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PROTEIN SUPPLEMENTATION

Influence of Addition of Certain Amino Acids and Vitamin B_{12} to Proteins in Enriched Milled Wheat Flour on Growth, Protein Efficiency, and Liver Fat Deposition

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The influence of addition of certain amino acids and vitamin B_{12} to proteins in enriched milled wheat flour on growth, protein efficiency, and liver fat deposition was investigated. The proteins in milled wheat flour were fed to albino rats at an 8% level for 10 weeks. The optimum gains in body weight and protein efficiency were secured by supplementation with 0.4% L-lysine, 0.2% DL-threonine, 0.4% DL-methionine, and vitamin B_{12}.

THE BENEFICIAL EFFECTS of additions of several essential amino acids and vitamin B_{12} to the proteins in cereal grains have been demonstrated (*7–11, 13, 15*). This study shows the influence of additions of methionine, and increasing amounts of lysine, threonine, and valine—in the presence and absence of vitamin B_{12}—to the proteins in enriched milled wheat flour on growth, protein efficiency, and liver fat deposition. This investigation was stimulated by the recent reports of Harper and associates that certain combinations of amino acids may produce imbalance, toxicities, and antagonisms (*4*). Elvehjem has recently reviewed the findings of his Wisconsin group on the effects of amino acid imbalance on maintenance and growth (*2*).

Recently Deshpande and associates (*1*) found in 2-week experiments that the retardation in growth caused by including 0.4% of L-lysine hydrochloride in a rice diet was prevented by increasing the levels of leucine, isoleucine, valine, and histidine. In longer experiments the growth-retarding effect of additional lysine disappeared without the addition of other amino acids in the diet. Supplementation of the rice diet with various combinations of amino acids, from which one or more of leucine, isoleucine, valine, and histidine had been omitted, resulted in a retardation of growth. In all instances in which the ration contained at least 0.4% of additional L-lysine hydrochloride, the fat content of the liver approached a normal value. Even after 7 weeks an accumulation of liver fat occurred, unless the rice diet was supplemented with this level of lysine.

As the Food and Nutrition Board of the National Research Council is now investigating the matter of protein nutrition in the United States and the advisability of amino acid enrichment of cereal foods, it was thought particularly essential to study the possible injurious effects of increasing concentrations of lysine, threonine, and valine to the proteins in enriched milled wheat flour fed at a higher level of protein intake, 8%, than Deshpande and associates used in their rice diets fed at a lower plane of protein intake, 5.9% (*1*).

Experimental Procedure and Materials

This study was carried out, for 10 weeks, on the Wistar strain of albino rats, which were 30 days old when started on experiments and weighed 50 to 54 grams each. There were 12 animals in each group, equally divided between male and female. The rations contained 8% of proteins (supplied entirely by enriched wheat flour); 2% of Cellu flour for roughage; 4% of Sure's salts No. 1 (*14*); 7% of hydrogenated vegetable shortening; 2% of cod liver oil; 1% of wheat germ oil; and the rest, percentagewise, glucose (Cerelose). The fat-soluble vitamins A, D, and E were supplied by cod liver oil and wheat germ oil in the rations. All rations were supplemented with a liberal supply of the B vitamins, separately, six times weekly with double doses on Saturdays (*12*). The vitamin B_{12} was prepared daily in aqueous solutions and administered to each animal in Petri dishes in a daily dose of 0.1 γ per animal per day. The animals were weighed once weekly and accurate records were kept of food consumption. From these data the protein efficiency ratios were calculated, and expressed as gains in body weight per gram of protein intake.

The amino acids added some nitrogen to the rations and the food consumption was multiplied by 8, which gives the total protein intake, but does not express the total nitrogen intake. However, it has been demonstrated (*9, 10*) that such amino acid additions contribute so little nitrogen that when calculations are made on gains in body weight per gram of nitrogen intake the relative results obtained approximate those of gains per gram of protein intake; hence figures for total nitrogen intake have not been included.

The liver fats were covered with 95% ethyl alcohol, dried overnight at 53° C., and extracted with petroleum ether for

Table I. Influence of Additions of Methionine, and Increasing Doses of Lysine, Threonine, and Valine, in Presence and Absence of Vitamin B_{12} to Proteins in Enriched Milled Wheat Flour on Growth, Protein Efficiency, and Liver Fat Deposition

Ration Number	Ration[a]	Gains in Body Wt., G.	Protein Intake, G.	PER[b]	Liver Fat Dry Wt., %	Change, %
1	Milled wheat flour	21.6 ± 2.1	35.7	0.60 ± 0.04	28.0 ± 1.24	...
2	+0.4% L-lysine	91.2 ± 8.4	52.0	1.75 ± 0.06	24.7 ± 1.60	−11.8
3	+0.4% L-lysine +0.1 γ B_{12}[c]	102.5 ± 9.3	55.8	1.84 ± 0.07	30.2 ± 1.38	+ 7.9
4	+0.4% L-lysine +0.4% DL-methionine	112.3 ± 10.1	54.8	2.05 ± 0.08	18.8 ± 1.21	−32.9
5	+0.6% L-lysine	87.3 ± 7.6	49.2	1.77 ± 0.06	21.4 ± 1.50	−23.6
6	+0.6% L-lysine +0.1 γ B_{12}[c]	89.3 ± 8.3	47.8	1.87 ± 0.05	20.0 ± 0.80	−28.6
7	+1.0 L-lysine	91.7 ± 8.9	46.3	1.98 ± 0.09	21.9 ± 1.03	−21.8
8	+1.0 L-Lysine +0.1 γ B_{12}[c]	95.3 ± 9.2	50.5	1.89 ± 0.07	26.4 ± 1.03	− 5.7
9	+0.4% L-lysine +0.2% DL-threonine	146.2 ± 10.1	58.7	2.49 ± 0.04	18.0 ± 1.10	−35.7
10	+0.4% L-lysine +0.2% DL-threonine +0.1 γ B_{12}[c]	148.8 ± 10.3	67.3	2.21 ± 0.05	18.4 ± 1.10	−34.3
11	+0.4% L-lysine +0.4% DL-threonine	139.9 ± 9.7	68.8	2.03 ± 0.09	18.0 ± 1.30	−35.7
12	+0.4% L-lysine +0.4% DL-threonine +0.1 γ B_{12}[c]	138.1 ± 9.9	65.3	2.11 ± 0.06	19.6 ± 0.80	−30.0
13	+0.6% L-lysine +0.6% DL-threonine	131.4 ± 6.9	60.4	2.17 ± 0.05	16.8 ± 0.70	−40.0
14	+0.6% L-lysine +0.6% DL-threonine +0.1 γ B_{12}[c]	143.3 ± 11.1	64.1	2.24 ± 0.08	15.6 ± 0.90	−44.3
15	+1.0 L-lysine +0.6% DL-threonine	132.2 ± 10.9	63.1	2.10 ± 0.09	17.5 ± 2.40	−37.5
16	+1.0 L-lysine +0.6% DL-threonine +0.1 γ B_{12}[c]	154.6 ± 12.3	69.9	2.21 ± 0.10	18.3 ± 1.30	−34.6
17	+0.4% L-lysine +0.2% DL-threonine +0.4% DL-valine	134.4 ± 9.2	64.2	2.10 ± 0.07	22.0 ± 0.85	−21.4
18	+0.4% L-lysine +0.2% DL-threonine +0.4% DL-valine +0.1 γ B_{12}[c]	170.0 ± 11.5	69.9	2.43 ± 0.10	22.7 ± 1.24	−18.9
19	+0.4% L-lysine +0.2% DL-threonine +0.7% DL-valine	127.7 ± 10.2	59.9	2.13 ± 0.09	15.8 ± 0.92	−43.6
20	+0.4% L-lysine +0.2% DL-threonine +0.7% DL-valine +0.1 γ B_{12}[c]	145.5 ± 11.3	68.0	2.14 ± 0.05	18.9 ± 0.76	−32.5
21	+0.4% L-lysine +0.2% DL-threonine +0.4% DL-methionine	138.6 ± 7.6	62.9	2.20 ± 0.06	19.5 ± 1.31	−30.3
22	+0.4% L-lysine +0.2% DL-threonine +0.4% DL-methionine +0.1 γ B_{12}[c]	192.8 ± 12.6	75.1	2.57 ± 0.12	21.2 ± 1.18	−24.3

[a] Fed at 8% protein level for 10 weeks. Average results per animal.
[b] Protein efficiency ratio, expressed as gains in body weight per gram of protein intake.
[c] Per animal per day.

4 hours in a Laboratory Construction Co. extractor. The dry weight of the livers was obtained after determining the moisture content after extraction at 103° C. The fat deposition is expressed as per cent in the dried livers.

The results of this study are summarized in Table I.

Discussion

As the liver is the central organ of protein metabolism and as protein malnutrition is intimately connected with pathological alterations of the liver associated with excessive fat accumulation and/or hemorrhages (*3*), liver fat deposition is another yardstick used in this study for evaluation of the nature of responses obtained by supplementing the proteins of enriched milled wheat flour with several essential amino acids and vitamin B_{12}. Increase of body weight accompanied by excessive accumulation of liver fat could not be considered a standard of nutritional improvement.

There is no parallelism between gains in body weight produced by the various amino acid and vitamin B_{12} additions and liver fat deposition (Table I)—i.e., nutritional improvement as manifested by growth and increase in protein efficiency is not always accompanied by reduction in liver fat. In many instances, there are marked reductions in liver fat accompanied by slight amino acid imbalances, as indicated by small losses of body weight and slight reduction in protein efficiency ratios.

The most efficient concentration of L-lysine in the ration was 0.4%. The supplementation of ration 2 containing 0.4% L-lysine with 0.1 γ of vitamin B_{12} per animal per day was accompanied by appreciable increased growth and increase in protein efficiency ratio. As vitamin B_{12} has been shown to be functioning in methionine synthesis (*5*, *6*) this vitamin supplementation was replaced by addition of 0.4% DL-methionine in ration 4. Probably part of the beneficial effects of vitamin B_{12} was due to methionine synthesis but the influence

on liver fat deposition was markedly different—i.e., a marked reduction due to methionine and a small increase due to vitamin B_{12} (ratios 3 and 4).

Vitamin B_{12} acted as an efficient supplement to other amino acid combinations, but its physiological mechanism is not evident from this study. The best illustration of the supplementary value of vitamin B_{12} can be noted in rations 21 and 22. The addition in ration 21 of 0.4% DL-methionine to ration 9 produced a growth of 138.6 grams and a protein efficiency ratio of 2.20, which is less than that secured on ration 9, but the fortification of this ration with vitamin B_{12} in ration 22 not only counteracted the slight amino acid imbalance but was responsible for the production of maximum gain in body weight, 192.8 grams, and an optimum protein efficiency ratio of 2.57. As ration 21 was fortified with methionine, it would appear that vitamin B_{12}, in addition to functioning in synthesis of this sulfur-containing amino acid, increases the utilization of other amino acid combinations as supplements to the proteins in enriched milled wheat flour by a mechanism which will probably be clarified by future research.

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NUTRIENTS IN COFFEE

Nutritional Evaluation of Coffee Including Niacin Bioassay

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The presence of approximately 10 mg. of niacin in 100 grams of ordinary retail coffees, as measured microbiologically, was confirmed by rat assay. Niacin level is dependent on degree of roasting. Experimental dark roasts contained up to 43 mg. of niacin per 100 grams of coffee and equally high levels were found in some specialty coffees obtained on the open market. The niacin is readily extracted in the preparation of beverage. Appreciable, but rather low, levels of seven B vitamins, other than niacin, were present in coffee beverage. Moderate amounts of extractable calcium and iron and low levels of sodium and fluorine were found in roasted coffee.

IT WAS REPORTED IN 1944 THAT ordinary retail roasted coffees tested in the United States contained about 10 mg. of niacin per 100 grams of coffee as measured by microbiological assay (*23*). The niacin was found to be easily extractable with water and samples of restaurant coffee obtained in mid-western United States contained approximately 1 mg. of niacin per 175-ml. cup of coffee. Attempts to carry out bioassays with the chick and the dog met with some difficulties, but the results suggested that the niacin in coffee is biologically active (*24*).

In these studies, comparative microbiological and rat assays were performed on coffee beverage. A survey was made of the niacin content of ordinary and specialty retail coffees marketed in the United States. The relatively low niacin content of green coffees as compared to roasted coffee had suggested that most niacin in roasted coffee is formed during the roasting process, possibly from trigonelline (*N*-methylbetaine of nicotinic acid), which is present at rather high levels in the coffee bean but has no biological niacin activity (*24*). The niacin content of various experimental roasts is reported and data are given on four minerals and eight B vitamins other than niacin.

Materials and Methods

Coffee Samples. All of the determinations of minerals and vitamins, other than niacin, were performed on single samples of: green coffee, a popular roasted retail coffee made from the same batch of green coffee (and with a typical niacin content), and beverage made from this roasted coffee. Various other roasted coffee samples were obtained for niacin assay only.

Experimental Roasts. One-pound batches of coffee were prepared in a Burns gas-fired laboratory roaster. The lightest roast required 8.5 minutes and was judged visually to be lighter than ordinary commercial roasts. The darkest roast required 23 minutes and was judged to be overroasted. The next to the darkest roast required 16 minutes and was judged to have a color comparable to the most heavily roasted commercial coffee.

Analytical Procedures. Microbiological assays were employed for niacin (*14*), choline (*8*), pantothenic acid (*20*),