INTERRELATIONS OF PHENYLALANINE AND TYROSINE IN THE CHICK

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It has previously been established that the chick requires phenylalanine in its diet (1, 2) as do the rat (3) and mouse (4), but for none of these animals is tyrosine an essential dietary constituent (2–6). Moss and Schoenheimer showed (7), by isotope tracer methods, that an appreciable amount of phenylalanine is converted to tyrosine in the rat even when a large amount of tyrosine is present in the diet. Bauer and Berg (4) considered that for the mouse a lack of tyrosine imposed an additional need for phenylalanine; Almquist and Grau (2) found that chicks fed diets containing 1 per cent DL-phenylalanine alone grew poorer than those fed diets which contained, in addition, 2 per cent L-tyrosine. A high level of DL-phenylalanine (3 per cent) obviated the need for tyrosine. Recently, Womack and Rose (8) demonstrated that in the rat tyrosine is capable of stimulating growth only when phenylalanine is furnished in suboptimum amounts. The present report is concerned with the results of feeding various levels of phenylalanine and tyrosine on the growth of chicks, and with determinations of the percentage retention for tissue formation of these components of the diet.

Methods

White Leghorn chicks were reared on a commercial type of diet until they were 2 weeks old, when they were segregated on the basis of weight and were given the experimental diets, which consisted of the following ingredients (in gm.): DL-alanine 1.5, L-arginine monohydrochloride 1.2, L-cystine 0.5, L-glutamic acid 5.0, glycine 1.8, L-histidine monohydrochloride monohydrate 0.8, DL-isoleucine 1.0, L-leucine 1.5, L-lysine monohydrochloride 1.4, DL-methionine 0.6, L-proline 2.0, DL-threonine 1.3, DL-tryptophan 0.5, DL-valine 1.5, sodium bicarbonate 1.5, cellulose\(^1\) 5, calcium gluconate 8, mineral mixture 3.8, crude soy bean oil 5, sardine oil (400 vitamin D and 3000 vitamin A units per gm.) 0.25, natural mixed tocopherols\(^2\) 0.05, choline chloride\(^3\) 0.2, inositol 0.1, cholic acid 0.1, 2-

\(^1\) Cellu flour.

\(^2\) Natural mixed tocopherols (15 per cent), Distillation Products, Inc.

\(^3\) Choline chloride was provided by the Lederle Laboratories Division, American Cyanamid Company, through the courtesy of Dr. T. H. Jukes.
methyl-1,4-naphthohydroquinone diacetate 0.001, thiamine 0.001, riboflavin 0.001, pyridoxine 0.001, nicotinic acid 0.003, calcium (d) pantothenate 0.003, biotin4 0.00001, pteroylglutamic acid5 0.0001, and glucose6 to make 100 gm. The mineral mixture contributed the following materials, in gm. to each 100 gm. of diet: tricalcium phosphate 2.0, dipotassium phosphate 0.5, potassium chloride 0.3, manganese 0.01, silicon 0.046, magnesium 0.048, aluminum 0.008, iron 0.014, copper 0.001, zinc 0.001, iodine 0.0008, and cobalt 0.0005. Supplements consisted of DL-phenylalanine or L-tyrosine or mixtures of these two amino acids. Feed and water were supplied ad libitum.

In Experiment 1, pairs of carefully selected chicks weighing 69 to 87 gm. were fed diets containing 1.0 per cent phenylalanine plus 2 per cent tyrosine; or 2 per cent phenylalanine; or 2 per cent tyrosine. The chicks were weighed daily for 8 days. In Experiments 2 and 3, each bird was kept in an individual wire-floored compartment of an electrically heated battery brooder in order that accurate records of feed intake might be obtained. Chicks and feed were weighed daily. After a total of 12 days7 on the diets, the chicks were killed and the carcasses were analyzed for phenylalanine, tyrosine, and nitrogen by methods previously described (9).

Results

Growth Rates—In Experiment 1, the two chicks fed a diet containing 1.0 per cent DL-phenylalanine and 2.0 per cent L-tyrosine gained 3.8 and 4.3 per cent per day,8 while four chicks fed 2.0 per cent phenylalanine but no tyrosine gained 3.4, 3.9, 3.9, and 6.1 per cent per day, respectively. These two diets were of approximately equal value in promoting growth. The four chicks fed 2.0 per cent tyrosine but no phenylalanine lost weight (growth rate of -2.5 per cent per day); thus these results confirmed the indispensable nature of phenylalanine in the chick (1, 2).

In Experiments 2 and 3, in which various levels of phenylalanine and tyrosine were fed, the growth rates obtained gave an indication of the levels of these amino acids required for optimum growth. In Experiment 2, a high level of tyrosine (2.2 per cent9) was kept constant in all diets,

4 Biotin was furnished by Merck and Company, Inc.
5 Pteroylglutamic acid was provided by the Lederle Laboratories Division, American Cyanamid Company, through the courtesy of Dr. E. L. R. Stokstad.
6 Cerelose.
7 In Experiment 3, one of each pair of chicks was kept for 24 days before it was killed.
8 Per cent gain per day = (average gain per day X 100)/(average weight during experiment).
9 This level of tyrosine is equivalent on a mole basis to 2.0 per cent phenylalanine. In both Experiments 2 and 3, the tyrosine levels used were 110 per cent of the phenylalanine levels.
while the phenylalanine level was varied. Fig. 1, A shows the growth rates obtained with the several levels of phenylalanine. Best growth was obtained when 0.6 to 0.8 per cent phenylalanine was present; at levels above 1.0 per cent, phenylalanine was found to have a distinctly depressing effect on growth. When tyrosine was kept constant at 1.1 per cent (Experiment 3), the minimum required level remained unchanged, and there was no growth-depressing effect of higher levels of phenylalanine. When phenylalanine was held constant at marginal (0.8 per cent) or submarginal (0.4 per cent) levels, while tyrosine was varied, tyrosine had a definite
growth-promoting effect (Fig. 1, B). Data are insufficient to show whether there is any difference in the tyrosine requirement between the two phenylalanine levels used.

The data presented in Fig. 1 show clearly that the chick has a definite requirement for phenylalanine (0.6 to 0.8 per cent), and that in addition tyrosine or phenylalanine must be supplied at an approximately equal level in order to satisfy the tyrosine requirement. This is analogous to the sulfur amino acid requirements, in which the cystine requirement can be satisfied by cystine or methionine, but only methionine can satisfy the methionine requirement for tissue formation.

Feed Consumption—In Experiment 2 feed consumption of all chicks was
low for the 1st day; thereafter there were individual daily fluctuations which are not of importance to this study. In Experiment 3 this lag in consumption was eliminated by feeding a diet of a consistency similar to the amino acid diet before the experiment was started. The actual amounts are important in so far as they allow calculation of the amino acid and nitrogen intakes.

Retention of Ingested Amino Acids—From the feed consumption data, it was possible to calculate the amounts of phenylalanine, tyrosine, and nitrogen ingested. From the initial chick weight, the amino acid content of each carcass was estimated (9), and the actual amount was determined by analysis at the end of the experiment. The difference between the initial and final contents (the amount retained) was then divided by the amount ingested and multiplied by 100 to give the per cent retained of nutrient consumed. Some of these percentages were negative, and some were indeterminate (when a phenylalanine-free diet was fed, the percentage of phenylalanine retained could not be calculated).

The retention values for the amino acids are actually minimum values for the efficiencies of utilization, for they are the recoveries of the amino acids themselves. Any phenylalanine transformed into tyrosine or other amino acids is not included in the percentage retained; yet this amount would have to be included if a value were to be called an efficiency of utilization.11

In Fig. 2 the results of these retention calculations are presented for Experiment 2, in which 2 per cent tyrosine (corrected) was a constant component of the diets. The curve for phenylalanine rose rapidly from a large negative value to about 35 per cent at 0.6 per cent phenylalanine, after which it decreased to about 7 per cent at 2 per cent phenylalanine. The nitrogen retention followed a similar course, but did not decrease as much as did phenylalanine at the higher levels. This level of tyrosine did not allow its efficient use at any level of phenylalanine; the highest value observed was 9 per cent.

When a lower level of tyrosine was employed (Experiment 3, Fig. 3), the contents of the carcasses at the end of the experiment were determined by analysis rather than by calculation from body weight and previously published data (9), because the effect of the diet on the composition of the body proteins was not known (cf. also "Constancy of tissue protein composition," below).

11 The work of Moss and Schoenheimer (7) indicated that more than 25 per cent of ingested phenylalanine is transformed to tyrosine and incorporated into the body proteins of growing rats fed a diet adequate in both these amino acids. A calculation of the chick results on the basis of this work increases the phenylalanine utilization from the retention value of 14 to 21 per cent, and decreases the tyrosine retention from 18 to 5 per cent. These results are calculated from the chicks of Experiment 3 which were fed 1.1 per cent tyrosine and 2.0 per cent phenylalanine.
the same curve forms were found. The phenylalanine retention decreased as the diet became overloaded with this amino acid, but the nitrogen retention did not decline. It will be recalled that rate of growth was adversely affected by high phenylalanine levels when tyrosine was present at the 2 per cent level, but not at the 1 per cent tyrosine level.

As would be expected, the tyrosine retention was higher than before, but still below the nitrogen retention.

In both experiments (Figs. 2 and 3) the optimum percentage retention of phenylalanine was practically identical with that of nitrogen. If only one of the phenylalanine isomers were being used for tissue formation, the phenylalanine retention would be only about half the nitrogen retention. Thus it appears that the isomers of phenylalanine are of equal value in promoting growth in the chick, as they are in the rat (10) and mouse (4).
These retentions were approximately equal to those obtained when the diets fed were of a purified type, or composed of natural feedstuffs.\textsuperscript{12}

When the phenylalanine level was suboptimum (0.4 per cent, Fig. 4), nitrogen retention was markedly increased as the dietary tyrosine level was increased, although this retention curve was definitely below that obtained with 0.8 per cent phenylalanine. The phenylalanine curves of

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4}
\caption{The effects of various levels of tyrosine (foot-note 9) on the percentage retention of nitrogen when the phenylalanine content of the diet was marginal (0.8 per cent) or submarginal (0.4 per cent).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig5}
\caption{The relation of the tyrosine content of the diet to the percentage retention of phenylalanine (Pa) at two phenylalanine levels.}
\end{figure}

Fig. 4 and Fig. 5 show that tyrosine aided in the retention of phenylalanine at both levels of phenylalanine, but that a greater effect was produced when the phenylalanine level of the diet was suboptimum.

\textsuperscript{12} A purified diet, in which all the 20 per cent protein was provided by a mixture of casein, arginine, cystine, and glycine, yielded the following retentions (the average and the range of four chicks): nitrogen 40 per cent (34 to 43); phenylalanine 32 per cent (27 to 35); tyrosine 20 per cent (18 to 22). Casein contributes slightly more phenylalanine to the diet than is necessary to satisfy the chick requirements; hence the phenylalanine retention is lower than that of nitrogen.
It was indicated in Figs. 2 and 3 that dietary tyrosine was never efficiently retained when high levels of tyrosine were present in all diets. When only a minimum level of phenylalanine was fed, an appreciable amount of ingested tyrosine was retained at low tyrosine levels (Fig. 6). The most efficient retention of tyrosine was found when the diet contained marginal phenylalanine and insufficient tyrosine for best growth.

**Constancy of Tissue Protein Composition**—The extent to which an animal existing under the stress of a deficient diet can change the amino acid contents of its tissue protein is not well known. Considerable work has been done on this subject, involving principally carcass analyses for nitrogen and sulfur rather than for individual amino acids. The early work (reviewed by Cathcart (11) and Lee and Lewis (12)) yielded no unequivocal answer. Consequently Lee and Lewis (12) investigated the problem by analyzing carcasses of rats that were fasted, or fasted and refed, or fed a cystine-deficient diet. The amino acids studied were cystine, tyrosine, and tryptophan, and in these no deviations from normal were found under any of these conditions.

Pertinent data obtained from Experiment 3 are presented in Table I, from which it is apparent that there is no consistent effect of the various dietary levels on the levels found in the protein. There are several conclusions to be drawn from this fact: (1) The animal must fit the amino acids available into a rigid pattern of amino acids in tissue protein; if the diet does not contain a particular amino acid, either the animal must manufacture it, or new tissue will not be formed, and old tissue will break down.

**Table I**

Relation of Diet to Amino Acid Composition of Carcass Crude Protein (N × 6.25)

<table>
<thead>
<tr>
<th>Phenylalanine</th>
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<td>Deficient</td>
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down. (2) There is no appreciable amino acid storage. This has long been apparent from the rapid effect on growth noted after an animal is placed on an amino acid-deficient diet. (3) When fed a diet deficient in an essential amino acid, the animal wastes all amino acids, for the proportions are kept constant in its tissues. (4) Finally, and perhaps most important, we are justified in considering the rate of growth as the most important criterion of dietary protein adequacy, because it is a measure of the actual amount of amino acids laid down in the tissue proteins.

**Fig. 6.** The relation of the tyrosine content of the diet to the percentage retention of tyrosine (Ty) at two phenylalanine levels.

**SUMMARY**

The chick requires 0.6 to 0.8 per cent DL-phenylalanine in its diet for optimum growth, plus an equal amount of either phenylalanine or L-tyrosine to provide for the tyrosine requirement. Tyrosine can thus spare phenylalanine only when marginal or submarginal levels of phenylalanine are present.

Under optimum conditions, approximately 35 per cent of the ingested phenylalanine is incorporated into the body proteins. Because this percentage is practically identical with the per cent of nitrogen retained, it is concluded that both optical isomers of phenylalanine are used by the chick, as they are in the rat and mouse. Tyrosine is not efficiently retained unless the phenylalanine level for growth is marginal and the tyrosine level is submarginal.

The phenylalanine and tyrosine contents of the diets had no effect on the levels of these amino acids in the tissue proteins.
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