tained from assays on 5 samples of dried chicken erythrocytes are presented in Table I. The values represent milligrams of amino acids per gram of dried erythrocytes.

A survey of the literature indicates rather meager information on the amino acids contained in erythrocytes of various species of animals. However, analyses have been reported for several hemoglobins and stroma.[8] It is of interest to note the similarity in the results presented in Table I with those reported by Stokes, Gunness, Dwyer, and Caswell[4] for "blood meal." The chicken erythrocytes are somewhat higher in arginine and threonine and lower in histidine, lysine, leucine, and phenylalanine.

Summary. The following amino acids contained in the chicken erythrocyte have been determined by means of microbiological assays and are expressed as percentages of the dry weight: histidine 3.75, arginine 4.35, lysine 6.81, leucine 9.20, isoleucine 3.20, valine 7.05, methionine 1.25, threonine 4.93, tryptophane 1.28, phenylalanine 4.25, and tyrosine 2.60.

8 Block, R. J., and Bolling, D., *The Amino Acid Composition of Proteins and Foods*, 1945, Springfield.

15914

Renal Clearance of Essential Amino Acids: Threonine and Phenylalanine.

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Protein hydrolysates or amino acid mixtures are being employed widely today in clinical medicine. Present practices of administering these preparations either orally or parenterally in relatively large amounts have emphasized the need for additional information concerning the extent to which the various amino acids are reabsorbed by the kidney tubules at elevated plasma levels.

Previous publications from this laboratory in which microbiological methods of assay for the specific determination of amino acids in blood and urine were employed have been concerned with the renal clearances in dogs of the amino acids leucine, isoleucine, valine and tryptophane[1] and arginine, histidine, lysine and methionine.[2] This paper describes the renal clearances of threonine and phenylalanine, the remaining 2 amino acids that are essential dietary components for one or more mammalian species.

Methods. The physiological procedures employed in these experiments have been described in previous publications.[1,2] Creatinine clearances were used as a measure of glomerular filtration rate while clearances of para-aminohippuric acid determined at low plasma levels were employed as a measure of minimal renal plasma flow.

The experiments involving threonine and phenylalanine were designed to determine the clearances of the individual amino acids at normal postabsorptive blood levels followed by similar clearance periods at elevated plasma levels. Increased plasma levels were obtained by the use of priming doses and constant intravenous infusions of the compounds.

Microbiological determinations of the amino acids in plasma were carried out on protein-free filtrates prepared according to the method of Dunn *et al.*[3] Determinations

1 Beyer, K. H., Wright, L. D., Russo, H. F., Skeggs, H. R., and Patch, E. A., *Am. J. Physiol.*, 1946, **146**, 330.

2 Wright, L. D., Russo, H. F., Skeggs, H. R., Patch, E. A., and Beyer, K. H., *Am. J. Physiol.*, 1947, **149**, 130.

TABLE I.
Renal Clearance Studies of *dl*-Threonine.

Time hr:min	Renal plasma flow PAH cc/min	Glomerular filtration rate cc/min	Urine flow cc/min	*dl*-Threonine: Plasma conc. mg/cc	Amt filtered mg/min	Amt reabsorbed mg/min	Amt excreted mg/min	Clearance cc/min
Dog 84, wt 17.0 kg.								
Control: Post-absorptive but after priming dose of water.								
0:10	183	72.0	4.45	0.057	4.10	4.07	0.03	0.52
0:20	208	68.6	4.45	0.058	3.98	3.96	0.02	0.41
Priming 4.0 mg/kg—Maintenance 4.0 mg/kg/min—Infusion 3 cc/min.								
0:50		77.9	4.20	0.239	18.62	18.37	0.25	1.05
0:60	187	67.3	5.65	0.280	18.84	18.55	0.29	1.05
Priming 6.0 mg/kg—Maintenance 10.0 mg/kg/min—Infusion 3 cc/min.								
1:30	195	77.8	7.55	0.709	55.16	54.02	1.14	1.61
1:40		69.4	7.35	0.829	57.53	56.16	1.37	1.66
Priming 8.0 mg/kg—Maintenance 14.0 mg/kg/min—Infusion 3 cc/min.								
2:10		68.8	8.20	1.274	87.65	85.23	2.42	1.90
2:20	165	66.2	8.30	1.512	100.09	97.65	2.44	1.62

TABLE II.
Renal Clearance Studies of *dl*-Phenylalanine.

Time hr:min	Renal plasma flow PAH cc/min	Glomerular filtration rate cc/min	Urine flow cc/min	*dl*-Phenylalanine: Plasma conc. mg/cc	Amt filtered mg/min	Amt reabsorbed mg/min	Amt excreted mg/min	Clearance cc/min
Dog 84, wt 17.3 kg.								
Control: Post-absorptive but after priming dose of water.								
0:20	209	70.3	1.10	0.037	2.60	2.58	0.02	0.56
0:30	198	73.5	3.10	0.042	3.09	3.07	0.02	0.55
Priming 6.0 mg/kg—Maintenance 8.0 mg/kg/min—Infusion 12 cc/min.								
0:60	150	69.9	4.80	0.349	24.39	22.82	1.57	4.50
1:10		76.1	4.95	0.436	33.18	30.90	2.28	5.24
1:20	160	81.4	5.45	0.439	35.73	33.29	2.44	5.56

on urine were carried out on suitable aliquots of the untreated material.

Threonine was determined microbiologically by the procedure of Stokes *et al.*[4] in which *Streptococcus fecalis* R was used as the assay organism. The extent of bacterial growth was determined turbidimetrically after an incubation period of 18-24 hours. Recovery of threonine from plasma was 105%.

The method of Stokes *et al.*[4] for the determination of phenylalanine was found satisfactory when applied to plasma but failed in the determination of phenylalanine in urine. There appears to occur in urine a material giving anomalous responses to phenylalanine with this medium such that quantitative recovery of the amino acid was not obtained. The method of Dunn *et al.*[5] employing a somewhat different basal medium was found satisfactory for the determination of phenylalanine in urine. A recovery of 100% was obtained.

It was necessary to use racemic amino acids in these experiments because the naturally-

[3] Dunn, M. S., Schott, H. F., Frankl, W., and Rockland, L. B., *J. Biol. Chem.*, 1945, **157**, 387.

[4] Stokes, J. L., Gunness, M., Dwyer, I. M., and Caswell, M. C., *J. Biol. Chem.*, 1945, **160**, 35.

[5] Dunn, M. S., Shankman, S., and Camien, M. N., *J. Biol. Chem.*, 1945, **161**, 643.

occurring forms were not available to us. The results have been expressed in terms of the dl products used in the infusion solutions and used as standards in the assays. *Streptococcus fecalis* R, and *Lactobacillus casei* utilize only the naturally-occurring enantiomorphs of the amino acids studied.[4,5] Two dogs were used in these experiments.

Results and Discussion. The results have been summarized as protocols in Tables I and II.

Plasma levels of threonine were attained such that 100 mg of the amino acid were calculated as filtered per minute at the glomerulus. It was not practicable to administer larger amounts of phenylalanine than that employed because of the relative insolubility of the amino acid, limitations in the amount of fluid that could be continuously infused, and the nausea that accompanied the administration of the amino acid in large amounts.

At postabsorptive plasma levels of threonine and phenylalanine less than 1.0% of either amino acid that was filtered at the glomerulus appeared in the urine. When the plasma level of threonine was raised to over 10 times that of the postabsorptive state, more than 97% of the filtered amino acid was reabsorbed by the tubules, and clearances of only 1.0-2.0 cc per minute were obtained.

The capacity of the tubules to reabsorb phenylalanine was not exceeded by the maximal doses that could be administered. However, at the higher blood levels studied reabsorption of phenylalanine was only about 85% complete. It is possible that at plasma levels only slightly higher than were attainable in these studies the maximal rate of tubular reabsorption (Tm) might have been attained.

Previously reported clearance data concerning the amino acids commonly referred to as essential have indicated that they may be classified in 2 groups with respect to their reabsorption by the kidney tubules of the dog.[1,2] Leucine, isoleucine, valine, tryptophane, histidine, and methionine, when administered singly, were completely reabsorbed at all plasma levels practical for study while arginine and lysine were not well reabsorbed at elevated plasma levels. Tm values of 11 and 13 mg per minute respectively were obtained for arginine and lysine. The present studies would place threonine and phenylalanine in that group of amino acids that are well reabsorbed and for which no well defined threshold appears to exist.

Had the naturally-occurring forms of threonine and phenylalanine been available, it would have been desirable to use these forms in the studies reported in this paper. It would be anticipated that if an unnatural enantiomorph of an amino acid influences reabsorption of the natural form it should inhibit rather than enhance the process.

Summary. Renal clearance studies of threonine and phenylalanine in dogs have shown that the maximal rate of tubular reabsorption of these amino acids could not be exceeded by the administration of an amount of each amino acid sufficient to raise the plasma level to a value over 10 times that of the postabsorptive state.