PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 167, 310–317 (1981)

Dietary Phenylalanine and Tyrosine Interrelationships in the Sprague–Dawley Rat[1] (41171)

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Abstract. Three experiments were conducted to investigate the effects of various levels of dietary phenylalanine (Phe) and tyrosine (Tyr) on weight gain, feed conversion, serum Tyr and Phe, and hepatic Phe hydroxylase and Phe-pyruvate transaminase activities in Sprague–Dawley rats. In general, the results indicated that these animals were not able to obtain Tyr from dietary Phe with 100% efficiency when the animals were fed diets devoid of Tyr. At or above the calculated requirement level of Phe (0.8% and greater), this phenomenon might be explained by substrate inhibition of Phe hydroxylase. Serum Tyr appeared to be a better indicator of the amount of dietary Phe and Tyr consumed than was serum Phe.

Recent studies in our laboratory (1) showed that phenylalanine (Phe) alone could not efficiently meet the chick's needs for tyrosine (Tyr) at dietary amounts below the aromatic amino acid (AAA)[2] requirement level. However, at or above the requirement level, Phe supported chick growth equal to that of birds fed the same total amount (moles) of AAA supplied either as a 50:50 or a 55:45 (mole:mole) mixture of Phe and Tyr. To our knowledge, this inefficiency of conversion of Phe to Tyr at dietary levels below the AAA requirement had not been previously reported for either the chick or rat, although Armstrong (2) observed that only one-half of the amount of Phe in excess of that required to fulfill the Phe requirement of Sprague–Dawley rats was converted to Tyr. The present study was undertaken to further investigate dietary Phe and Tyr interrelationships in the rat.

Materials and Methods. *Animals.* Male weanling rats of the Sprague–Dawley strain[3] were individually caged in a temperature-controlled room (22°) with a fixed 12-hr artificial light–dark cycle. Animals were assigned to their respective treatments such that the average initial weights of rats on each treatment were approximately equal.[4] During each experiment, feed and water were provided *ad libitum* for a 28-day period. There were 10 rats per treatment in all experiments, and individual weight gain and feed consumption were determined.

Diets. The crystalline amino acid basal diet used in all experiments is shown in Table I. Indispensable amino acids were added in amounts suggested by the National Research Council (NRC) (3) with the exception of Phe and Tyr which were variable. The dispensable amino acids were added at levels used by Sowers *et al.* (4). Within each experiment, all diets were made isonitrogenous by the addition of L-glutamic acid.

Serum amino acids. At the conclusion of each experiment, rats were killed by decapitation and blood samples were collected. Serum Tyr was determined by the method of Udenfriend and Cooper (5) as modified by Nielson (6). Serum Phe was determined by the method of McCaman and Robins (7).[5]

Enzyme assays. Immediately following decapitation, rat livers were excised and chilled in cold 0.15 *M* KCl. They were then

[1] Journal Paper No. 8313, Purdue University Agricultural Experiment Station.

[2] In this paper aromatic amino acid (AAA) refers to Phe and Tyr only. The authors do recognize, however, that tryptophan and, in most chemists' views, histidine are also considered aromatic amino acids.

[3] Murphy Breeding Lab., Inc., Plainfield, Ind.

[4] The average initial weights (weight ranges in parentheses) for Experiments 1, 2, and 3 were 75 (65–86), 66 (51–84), and 69 g (56–83), respectively.

[5] Sigma Technical Bulletin No. 60F, Sigma Chemical Co., St. Louis, Mo.

0037-9727/81/070310-08$01.00/0

TABLE I. COMPOSITION OF CRYSTALLINE AMINO ACID BASAL DIET

Ingredient	Percentage
DL-Methionine	0.44
L-Cystine	0.23
L-Arginine–HCl	0.81
L-Histidine–HCl–H_2O	0.45
L-Lysine–HCl	1.25
Glycine	1.39
L-Tryptophan	0.17
L-Threonine	0.56
L-Leucine	0.83
L-Isoleucine	0.61
L-Valine	0.67
L-Proline	0.44
L-Asparagine	0.44
L-Alanine	0.23
L-Aspartic acid	0.23
L-Serine	0.23
L-Phenylalanine	0.22–1.04
L-Tyrosine	0.00–0.51
L-Glutamic acid	4.40–4.83
Oil[a]	10.00
Sodium bicarbonate	1.00
Mineral premix[b]	4.75
Vitamin premix[b]	2.75
Cellulose	5.00
Corn starch	To 100.00

[a] Corn oil (Experiments 1 and 2), soybean oil (Experiment 3).
[b] Sell *et al.* (29).

trimmed, blotted, and weighed. Three-gram portions from each liver were homogenized with 21 ml of cold 0.15 *M* KCl with a Potter–Elvehjem tissue grinder tube and a Teflon pestle. Homogenates were centrifuged at 16,000*g* for 15 min in a refrigerated centrifuge at 4°.

Phenylalanine hydroxylase (Phe OHase) activity was assayed by the method of McGee *et al.* (8). However, the final concentration of 6,7-dimethyl-5,6,7,8-tetrahydropterine in the reaction mixture was increased to 0.002 *M*.

Phenylalanine-pyruvate transaminase (PPT) activity was determined by the procedure of Lin *et al.* (9). Tissue preparation for the PPT assay was similar to that for Phe OHase except that the livers were homogenized in cold 0.15 *M* KCl solution containing 0.005 *M* NaOH and that the homogenates were centrifuged for 40 min. Both Phe OHase and PPT activities were measured in 0.3-ml portions of the supernates within 1–3 hr after obtaining the tissues. Supernate protein concentrations were determined by the procedure of Lowry *et al.* (10) using bovine serum albumin as the standard.

For both enzymes, 1 unit of activity is equal to 1 nmole of product formed per minute. Enzyme activities are expressed both as units per 100 g body wt and as units per milligram of liver protein (specific activity or sp act). The former representation apparently serves as a more accurate expression of an animal's capacity for the metabolism of substrates by the particular enzyme being studied (11–13).

Statistical methods. The data were statistically analyzed by analysis of variance (14). Individual treatment differences were tested by the Newman–Keuls multiple-range test (14).

Results and Discussion. In all experiments, the diet containing 100% of the AAA requirement as 0.44% L-Phe and 0.40% L-Tyr was considered to contain the required amounts of all indispensable amino acids and dispensable amino acid nitrogen (3, 4). In the absence of dietary Tyr, 100% of the AAA "requirement" was considered to have been met by 0.8% Phe, which was equimolar to 0.44% Phe plus 0.40% Tyr. The 0.8% figure is the NRC requirement value for the growing rat for total dietary Phe plus Tyr on an "as is" basis (3). NRC states that one-third to one-half of the requirement may be supplied by Tyr. In concordance with this, it has been reported by Stockland *et al.* (15) that 45% of the total Phe plus Tyr requirement could be met by Tyr. On an equimolar basis, 0.40% Tyr supplies 45% of the Phe requirement.

The results of Experiment 1 (Table II) suggest that the Sprague–Dawley rat may not be able to convert dietary Phe to Tyr with complete efficiency as evidenced by the greater weight gains of rats fed a 55:45 (mole:mole) mixture of Phe and Tyr (Phe–Tyr mixture) as compared with rats fed the same amount (moles) of AAA as Phe only (diets 2 vs 1, 4 vs 3, and 6 vs 5), although the only significant difference within a level was at 100% of the AAA requirement. Rats fed this level of AAA ex-

TABLE II. INFLUENCE OF VARIOUS DIETARY LEVELS OF PHENYLALANINE (Phe) AND TYROSINE (Tyr) ON 28-DAY RAT WEIGHT GAIN, FEED CONVERSION, SERUM Tyr, AND HEPATIC Phe HYDROXYLASE ACTIVITY (EXPERIMENT 1)

Diet	Percentage of TAAA requirement[a]	Treatment (%)		Weight gain[b]	Gain (g)/feed (g)[b]	Serum Tyr[c]	Hepatic Phe hydroxylase activity[c,d]	
		L-Phe	L-Tyr	(g)		(μg/ml)	Units/100 g body wt	Units/mg protein
1	100	0.80	—	165[e]	0.36[e]	9.1[e]	814[f]	1.7[e]
2	100	0.44	0.40	193[f]	0.40[f]	16.2[f]	934[f]	2.2[e,f]
3	75	0.60	—	123[g]	0.32[g]	5.4[e]	1039[f]	2.2[e,f]
4	75	0.33	0.30	137[g]	0.35[e]	9.1[e]	854[f]	2.1[e,f]
5	50	0.40	—	42[h]	0.17[i]	6.3[e]	1027[f]	2.8[f]
6	50	0.22	0.20	49[h]	0.20[h]	6.4[e]	891[f]	2.5[e,f]
Pooled SE				8	0.01	0.9	76	0.2

[a] The even numbered diets contained a 55:45 (mole:mole) mixture of L-Phe and L-Tyr and were equimolar in total aromatic amino acids (TAAA) with the preceding diet containing only L-Phe. One hundred percent of the TAAA requirement was considered to have been met by 0.80% L-Phe.
[b] Means of 10 rats per diet.
[c] Means of individual samples from 6 rats per diet.
[d] One unit of activity is equal to the production of 1 nmole of Tyr/min at 25°.
[e,f,g,h,i] Column means with different superscripts are significantly different ($P < 0.05$).

hibited significantly ($P < 0.05$) better weight gains than those fed the lower levels of AAA. In addition, rats fed 75% of the AAA requirement (diets 3 and 4) gained significantly ($P < 0.05$) more weight than those fed 50% of the AAA requirement (diets 5 and 6). Feed efficiency values followed a similar trend. However, within all dietary AAA levels, rats fed the Phe–Tyr mixture gained significantly ($P < 0.05$) more weight per gram of feed consumed than rats fed the same amount (moles) of AAA as Phe alone. When weight gain and feed efficiency data were analyzed as a factorial arrangement of treatments (factorial analysis), significant ($P < 0.05$) effects due to both the level of dietary AAA and the amino acid type (Phe vs the Phe–Tyr mixture) were obtained.

These findings with rats are in contrast to the results of similar studies with broiler chicks (1) which showed that at or above the dietary AAA requirement level, Phe could completely meet the chick's needs for Tyr, while below this level chicks fed the Phe–Tyr mixture exhibited significantly greater weight gains and feed conversion values than those fed the same dietary amount of AAA as Phe alone.

At each level of AAA, serum Tyr values were consistently higher with the Phe–Tyr mixture as compared with Phe alone, although the difference was statistically significant ($P < 0.05$) only at 100% of the AAA requirement (Table II). There also appeared to be a progressive decline in serum Tyr as the AAA level was reduced in rats fed the Phe–Tyr mixture (diets 2, 4, and 6). This is in agreement with previous results obtained with chicks (1). Factorial analysis of the serum Tyr data revealed significant ($P < 0.05$) effects due to AAA level, amino acid type, and the interaction.

Hepatic Phe OHase values, when expressed on a body weight basis, were not significantly affected by dietary treatment although the highest activities were noted in rats fed the lower levels of Phe in diets devoid of Tyr (diets 3 and 5, Table II), while the lowest activity was noted in rats fed the highest level of Phe with no Tyr (diet 1). Enzyme sp act was highest in rats fed 50%

of the AAA requirement as Phe alone (diet 5). In comparison to these animals, rats fed the highest level of Phe alone (diet 1) had a significantly ($P < 0.05$) lower sp act value. Factorial analysis showed a significant ($P < 0.05$) effect of dietary AAA level on this parameter.

The poorer growth and feed conversion noted in rats fed diet 1 as compared to those fed diet 2 might possibly be attributed to the inability of the former to maximally convert Phe to Tyr, as evidenced by their reduced liver Phe OHase activities. This is not an unreasonable suggestion since high dietary levels of Phe have been shown to decrease rat hepatic Phe OHase activity *in vitro* (16–25). In addition to Phe OHase, a variety of enzymes have been reported to be inhibited by substrate (26).

Experiment 2 was conducted to study the efficiency of Phe alone versus a Phe–Tyr mixture in meeting the rat's needs for Tyr at 130, 115, 100, 85, and 70% of the AAA requirement level (Table III). In contrast to the previous experiment, there was no significant difference in weight gain between rats fed Phe alone and rats fed the Phe–Tyr mixture at 100% of the AAA requirement. However, at all other levels of AAA, weight gains tended to be slightly greater in rats fed the Phe–Tyr mixture as compared with rats fed Phe as the sole source of AAA, although none of the differences were significant within a given level. At both the 85 and 70% levels of AAA, growth was depressed as compared with higher levels, but the differences were significant only with the 70% level. Only the effect of dietary AAA level on weight gain was significant ($P < 0.01$) when the data were analyzed as a factorial arrangement of treatments.

Within all AAA levels, feed conversion was better in rats fed the Phe–Tyr mixture as compared with rats fed Phe alone and the trend was similar to that noted for weight gain. By factorial analysis, there was a significant effect of both AAA level ($P < 0.01$) and amino acid type ($P < 0.05$) on this parameter.

As in the previous experiment, serum Tyr values were higher in rats fed the Phe–Tyr mixture as compared to rats fed Phe alone within each dietary AAA level. Factorial analysis of the data revealed a significant ($P < 0.01$) effect due to AAA level, amino acid type, and the interaction. Although increasing Phe above the requirement elevated serum Tyr levels in rats fed diets devoid of Tyr (diets 1 and 3 vs diet 5), these values were still slightly lower than that noted for rats fed the Phe–Tyr mixture at 100% of the requirement (diet 6).

Phe OHase activity, expressed either on a body weight basis or as sp act, was significantly ($P < 0.05$) higher in rats fed the lowest level of Phe in a diet devoid of Tyr (diet 9). The lowest values were once again observed in rats fed the highest level of dietary Phe alone (diet 1). Factorial analysis of the enzyme data showed significant effects of both dietary AAA level ($P < 0.01$) and the level × amino acid interaction ($P < 0.05$) on this parameter expressed either way.

The results of the first two experiments raised a question as to whether Phe was somewhat "toxic" at or above 100% of the AAA requirement in diets devoid of Tyr. If the effects of high dietary levels of Phe were mediated via the inhibition of Phe OHase, then supplemental Tyr would be expected to improve rat performance. Thus, Experiment 3 was conducted to investigate the effect of L-Tyr additions to diets containing 120% of the AAA requirement as Phe alone. In addition to the aforementioned diets, two other levels of dietary AAA supplied either as Phe or as a Phe–Tyr mixture were included in this study.

As shown in Table IV, there was no significant difference in weight gain between rats fed any of the diets containing 100% or more of the AAA requirement (diets 1–6). Both diets containing the 80% level of AAA reduced growth, but the difference was significant only with the all Phe diet. With the exception of the 120% AAA level, the Phe–Tyr mixture again tended to produce greater gains than Phe alone. The lack of response to the mixture at 120% of the requirement does not agree with the previous experiment where there appeared to be some beneficial effect of Tyr at AAA levels above 100%. The addition of 0.05% Tyr to the Phe diet at 120% of the AAA require-

TABLE III. EFFECT OF VARIOUS DIETARY LEVELS OF PHENYLALANINE (Phe) AND TYROSINE (Tyr) ON 28-DAY RAT WEIGHT GAIN, FEED EFFICIENCY, SERUM Tyr, AND HEPATIC Phe HYDROXYLASE ACTIVITY (EXPERIMENT 2)

Diet	Percentage of TAAA requirement[a]	Treatment (%)		Weight gain[b] (g)	Gain (g)/ feed (g)[b]	Serum Tyr[c] (μg/ml)	Hepatic Phe hydroxylase activity[c,d]	
		L-Phe	L-Tyr				Units/100 g body wt	Units/mg pro
1	130	1.04	—	164^{e,f}	0.39^{e,f}	12.3^{f}	654^{f}	1.7^{f}
2	130	0.57	0.51	182^{e,f}	0.41^{e,f}	20.5^{e}	746^{f}	1.9^{f}
3	115	0.92	—	170^{e,f}	0.39^{e,f}	11.8^{f,g}	—	—
4	115	0.51	0.45	199^{e}	0.43^{e}	22.6^{e}	—	—
5	100	0.80	—	175^{e,f}	0.40^{e,f}	8.1^{g,h}	—	—
6	100	0.44	0.40	174^{e,f}	0.41^{e,f}	14.2^{f}	—	—
7	85	0.68	—	156^{f}	0.38^{f}	6.8^{h}	—	—
8	85	0.37	0.34	158^{e,f}	0.39^{e,f}	11.4^{f,g}	—	—
9	70	0.56	—	102^{g}	0.30^{g}	4.9^{h}	1000^{e}	2.7^{e}
10	70	0.31	0.28	110^{g}	0.32^{g}	7.3^{h}	767^{f}	2.2^{f}
Pooled SE				10	0.01	1.1	57	0.1

[a] See footnote a, Table II.
[b] See footnote b, Table II.
[c] See footnote c, Table II.
[d] See footnote d, Table II.
[e,f,g,h] Column means with different superscripts are significantly different ($P < 0.05$).

ment did give a small growth response, but there was no improvement when 0.10% Tyr was added to this ration. Feed efficiency values followed a trend similar to the weight gain data, and factorial analyses of these two parameters revealed a significant ($P < 0.05$) effect due to AAA level only. The lack of a consistent response to supplemental Tyr in a diet containing 0.96% Phe is difficult to explain.

In order to investigate the overall effect of Phe versus the Phe–Tyr mixture at all dietary AAA levels across all three experiments, weight gain and feed conversion data were analyzed as a nested factorial arrangement of treatments with levels nested within experiments and amino acid types (Phe vs the Phe–Tyr mixture) factorial to levels. It was found that only amino acid type significantly affected weight gain ($P < 0.05$) and feed efficiency ($P < 0.01$) and there were no significant interactions. Thus, when considering all three experiments, rats fed Phe alone did not gain as much weight and were less efficient in terms of feed conversion than rats fed the same total amount (moles) of AAA as the Phe–Tyr mixture.

Within all levels, serum tyrosine concentrations were once again higher in rats fed the Phe–Tyr mixture as compared with rats fed Phe as the sole AAA source (Table IV). Unlike the previous experiment, increasing dietary Phe from 100% of the requirement to a supraoptimal level in diets devoid of Tyr had little effect on serum Tyr. However, supplementing the diet containing 0.96% Phe with either 0.05 or 0.10% Tyr elevated serum Tyr levels in proportion to the amount of dietary Tyr added.

Serum Phe did not serve as a good indicator of the level of dietary Phe consumed. This is in agreement with the work of Stockland *et al.* (15) and Dierks-Ventling *et al.* (24). Serum Tyr, therefore, appears to be a better indicator of the amount of dietary Phe and Tyr consumed, although within an AAA level serum Phe values were higher in rats fed Phe alone versus the Phe–Tyr mixture. This difference between Phe and the Phe–Tyr mixture was significant ($P < 0.05$) when analyzed across AAA levels.

The effects of various dietary Phe and Tyr levels on the activities of hepatic Phe OHase and PPT appear in Table V. PPT was studied in an attempt to determine if any of the Phe that was presumably not hydroxylated in rats fed high Phe diets was channeled into an alternate degradative pathway.

Concerning Phe OHase, lower total and sp act were once again seen in rats fed Phe as the sole source of AAA at a supraoptimal

TABLE IV. INFLUENCE OF VARIOUS DIETARY LEVELS OF PHENYLALANINE (Phe) AND TYROSINE (Tyr) ON 28-DAY RAT WEIGHT GAIN, FEED EFFICIENCY, SERUM Tyr, AND SERUM Phe (EXPERIMENT 3)

Diet	Percentage of TAAA requirement[a]	Treatment (%) L-Phe	Treatment (%) L-Tyr	Weight gain[b] (g)	Gain (g)/feed (g)[b]	Serum Tyr[c] (μg/ml)	Serum Phe[c] (μg/ml)
1	120++	0.96	0.10	180^{d,e}	0.39^{d}	16.7^{d}	25.2^{d,e}
2	120+	0.96	0.05	192^{d}	0.40^{d}	13.9^{e,f}	24.0^{d,e}
3	120	0.96	—	181^{d,e}	0.39^{d}	12.0^{f}	25.7^{d,e}
4	120	0.53	0.47	182^{d,e}	0.38^{d,e}	17.8^{d}	22.7^{d,e}
5	100	0.80	—	176^{d,e}	0.38^{d,e}	11.9^{f}	26.3^{d,e}
6	100	0.44	0.40	183^{d,e}	0.39^{d}	15.5^{d,e}	23.8^{d,e}
7	80	0.64	—	150^{f}	0.33^{f}	7.5^{g}	27.4^{d}
8	80	0.35	0.32	156^{e,f}	0.36^{e}	8.7^{g}	21.9^{e}
Pooled SE				7	0.01	0.8	1.1

[a] See footnote a, Table II, for diets 3–8. Diets 1 and 2 contained 120% of the TAAA requirement as Phe plus 0.10 (++) or 0.05 (+) % L-Tyr, respectively.
[b] See footnote b, Table II.
[c] See footnote c, Table II.
[d,e,f,g] Column means with different superscripts are significantly different ($P < 0.05$).

TABLE V. EFFECT OF VARIOUS DIETARY LEVELS OF PHENYLALANINE (Phe) AND TYROSINE (Tyr) ON RAT HEPATIC Phe HYDROXYLASE AND Phe-PYRUVATE TRANSAMINASE ACTIVITIES (EXPERIMENT 3)

Diet	Percentage of TAAA requirement[a]	Treatment (%)		Phe hydroxylase[b,c]		Phe-pyruvate transaminase[b,d]	
		L-Phe	L-Tyr	Units/100 g body wt	Units/mg protein	Units/100 g body wt	Units/mg protein
1	120++	0.96	0.10	731[e]	1.5[e,f]	2103[e]	4.9[e]
2	120+	0.96	0.05	683[e]	1.4[f]	1850[e]	4.6[e]
3	120	0.96	—	660[e]	1.4[f]	2151[e]	5.3[e]
4	120	0.53	0.47	798[e]	1.8[e,f]	1802[e]	4.5[e]
5	100	0.80	—	—	—	—	—
6	100	0.44	0.40	—	—	—	—
7	80	0.64	—	879[e]	1.9[e]	2009[e]	4.7[e]
8	80	0.35	0.32	850[e]	1.9[e]	2095[e]	5.4[e]
Pooled SE				54	0.1	93	0.2

[a] See footnote a, Table IV.
[b] See footnote c, Table II.
[c] See footnote d, Table II.
[d] One unit of activity is equal to the production of 1 nmole of phenylpyruvate/min at 23°.
[e,f] Column means with different superscripts are significantly different ($P < 0.05$).

AAA level (diet 3) as compared with rats fed suboptimal amounts of AAA (diets 7 and 8). This is in agreement with the results of the previous two experiments. In addition, when the values were analyzed as a factorial arrangement of treatments, there was a significant ($P < 0.05$) effect of dietary AAA level on Phe OHase activities expressed either way.

There were no significant differences due to dietary treatment on PPT activities expressed either on a body weight basis or on a sp act basis. This is in agreement with the results of Boggs and Waisman (20) who reported that the activity of this transaminase was not increased by feeding rats excess dietary Phe. They concluded that this alternate pathway was not adequate for the disposition of Phe. In addition, Auerbach and Waisman (27) reported that PPT activity in rats did not increase following an ip injection of Phe.

It should be noted that hepatic PPT activities were approximately two to three times greater than hepatic Phe OHase activities. This was surprising since Haley and Harper (28) reported that 24 hr following the administration of a Phe load which was 1.5 times the daily requirement for the rat, 75% was metabolized via Phe OHase, 6% was either transaminated or decarboxylated, 2% was excreted as Phe, Tyr, or various other metabolites, and 17% remained in the carcass. However, it has been reported that *in vitro* enzyme activity measurements "cannot be considered to provide a reliable index of the magnitude of the flux of substrate through various steps in a given pathway" (13). In addition, it has been suggested that Phe OHase activity may be significantly greater *in vivo* than *in vitro* (13). Furthermore, rat kidney also contains significant Phe OHase activity (8).

The results of the present studies suggest that the male Sprague–Dawley rat may not be able to obtain Tyr from dietary Phe with complete (100%) efficiency when fed diets devoid of Tyr. In rats fed at least 0.8% Phe, this occurrence may be partially explained by the fact that there appeared to be a slight, but in some cases significant, inhibition of Phe OHase by substrate.

1. Elkin, R. G., Featherston, W. R., and Rogler, J. C., J. Nutr. **110**, 130 (1980).
2. Armstrong, M. D., J. Biol. Chem. **213**, 409 (1955).
3. National Research Council, "Nutrient Requirements of Laboratory Animals," Nat. Acad. Sci., Washington, D.C. (1972).
4. Sowers, J. E., Stockland, W. L., and Meade, R. J., J. Anim. Sci. **35**, 782 (1972).
5. Udenfriend, S., and Cooper, J. R., J. Biol. Chem. **196**, 227 (1952).
6. Nielsen, K. H., Eur. J. Biochem. **7**, 360 (1969).
7. McCaman, M. W., and Robins, E., J. Lab. Clin. Med. **59**, 885 (1962).
8. McGee, M. M., Greengard, O., and Knox, W. E., Biochem. J. **127**, 669 (1972).
9. Lin, E. C. C., Pitt, B. M., Civen, M., and Knox, W. E., J. Biol. Chem. **233**, 668 (1958).
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).
11. Freedland, R. A., Krakowski, M. C., and Waisman, H. A., Amer. J. Physiol. **202**, 145 (1962).
12. Boctor, A. M., and Harper, A. E., J. Nutr. **95**, 535 (1968).
13. Brand, L. M., and Harper, A. E., Biochem. J. **142**, 231 (1974).
14. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics." McGraw–Hill, New York (1980).
15. Stockland, W. L., Lai, Y. F., Meade, R. J., Sowers, J. E., and Oestemer, G., J. Nutr. **101**, 177 (1971).
16. Freedland, R. A., Krakowski, M. C., and Waisman, H. A., Fed. Proc. **19**, 7 (1960).
17. Huang, I., Tannenbaum, S., Blume, L., and Hsia, D. Y.-Y., Proc. Soc. Exp. Biol. Med. **106**, 533 (1961).
18. Wang, H. L., and Waisman, H. A., Proc. Soc. Exp. Biol. Med. **108**, 332 (1961).
19. Freedland, R. A., Biochim. Biophys. Acta **73**, 71 (1963).
20. Boggs, D. E., and Waisman, H. A., Arch. Biochem. Biophys. **106**, 307 (1964).
21. Woods, M. N., and McCormick, D. B., Proc. Soc. Exp. Biol. Med. **116**, 427 (1964).
22. Freedland, R. A., Krakowski, M. C., and Waisman, H. A., Amer. J. Physiol. **206**, 341 (1964).
23. Kerr, G. R., and Waisman, H. A., J. Nutr. **92**, 10 (1967).
24. Dierks-Ventling, C., Wapnir, R. A., and Braude, M. C., Proc. Soc. Exp. Biol. Med. **127**, 121 (1968).
25. Wapnir, R. A., Hawkins, R. L., and Lifshitz, F., Amer. J. Physiol. **223**, 788 (1972).
26. Webb, J. L., "Enzyme and Metabolic Inhibitors," Vol. 1, p. 111. Academic Press, New York (1963).
27. Auerbach, V. H., and Waisman, H. A., Proc. Soc. Exp. Biol. Med. **98**, 123 (1958).
28. Haley, C. J., and Harper, A. E., Arch. Biochem. Biophys. **189**, 524 (1978).
29. Sell, D. R., Featherston, W. R., and Rogler, J. C., Poul. Sci. **59**, 1878 (1980).

Received November 24, 1980. P.S.E.B.M. 1981, Vol. 167.