Tryptophan Content of Casein as Determined by a Rat Growth Method.* (22783)

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Extreme variations in values for the tryptophan content of casein have appeared in the literature(1). These have ranged from 1.68%, obtained by applying a colorimetric method to the intact protein(2) to 0.92% listed in(3). Using the colorimetric method referred to above, analysis of alkaline hydrolysates gave a value of 1.24%(4).† The latter value corresponds closely to those found by microbiological assay of alkaline hydrolysates, 1.20%(5), 1.24%(6), 1.18-1.24%(7), or enzyme hydrolysates 1.28%(6), 1.31%(8), and 1.20%(1). Lower values can be attributed to the extreme ease with which tryptophan is destroyed by hydrolysis procedures or to incomplete hydrolysis in the case of enzymatic methods. The higher values may result from lack of specificity of the chemical methods which have been used. In this connection, it is interesting to note that a bioassay with *Tetrahymena geleii*(9), in which preliminary hydrolysis is not required, has given results which agree closely with those of most microbiological assays.

In view of this extreme variation, probably due largely to difficulties in hydrolysis, it seemed desirable to apply an animal assay to this problem. Such assays have been widely used for most essential nutrients and it seemed particularly applicable to tryptophan, since the report by Oesterling and Rose(10) indicated that the rat's weight gain is related linearly to the L-tryptophan dosage, when the latter is varied from 0.10 to 0.15% of the diet. The chief source of error in this assay method might result from incomplete hydrolysis of the protein with accompanying incomplete absorption of tryptophan from the small intestine. This would tend to give low values but the same errors would be encountered whenever casein is employed in rat feeding experiments. On the other hand, high values might result if the free tryptophan used as the standard were not utilized as well as that released from the digested casein.

Methods. Female weanling rats weighing 55 to 65 g were obtained from the local stock colony. The experimental period was 4 weeks during which time the animals were housed in individual cages in an air-conditioned room and given food and water *ad libitum*. Weekly weight gains and daily food consumption were recorded. The basal diet contained 15% acid-hydrolyzed casein. This preparation, previously described(11), had the following amino acid composition: phenylalanine, 4.0%; histidine, 1.1%; methionine, 2.7%; arginine, 2.6%; valine, 6.2%; leucine, 8.0%; threonine, 2.9%; isoleucine, 4.7%; lysine, 5.9%; total nitrogen, 13.28%. The remaining components of the basal diet were: 4% Salts IV(12), 2% vitamin mixture, 0.1% L-histidine, 0.2% L-cystine, 5% corn oil and sucrose to make 100%. The vitamin mixture provided the following quantities per kg of diet: thiamine • HCl 2 mg, riboflavin 3 mg, pyridoxine • HCl 2.5 mg, calcium pantothenate 20 mg, choline chloride 1 g, inositol 100 mg, biotin 0.1 mg, folic acid 0.2 mg, vit. B_{12} 17 μg, and niacin 25 mg. Levels of L-tryptophan from 0.04 to 0.15% or of casein from 4 to 9% were incorporated into the basal diet with suitable reductions in the acid-hydrolyzed casein to maintain the nitrogen level of the diet at 1.99%. The L-tryptophan used was obtained from the Nutritional Biochemicals Corp., Cleveland, Ohio. The casein employed in these experiments was vitamin-free Labcocasein (Borden Co., N. Y.) and con-

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† The recent use of the higher values in the design and interpretation of nutritional experiments prompted the studies reported here. See Ebisuzaki, K., Williams, J. N., Jr., and Elvehjem, C. A., *J. Biol. Chem.*, 1952, v198, 63, and Hopper, J. H., and Johnson, B. C., *J. Nutrition*, 1953, v56, 303.

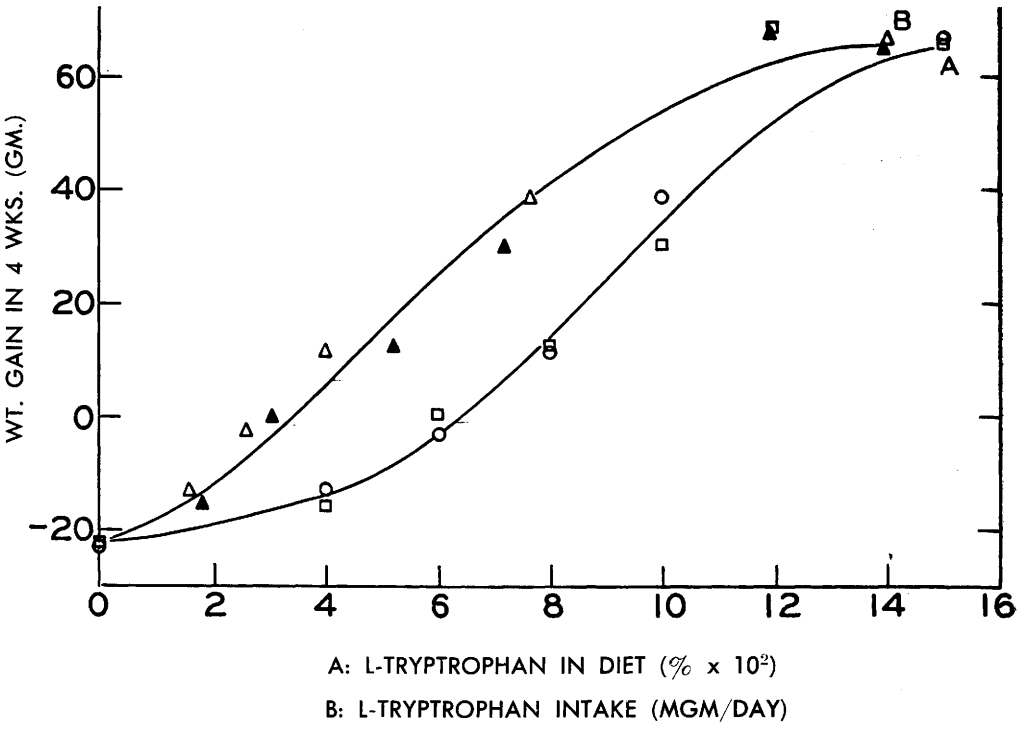

FIG. 1. Standard dose-response curves for tryptophan expressed as % of diet and as intake in mg/day.

tained 14.64% nitrogen on a moisture-free basis.

Results. Fig. 1 presents standard curves representing the data from two experiments. From the daily food consumption and the level of tryptophan in each diet, it was possible to calculate the daily L-tryptophan intake which is plotted against the growth rate in curve B. Curve A was constructed by plotting the growth rate versus the level of L-tryptophan in the diet. The experimental points from both studies are presented, but since the results were in good agreement, single standard curves were drawn. In each experiment the increase in body weight during 4 weeks for each level of casein was measured; these growth rates in conjunction with curve A provide the basis for the calculation of the per cent tryptophan in these diets and, from this, the per cent tryptophan in the casein (Column A, Table I). The most nearly linear portion of the dosage-response curve (A), which gives the most reliable value for the tryptophan present in casein, lies between 0.05 and 0.12% L-tryptophan. Food consumption data on the same animals were

TABLE I. L-Tryptophan Content of Casein.*

Exp.	Casein in diet, %	Avg wt gain 4 wk, g	Tryptophan in casein (%) A†	B†
I	4	− .5	1.56‡	1.82‡
	6	7.5	1.22	1.32
	8	36.5	1.28	1.33
		Avg	1.35 ± .08§	1.49 ± .29
II	5	−4.0	1.16	1.33
	6	8.5	1.23	1.28
	7	14.5	1.06	1.09
	8	38.0	1.29	1.20
	9	58.5	1.44‖	1.45‖
		Avg	1.24 ± .06	1.27 ± .14

* Two animals received each level of casein in both experiments.

† Values in column A are based on Curve A; those in column B are based on Curve B (Fig. 1).

‡ Growth data yielding these values involves consideration of the less reliable lower portion of Curves A and B.

§ Stand. error of the mean.

‖ Calculation of these values involves the less reliable upper portions of Curves A and B.

employed to calculate the tryptophan content of casein from curve B.

The highest values (Table I) calculated from the growth rates with 4 and 9% casein diets appear to result from the use of less reliable portions of the dose-response curves, near or beyond the inflection points. In contrast to these seemingly unreliable values, extremely good agreement between the two experiments was obtained on the 6% and 8% casein diets. All of these values were in the range from 1.22 to 1.33% whether calculated on the basis of percentage of the diet (Column A) or actual tryptophan ingested (Column B). From the most reliable data of Experiment I and all of the results in Exp. II, the best value for the tryptophan content of casein appears to be 1.25%.

Further support for this value is given by amino acid imbalance studies. Experiments in this area have shown that rats develop a niacin deficiency on either a 9% casein diet (13) or purified amino acid diets containing 0.10% to 0.11% of tryptophan and levels of other essential amino acids approaching those required for optimum growth(14). If threonine or another essential amino acid is reduced to a level which is more limiting than tryptophan, the growth rate is improved. It has been found that the tryptophan content of the diet is critical in obtaining such unusual imbalance effects(11). The narrow range, 0.10 to 0.11% of tryptophan or 9% casein in the diet, over which these effects are observed may be used as a basis for roughly calculating the tryptophan content of casein. Thus a value of 1.11 to 1.22% is indicated from the data reported.

For the purposes of comparison with other values in the literature the one arrived at here corresponds to 1.37% when converted to the hypothetical 16% nitrogen basis or to 8.52 mg of tryptophan per 100 mg of casein nitrogen.

Summary. The tryptophan content of casein has been estimated using a rat growth assay method. Whether the results were calculated on the basis of the percentage of tryptophan in the diet or by using food consumption data to calculate the daily intake of tryptophan, essentially the same results were obtained. These animal assays indicate that casein (14.64% N) contains 1.25% tryptophan which is in good agreement with many reports based on microbioassays of alkaline and enzyme hydrolysates of this protein, but much lower than many commonly accepted figures obtained with chemical methods.

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