortex without O_2.

Exchange diffusion

Exchange diffusion, a characteristic of carrier-mediated transport, was studied in both papillary and cortical slices by comparing the amino acid uptake of pre-loaded with unloaded cells. Exchange diffusion, as manifested by an increase in the distribution ratio in pre-loaded cells, was present in cortical slices at 142 and 439 mequiv/l sodium concentration. No such increase was present in the papilla at either of the sodium concentrations. Neither papilla nor cortex manifested exchange diffusion in 850 mosmolar sucrose buffers.

TABLE III

EFFECT OF ANAEROBIOSIS ON AMINO ACID UPTAKE IN RENAL PAPILLARY SLICES

Slices of renal papilla were incubated for 90 min at 37° either in Krebs–Ringer buffer of pH 7.4 (—) or with NaCl added to a final concn. of 438 mequiv/l of Na^+ (+). Oxygen (+) indicates that flasks and buffer were gassed with O_2–CO_2 (95:5, v/v) prior to incubation. Oxygen (—) indicates that buffer was prepared and flasks were gassed with N_2–CO_2 (95:5, v/v) prior to incubation. Slices were incubated for 30 min in the buffers before the labelled amino acid was added. 0.2 μC of either [^{14}C]lysine or [^{14}C]aminoisobutyric acid was added to all the buffers. The distribution ratio was calculated as the ratio of labelled amino acid in the intracellular fluid to that in the extracellular fluid.

High sodium media	*Oxygen*	*Distribution ratio*	
		α-Aminoisobutyric acid	*Lysine*
+	+	10.06 ± 1.14	6.69 ± 0.406
+	—	4.10 ± 0.234	5.59 ± 0.042
—	+	7.30 ± 0.868	6.88 ± 1.12
—	—	5.57 ± 0.856	5.72 ± 0.902

TABLE IV

EXCHANGE DIFUSSION OF LYSINE IN RENAL PAPILLARY AND CORTICAL SLICES

Slices of renal papilla or cortex were placed in Krebs–Ringer-bicarbonate, Krebs–Ringer-bicarbonate buffer with sodium added to a final concn. of 439 mequiv/l or Krebs–Ringer-bicarbonate buffer with sucrose added to a final concn. of 850 mosmoles/l. These were called the control media. Other flasks had the same media with 30 mM lysine added. The flasks were incubated at 37° in O_2–CO_2 (95:5, v/v) for 30 min, then incubated for 5 min in flasks containing the respective control media *plus* 0.2 μC of [^{14}C]lysine at 65 μM. The initial uptake of 30 mM lysine was determined by incubating slices for 30 min in 30 mM lysine with 2 μC of [^{14}C]lysine added. Each value represents the average of 9 rats, pooled into groups of 3.

	*Distribution ratio**					
	Papilla			*Cortex*		
	mM lysine in loaded cells	*Control*	*Loaded*	*mM lysine in loaded cells*	*Control*	*Loaded*
Krebs–Ringer-bicarbonate buffer	34.2	1.47	1.43	60	1.07	4.12
439 mequiv/l	37.5	2.22	2.81	24	1.54	2.68
850 mosmoles/l	27.0	1.48	0.36	10.5	0.30	0

* Distribution ratio = $\frac{\text{ratio of counts/min per ml intracellular fluid}}{\text{counts/min per ml medium}}$

Effect of aldosterone and vasopressin on amino acid uptake in the papilla

Slices of papilla or cortex were incubated for up to 120 min with aldosterone at a final concn. of 10^{-9} to 10^{-5} M in media with sodium concentrations between 142 and 438 mequiv/l. Aldosterone did not affect the uptake of α-aminoisobutyric acid or cycloleucine under the experimental conditions. In similar series of experiments, lysine-8-vasopressin added to media of various sodium concentrations at final concentration of 0.01 to 2 munits/ml did not alter the uptake of α-aminoisobutyric acid, cycloleucine, or valine in papillary and cortical slices.

DISCUSSION

The results of this study indicate that the papilla is able to transport amino acids *in vitro* under conditions which are detrimental to the accumulation of amino acid in the renal cortex. These conditions, which consist of high osmolality, high sodium concentrations, and low O_2 tensions, are present *in vivo* in the papilla. The papilla consists of loops of Henle, which act as a counter-current-multiplier system[1]. The osmolality gradually increases from the corticol-medullary junction to the tip of the papilla. This osmotic gradient, which is probably produced by the active reabsorption of sodium and the passive reabsorption of water from the ascending loop of Henle, occurs as well in the tissues, the collecting ducts, the interstitial fluid, and the medullary blood[1,4]. Along with the increased osmotic gradient, there is an increased concentration of urea, sodium, and other ions[2,3], in the papillary structures. The protein concentration is also increased in the papilla[8]; presumably the free amino acids are also increased, although this has not been measured.

At the tip of the papilla during anti-diuresis, tissue concentrations of urea may reach 840 mM and those of sodium, 425 mequiv/l. These values rapidly reach nearly isosmotic levels during osmotic diuresis[9]. It is obvious that the papilla can function in a wide range of sodium concentrations *in vivo* and the present study confirms that the papilla functions well *in vitro* under similar conditions, since concentrations of non-electrolyte and sodium used are similar to those found *in vivo*.

The cortex, on the other hand, exists in extracellular fluid which is isosmotic and has fairly similar electrolyte and urea concentrations to plasma. When some of the extracellular conditions surrounding the papilla were duplicated *in vitro*, the cortex was unable to function normally in regard to the transport of amino acids. Sodium is the major extracellular ion necessary for the transport of many amino acids into tissues: for example, the transport of glycine and α-aminoisobutyric acid into kidney cortex[6], of glycine into Ehrlich ascites tumor cells[10,11] and of alanine, tyrosine, and phenylalanine into the small intestine[12,13]. Although α-aminoisobutyric acid accumulation depends on the presence of Na^+ (ref. 7), α-aminoisobutyric acid accumulation into cortex slices decreased with the high sodium concentrations in the medium employed in the present investigation. KLEINZELLER, NEDVIDKOVA AND KNOTKOVA[14] have found that when the osmolarity of the medium was increased up to 420 mosmoles by the addition of sucrose or mannitol, the electrochemical gradient and the membrane potential decreased in slices of rabbit kidney cortex. The changes which affect sodium transport may thus in turn affect the transport of α-aminoisobutyric acid in the cortex. Such membrane changes may not be as marked in the papilla, which continues to accumulate α-aminoisobutyric acid at high sodium concentrations, as shown in the present study.

Under anaerobiosis there was only slight swelling of the tissue water spaces in the cortex similar to but not as marked as the 15 % increase in tissue water previously observed in rat renal cortex slices incubated anaerobically by GORDON AND MAEIR[15]. The papilla showed little or no increase in tissue water. Without O_2, the accumulation of α-aminoisobutyric acid has been found to cease and that of lysine to diminish in the renal cortex[6,16]. However, in the papilla, the accumulation of α-aminoisobutyric acid fell only slightly in O_2-deficient media and the accumulation of lysine remained the same. The combination of both O_2 lack and a sodium concentration of 438

mequiv/l in the medium produced a fall in the accumulation of α-aminoisobutyric acid but not of lysine.

The water spaces of the papillary slices were greater than those of the cortex and the increase was manifest by an increased amount of extracellular fluid. The water spaces were similar to those found in the sheep and dog[17]. Increases in osmolality in the medium would be expected to decrease the intracellular fluid space of both cortex and papilla, and both types of tissue behaved similarly. This indicates that although the papilla can exist at high osmolalities, it follows the same osmotic properties as does the cortex and other tissues that normally exist at 300 mosmoles/l but which cannot function at high osmolalities.

Our observations on concentrative uptake of amino acids is somewhat at variance with GANS, BAILIE AND BIGGS[18] who reported that the papilla was not capable of actively accumulating the amino acids glutamine, lysine, leucine, and phenylalanine. However, their data can be interpreted to indicate that both lysine and glutamine were accumulated against a concentration gradient, since the calculated distribution ratios were greater than one.

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REFERENCES

1 H. WIRZ, B. HARGITAY AND W. KUHN, *Helv. Physiol. Pharmacol. Acta*, 9 (1951) 196.
2 K. J. ULLRICH AND K. H. JARAUSCH, *Arch. Ges. Physiol.*, 262 (1956) 537.
3 K. J. ULLRICH, F. O. DRENCKHAHN AND K. H. JARAUSCH, *Arch. Ges. Physiol.*, 261 (1955) 62.
4 H. WIRZ, *Helv. Physiol. Pharmacol. Acta*, 11 (1953) 20.
5 G. A. BRAY, *Am. J. Physiol.*, 199 (1960) 915.
6 L. E. ROSENBERG, A. BLAIR AND S. SEGAL, *Biochim. Biophys. Acta*, 54 (1961) 479.
7 M. FOX, S. THIER, L. E. ROSENBERG AND S. SEGAL, *Biochim. Biophys. Acta*, 79 (1964) 167.
8 M. SERRATTO, C. L. FLANAGAN AND D. P. EARLE, *Am. J. Physiol.*, 202 (1962) 805.
9 R. L. MALVIN AND W. S. WILDE, *Am. J. Physiol.*, 197 (1959) 177.
10 H. N. CHRISTENSEN, T. R. RIGGS, H. FISCHER AND I. M. PALATINE, *J. Biol. Chem.*, 191 (1952) 1.
11 E. HEINZ AND J. T. HOLDEN, in J. T. HOLDEN, *Amino Acid Pools*, Elsevier, Amsterdam, 1962, p. 539.
12 T. Z. CZAKY, *Federation Proc.*, 20 (1961) 139.
13 M. FIELD, S. G. SCHULTZ AND P. F. CURRAN, *Biochim. Biophys. Acta*, 135 (1967) 236.
14 A. KLEINZELLER, J. NEDVIDKOVA AND A. KNOTKOVA, *Biochim. Biophys. Acta*, 135 (1967) 286.
15 E. E. GORDON AND D. M. MAEIR, *Am. J. Physiol.*, 207 (1964) 71.
16 S. SEGAL, L. SCHWARTZMAN, A. BLAIR AND D. BERTOLI, *Biochim. Biophys. Acta*, 135 (1967) 127.
17 J. H. GANS, D. WAKEFIELD AND G. KILSHEIMER, *Proc. Soc. Exptl. Biol. Med.*, 122 (1966) 624.
18 J. H. GANS, M. D. BAILIE AND D. L. BIGGS, *Am. J. Physiol.*, 211 (1966) 249.