Effects of Marginal and Optimal Intakes of B Vitamins on Protein Utilization by the Growing Rat from Varied Dietaries[1]

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The adverse effect of uneven distribution of the protein and carbohydrate moieties of the diet on nitrogen balance has been well demonstrated in humans (Cuthbertson and Munro, '39; Munro and Wikramanayake, '54), dogs (Larson and Chaikoff, '37; Munro and Wikramanayake, '54), and rats (Cuthbertson, McCutcheon and Munro, '40; Munro, '49; Lathe and Peters, '49; Geiger, Bancroft and Hagety, '50; Munro and Wikramanayake, '54). It has also been shown that with casein (Sure and Romans, '48) and fibrin (Sure, '50) diets, increasing concentrations of B vitamins increase growth and protein utilization. Impaired reproductive function in rats on low-protein diets is known to be improved by raising the level of dietary B vitamins (Nelson and Evans, '53). Equitable distribution of vitamin supplements is also apparently essential for effective feed utilization in poultry (Waibel, Cravens, Bird and Baumann, '54).

It was therefore of interest to ascertain if, in split feeding of the protein and non-protein components of diet, variations in levels of B vitamins have a functional significance in the utilization of proteins in rats. Such considerations will have implications in practical human nutrition where widespread extremes may often exist in the composition and distribution of individual meals (Platt and Miller, '58).

EXPERIMENTAL AND RESULTS

Split feeding of protein and other caloric components of a diet

Adult male Wistar rats 200 to 210 gm in weight were used. The composition of the experimental diets is presented in table 1.

The animals were fed a protein-free "depletion" diet (diet 1), with fasting on alternate days to accelerate mobilization of body protein. After 12 days, when the animals weighed approximately 170 gm, they were divided into 4 comparable groups, receiving the composite (diet 2), or split (diets 3 and 4) rations supplemented with B vitamins at one of two levels, a low minimal and a high optimal. The vitamin and mineral additions were more or less proportionally distributed in the protein and non-protein caloric moieties of the diet in the split-fed groups.

The animals had access to the diets in excess quantities and in scatter-proof cups twice a day at 9.30 A.M. and at 4.30 P.M. The order of feeding the two portions of the split-fed diet was reversed on successive days. Feed-cups were removed after one-half hour and the residues weighed.

Nitrogen retention studies were made during 4, 4-day periods. Animals were placed in individual, round metabolism cages. Urine and feces were collected before the morning feeding. Urine was stored with the addition of a few drops of sulphuric acid and toluene. Feces were dried at 80°C for 24 hours and weighed. The nitrogen content of the excreta pooled for each 4-day period was determined by the Kjeldahl method. In table 2 are presented the data on nitrogen retention and efficiency.

In order to obviate the effects of differences in food intake, the experiment was repeated with isocaloric feeding. The protein-depleted animals were fed 10 gm of

Received for publication May 25, 1959.

[1] This investigation was supported by a research grant from the Williams-Waterman Fund, Research Corporation, New York.

the diets daily. The split-fed groups received this quantity in two installments, 2.3 gm of the protein moiety (diet 3) in one meal and 7.7 gm of the caloric moiety (diet 4) in the following meal; both portions were proportionately represented with respect to the vitamin-mineral supplements (table 1). The results are included in table 2.

In animals fed ad libitum and given the minimal as contrasted with optimal amounts of B vitamins (table 2), split feeding markedly influenced the rate of protein repletion, evidenced by the well-defined differences in the data for nitrogen retention, weight gain and nitrogen efficiency, the increases being 3.5, 4.7 and 1.5 times the "minimal" values, respectively. This was also true under the conditions of restricted (isocaloric) feeding, but in this case the increases were more nearly the same, being 2.1, 2.8 and 2.7 times the "minimal" values for nitrogen retained, weight gained and nitrogen efficiency, respectively.

These studies were extended to a rice-legume diet based on a typical Indian vegetarian meal (table 1). The composite

TABLE 1

Composition of the diets used

Component	Diet no.[1]									
	Depletion	Experimental								
	1	2	3	4	5	6	7	8	9	10
	gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
Devitaminized casein	—	20	20	—	—	—	—	10	7.7	—
Corn starch	65	65	—	65	—	—	—	—	19.3	—
Cellulose[2]	5	5	1.2	3.8	—	—	—	—	—	—
Dextrin	20	—	—	—	—	—	—	—	—	—
Vitaminized sucrose‡	1	1	0.2	0.8	—	—	—	1	1	1
Vitaminized arachis oil†	5	5	1.2	3.8	5	2.55	2.45	5	5	5
Salt mixture[3]	4	4	0.9	3.1	—	—	—	4	4	4
Rice	—	—	—	—	37.5	—	37.5	45	35	90
Legume[4]	—	—	—	—	30	30	—	35	28	—
Fresh milk	—	—	—	—	10	10	—	—	—	—
Non-leafy vegetables[5]	—	—	—	—	10	5.1	4.9	—	—	—
Leafy vegetables[6]	—	—	—	—	5	2.55	2.45	—	—	—
Spice mixture[7]	—	—	—	—	2.5	1.3	1.2	—	—	—

‡ B vitamins per gm sucrose	Optimal	Minimal
	mg	*mg*
Thiamine·HCl	0.3	0.015
Riboflavin	0.4	0.015
Pyridoxine·HCl	0.3	0.015
Ca pantothenate	1.0	0.1
Niacin	2.0	0.1
Biotin	0.05	nil
Folic acid	0.1	0.003
Vitamin B_{12}	0.015	nil
Inositol	20.0	5.0
Choline Cl	20.0	5.0
Menadione	0.5	0.5

† Fat-soluble vitamins per 5.0 gm arachis oil	
	mg
Vitamin A acetate	0.31
Vitamin D (calciferol)	0.0045
Alpha tocopherol	5.0

[1] Additions of B vitamins to diets 2, 3, 4 and 8, 9, 10 were either at optimal or at minimal levels. Diets 5, 6, 7 were with or without supplements of B vitamins at the optimal level. B vitamins in the "depletion diet" were at optimal levels.

[2] Acid-washed powdered filter paper.

[3] U.S.P. XIV salt mixture.

[4] *Cajanus indicus.*

[5] The non-leafy vegetables used were edible portions of eggplant and okra in equal proportion.

[6] Leafy vegetables included amaranth and spinach in equal proportion.

[7] The spice mixture consisted of (parts): sodium chloride, 1; tamarind, 0.7; pepper, 0.1; chillies, 0.1; tumeric, 0.1; and cinnamon, 0.1; with additions of calcium (as calcium lactate) and iron (as ferrous ammonium sulphate) at levels of 50 mg and 2 mg respectively per 100 gm of the spice mixture.

TABLE 2

Effect of variations in dietary levels of B vitamins on nitrogen balance and nitrogen efficiency in protein-depleted rats refed the protein and caloric moieties of the diet separately at intervals (split-ration) or concurrently (composite ration)

Ration	Level of B vitamins[1]	Nitrogen balance[2]			Weight gain[3]	Nitrogen efficiency[4]
		Nitrogen intake	Caloric intake	Nitrogen retained		
		mg	*Cal.*	*mg*	*gm*	
		Ad libitum feeding				
Split	Minimal (6)	355 ± 21[5]	41 ± 2	58 ± 13	27 ± 3	4.8 ± 0.9
Split	Optimal (5)	1088 ± 75	68 ± 3	204 ± 37	128 ± 7	7.3 ± 0.7
Composite	Minimal (6)	342 ± 21	44 ± 3	91 ± 14	46 ± 5	8.3 ± 0.7
Composite	Optimal (5)	567 ± 14	71 ± 2	222 ± 13	137 ± 6	15.1 ± 1.1
		Restricted (isocaloric) feeding[6]				
Split	Minimal (6)	306 ± 13	38 ± 2	52 ± 8	20 ± 3	4.1 ± 0.3
Split	Optimal (6)	320 ± 4	40 ± nil	108 ± 3	56 ± 6	10.9 ± 0.8
Composite	Minimal (6)	301 ± 9	38 ± 1	92 ± 8	24 ± 3	5.0 ± 0.4
Composite	Optimal (6)	320 ± 4	40 ± nil	121 ± 4	88 ± 8	17.1 ± 0.7

[1] Figures within parentheses indicate the number of animals in each group.

[2] Figures represent mean values per rat per day and are calculated from data obtained for each animal of the group over 4 separate periods of 4 days' duration each.

[3] Mean gains per rat over entire period of 16 days of nitrogen balance study.

[4] Mean grams gain per day per gram of nitrogen consumed.

[5] Standard error of the mean.

[6] The small differences in caloric intake are due to traces of left-over food.

diet (diet 5) provided 320 Cal. per 100 gm and 15.1 % of protein, which was split between the predominantly protein component (legume-milk) (diet 6) and the predominantly carbohydrate component (rice) (diet 7) in the proportion 11.2:3.9. The composite and split rations were fed with or without a supplement of B vitamins at the optimal level, as indicated in table 1. Distribution of the vitamin supplement between the protein and carbohydrate moieties of the split ration was in the proportion of 1:4. During the experimental regeneration period feeding was ad libitum.

The data presented in table 3 again indicated an impairment of nitrogen retention as a result of separating the major protein and carbohydrate moieties of the diet. A similar effect, although less marked, was manifested in the growth rate. Nitrogen retention was improved markedly with vitamin supplementation and the deleterious effect of split feeding was reduced simultaneously. Nitrogen efficiency was, apparently, not influenced by the feeding procedure.

Intermittent feeding of proteins from a rice-legume-casein diet

In the next experiment, the effects of alterations in the level of protein feeding from day to day was studied in relation to dietary levels of B vitamins. The composition of the diets used is shown in table 1. Weanling male rats, 40 gm in weight, were fed alternately on diets with 18% of protein (diet 8) and 6% of protein (diet 10) on successive days. Such short-term protein restriction does not create a specific protein hunger in the rat (Geiger, Rawi and Thomas, '55). A ration providing 14% of protein (diet 11) was offered daily to the control group. The diets were based on rice for the low-protein ration, and on suitable proportions of rice, legume and casein for the two higher protein ones. After thorough mixing, the diets were steam-cooked for 45 minutes, dried at 70°C under vacuum and powdered. Comparable groups of animals received these diets ad libitum, varied with respect to the two levels, minimal and optimal, of the B vitamins. Food consumption and body weight records were recorded throughout the 6-week experimental period.

At the end of 6 weeks blood and sections were removed from the animals under ether anesthesia. Blood obtained from the hepatic portal vein was quickly heparinized and the plasma separated by centrifuging in the cold. Protein-free plasma filtrate was prepared by the procedure of Hier and Bergeim ('45); total and non-protein nitrogen was determined by direct Nesslerization following its initial liberation by Kjeldahl micro-digestion (Umbreit, '46). Nitrogen in liver homogenates and in protein-free preparations (obtained by trichloroacetic acid precipitation of the proteins) of the tissue was similarly determined. Total liver lipide was estimated by the method of Sperry ('54).

TABLE 3

Utilization of a rice-legume diet as influenced by split-feeding of its major protein and carbohydrate moieties and the effects of additional ingestion of a mixture of B vitamins[1]

Ration	B vitamins[2]	Nitrogen balance[3]			Gain in weight[4]	Nitrogen efficiency[5]
		Nitrogen intake	Caloric intake	Nitrogen retained		
		mg	*Cal.*	*mg*	*gm*	
Split	−	211 ± 15	28 ± 3	55 ± 8	33 ± 6	13.0 ± 0.4
Split	+	271 ± 16	41 ± 3	127 ± 9	48 ± 4	14.6 ± 0.3
Composite	−	262 ± 19	35 ± 3	94 ± 10	40 ± 4	12.6 ± 0.5
Composite	+	296 ± 17	40 ± 4	146 ± 9	53 ± 5	14.8 ± 0.5

[1] The data were obtained with 6 animals per group.

[2] The diets were with (+) or without (−) a supplement of B vitamins at optimal levels as indicated in table 1.

[3] Figures are mean values per rat per day with standard errors, calculated from data obtained for each animal of the group over three separate periods of 4 days' duration each.

[4] Mean weight gains per rat with standard errors (12 days).

[5] As defined in table 2.

TABLE 4

Influence of dietary B vitamins on protein efficiency and on certain tissue constituents as affected by uneven feeding of protein from a rice-legume-casein diet[1]

Diet description		Protein efficiency (5 weeks)			Tissue constituents				
					Liver[4]			Plasma	
Protein content[2]	Level of B vitamins	Protein intake	Weight gain	PER[3]	Total nitrogen	Non-protein nitrogen	Total lipids	Total nitrogen	Non-protein nitrogen
%		*gm*	*gm*		*mg/gm*	*mg/gm*	%	*mg/ml*	*mg/ml*
14	Minimal	58 ± 7	70 ± 6	1.21 ± 0.03	90 ± 4	8.8 ± 0.4	28 ± 2	7.3 ± 0.3	0.75 ± 0.04
14	Optimal	61 ± 5	128 ± 8	2.10 ± 0.21	99 ± 4	11.0 ± 1.0	39 ± 2	8.8 ± 0.2	0.49 ± 0.05
18 or 6	Minimal	55 ± 3	45 ± 4	0.81 ± 0.07	88 ± 3	11.1 ± 0.7	34 ± 3	6.9 ± 0.4	1.01 ± 0.04
18 or 6	Optimal	56 ± 5	84 ± 5	1.50 ± 0.12	95 ± 4	11.5 ± 0.5	40 ± 4	7.8 ± 0.2	0.66 ± 0.03

[1] Figures represent in each case, the mean of at least 5 replicates with its standard error.
[2] The animals were maintained either on a uniform diet with 14% protein (diet 9, table 1) or, interchangeably, on diets with 18% protein (diet 8) and 6% protein (diet 10) on successive days.
[3] Protein efficiency ratio, expressed as grams gain per gram of protein consumed.
[4] Values are expressed on the dry weight of the tissue.

The data on protein utilization and on plasma and liver constituents are presented in table 4. With both uniform and varied dietaries an increased intake of the B vitamins caused marked improvements in growth rate and efficiency of protein utilization. Further, these were associated with an increase in protein-nitrogen in liver and in plasma, increase in liver lipides and a decrease in plasma non-protein nitrogen. A varied intake of protein resulted in impaired protein utilization with consequent retardation of growth rate. These effects were not appreciably counteracted by B vitamins offered in optimal amounts. However, the non-uniform intake of protein caused an elevation of non-protein nitrogen in liver and in plasma as also in liver lipides and these effects were considerably suppressed with the higher intake of the vitamins. The reduced content of non-protein nitrogen in liver and in plasma and, hence, a possible reduction of free amino acids in these tissues with higher intake of B vitamins may point to a more efficient utilization of dietary amino acids. A similar specific effect of vitamin B_{12} has been reported in chicks (Charkey, Wilgus, Patton and Gassner, '50).

DISCUSSION

The two levels of B vitamins chosen in the present experiments were close to the optimal and minimal requirements of the animal and, hence, significant differences were noticeable in growth rate and in certain tissue constituents of the animals receiving the two supplements. However, no animal showed symptoms of avitaminosis through a period of 8 to 10 weeks. While, therefore, the greater food consumption of animals receiving the higher vitamin supplement can be attributed to a general improvement in these animals, this cannot be correlated specifically with any single vitamin deficiency *per se*.

The observations of Larson and Chaikoff ('37) and Munro ('49) indicate that deterioration in nitrogen retention as a result of separation of the protein and carbohydrate moieties of a purified diet is transitory and that very soon the animals reach a state of equilibrium. However, Geiger et al. ('50), using protein-depleted rats found that the differences in nitrogen utilization persisted during a 14-day period. The present work confirms these findings.

Cuthbertson and Munro ('39) noted that the association of a small fraction of dietary nitrogen with the carbohydrate is sufficient to maintain nitrogen equilibrium in humans. In an extension of this work, Munro and Wikramanayake ('54) concluded that protein utilization is influenced favorably by some carbohydrate in the protein-containing meals. The present work with the rice-legume diets suggest that with marginal intake of vitamins, any sort of non-uniformity or irregularity in the daily diet will adversely affect protein utilization. This appears to be so even with intermittent feeding of high- and low-protein dietaries (Geiger et al., '55; Anna, Dam-Bakker, De Groot and Luyken, '58), apparent when two deficient proteins, which in combination, form a good protein mixture, or a deficient protein and the lacking amino acid, are administered intermittently (Henry and Kon, '46; Geiger, '47, '48).

Even in such cases of imbalance, higher levels of dietary B vitamins may to some extent bring about better protein utilization, as shown. This is attributable in part, perhaps, to a more balanced selection of food components by the animal. Richter and Hawkes ('41) showed, for example, that rats select varying proportions of protein, fat and carbohydrate when supplied with different B vitamins, presumably adjusting the intake to actual needs.

The favorable influence of carbohydrate association on nitrogen retention is not related to their caloric function, as this property is not shared by fats (Thomson and Munro, '55). In other work,[2] deterioration in nitrogen retention caused by substitution of fat for dietary carbohydrate calls for altered requirement of certain of the B vitamins has been observed. Obviously, there is a good deal of flexibility in nutritional needs based upon the stresses to which the organism is exposed (Elvehjem and Krehl, '55).

SUMMARY

Adult protein-starved rats were used to compare the influence of marginal and optimal intakes of the B vitamins (thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, biotin, folic acid, vitamin B_{12}, choline and inositol) on protein utilization from diets with uneven distribution of protein and caloric moieties or with intermittently changing levels of protein.

When the protein and caloric components of a purified 20% casein diet were fed separately with a 6-hour intervening period each day, a lowering in nitrogen retention resulted as well as efficiency of protein utilization when compared to the effect of the composite diet. This effect, persisting over a three-week period, was greater in the groups receiving minimal levels of B vitamins and was somewhat offset by the inclusion of optimal amounts of B vitamins in the diet. These changes were noted in studies with animals fed either ad libitum or with restriction.

A similar effect due to B vitamins was observed in split feeding of the major protein and carbohydrate moieties of a mixed rice-legume diet at a 15% level of protein.

With weanling rats, intermittent feeding on successive days of an 18% protein ration based on rice, a legume and casein and a 6% rice-protein ration lowered the protein efficiency when compared with that observed with a uniform intake of a 14% protein diet with rice, legume and casein. This effect, again, was a little more prominent when the intake of B vitamins was minimal. The beneficial effects due to B vitamins were associated with a decrease in the non-protein content of liver and plasma and a reduction in liver lipids.

[2] Fatterpaker and Sreenivasan, unpublished data.

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