Effects of Suboptimal and Optimal Intakes of B Vitamins on Protein Utilization by the Growing Rat from Diets Containing Single and Mixed Proteins[1]

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It is recognized that vitamins may, to some extent, influence the minimal and optimal requirements of individual amino acids (Brock, '55). Sure and Romans ('48) and Sure ('50) observed an improvement in the protein efficiency of casein and fibrin with higher intakes of B vitamins. Nelson and Evans ('53) reported that impairment in the reproductive ability in rats on low-protein diets is partially corrected by doubling the vitamin supplements and the addition of vitamin B_{12}. In a previous publication (Marfatia and Sreenivasan, '60), it was demonstrated that, in the rat, B vitamins administered at optimal levels counteract the deterioration in nitrogen retention and growth rate arising from split feeding of the protein and carbohydrate moieties of the diet. The efficacy of higher dietary levels of B vitamins may be expected to differ with the over-all dietary protein quality and has now been ascertained, employing single- and mixed-protien diets derived from casein, egg albumin and wheat gluten and with variations in levels of thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, folic acid and vitamin B_{12}.

EXPERIMENTAL

Male albino rats of the Wistar strain were used. Prior to the experimental period these were reared on a balanced laboratory ration consisting of (percentages by weight): whole wheat flour, 75; casein, 12; whole milk powder, 2; yeast powder, 2; sodium chloride, 2; calcium carbonate, 2; arachis oil, 3; and shark liver oil, 2.

The composition of the experimental diets is given in table 1. Animals were housed individually in raised mesh-bottom cages and had free access to food and water.

Experiment 1. Trials with casein and egg albumin

In the first series, 32 rats with an average weight of 100 gm were used. The animals were divided into 4 comparable groups receiving diets containing purified egg albumin (diet A) or ethanol-extracted casein (diet B) as sole source of protein at the 10% level and supplemented with the B vitamins at one of two levels, a low sub-optimal and a high optimal. Growth was observed for a period of 5 weeks. At the end of this period the casein-fed animals were exsanguinated and the visceral organs collected and analyzed, individually and pooled, for total nitrogen and lipids.

In the second series of experiments, weanling rats, approximately 40 gm in weight, were similarly grouped and fed diets identical to the above except that the protein content was raised to the 18% level at the expense of starch. Growth was recorded at weekly intervals for a period of 6 weeks, at the end of which the animals were killed under ether anesthesia. Blood was collected from the hepatic portal vein, heparinized, and centrifuged in the cold to obtain the plasma. This was analyzed for total and non-protein nitrogen. Livers were quickly excised and chilled in cracked ice and determinations were made of total and non-protein nitrogen and total lipids.

Received for publication May 25, 1959.

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

TABLE 1
Composition of experimental diets[1]
(Quantities for 100 gm of diet)

Component	Experiment 1		Experiment 2		
	Diet A	Diet B	Diet C	Diet D	Diet E
	gm	*gm*	*gm*	*gm*	*gm*
Vitamin-free casein		10(or 18)			
Purified egg albumin[2]	10(or 18)		18	14	
Corn starch	80(or 72)	80(or 72)	72		
Whole wheat flour				76	76
Purified wheat gluten[2]					14
Vitaminized sucrose*	1	1	1	1	1
Vitaminized arachis oil†	5	5	5	5	5
Salt mixture, U.S.P. XIV	4	4	4	4	4

*B vitamins per gram sucrose

	Optimal	Sub-optimal
	mg	*mg*
Variable additions		
Thiamine·HCl	0.3	0.075
Riboflavin	0.4	0.1
Pyridoxine·HCl	0.3	0.075
Ca pantothenate	1.0	0.25
Niacin	2.0	0.5
Folic acid	0.1	0.025
Vitamin B_{12}	0.015	nil

Common additions	*mg*
Para-amino benzoic acid	10.0
Biotin	0.05
Inositol	20.0
Choline Cl	20.0
Menadione	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] Each of the diets was modified further with additions in sucrose of 7 of the B vitamins either at optimal or at sub-optimal levels as indicated.
[2] Egg albumin and wheat gluten, Nutritional Biochemicals Corporation, Cleveland.

Experiment 2. Trials with wheat, egg albumin and mixed dietaries

In this setup the effect of two levels of B vitamins on the utilization of a wheat diet (diet E) was studied in comparison with the effect of diets containing either egg albumin (diet C) or mixed proteins (diet D). The total protein was at the 18% level in all diets and in the wheat diet this was adjusted with purified wheat gluten.

Six groups of weanling, 40 gm rats were fed these diets supplemented, as in experiment 1, with the B vitamins either at an optimal or a sub-optimal level. Body-weight records were maintained throughout the experimental period of 8 weeks. The animals were decapitated at the end of this period and livers analyzed for total and non-protein nitrogen, total lipids, total riboflavin and flavin adenine dinucleotide (FAD), pyridine nucleotides, coenzyme A (CoA), folic acid and vitamin B_{12}.

METHODS

The organs were blotted and weighed immediately after they were excised. Suitable portions were later made into 20% homogenates with ice-cold distilled water using a Potter-Elvehjem glass homogenizer. Aliquots of the homogenates were used for subsequent determinations.

Protein-free filtrates of plasma were prepared according to the procedure of Hier and Bergeim ('45); those of liver were obtained using trichloroacetic acid for protein precipitation. Nitrogen in plasma and in tissue homogenates and their protein-free preparations was determined, after liberation by microKjeldahl digestion, by direct Nesslerization as described by Umbreit ('46). The determination of total lipids in tissue homogenates was done as described by Marfatia and Sreenivasan ('60). FAD and total riboflavin in liver were determined fluorimetrically by the procedure of Bessey, Lowry and Love ('49). Differential determination in liver of oxidized (PN) and reduced (PNH) pyridine nucleotides was carried out fluorimetrically by the procedure outlined by Dianzani ('55). For the assay of liver CoA the method of Kaplan and Lipmann ('48) was followed. Vitamin B_{12} in liver was liberated with papain (25 mg/gm of fresh liver) in an overnight incubation under toluene in acetate buffer, pH 4.6. The liberated vitamin was assayed using *Euglena gracilis* as the test organism (Hoff-Jorgensen, '54). Folic acid was liberated by autolysis of liver homogenates in 0.1 M phosphate buffer at pH 7.2 and was determined by the assay procedure of Mitbander and Sreenivasan ('54) using *Streptococcus faecalis R.*

RESULTS AND DISCUSSION

Utilization of casein and egg albumin

At the 10% level of protein, the growth rate with egg albumin was, as may be expected, consistently higher than with casein throughout the 5-week period, irrespective of the level of B vitamins in the diet. The average weight gains at the end of 5 weeks at sub-optimal and optimal intakes of the B vitamins were respectively 28 and 62 gm with casein diets and 68 and 78 gm with egg albumin diets. The growth response to B vitamins was thus considerably greater with casein diets in comparison with that obtained with diets containing egg albumin, so that differences in the growth rate due to type of protein narrowed with optimal intake of the vitamins.

In a similar study, Sure and Romans ('48) found that growth of rats on a low-casein (7.1%) diet was possible only when the various components of the vitamin B complex were raised to high concentrations and that with low intakes of the vitamins only maintenance could be achieved; the weight gained by the animals receiving the higher amounts of the B vitamins was mainly fat rather than protein. Sarett and Perlzweig ('43) also reported that, on low-protein diets, supplementation with B vitamins increased liver and body fat. The growth-promoting effect of vitamin B_{12} in rats deficient in this vitamin has also been attributed to increased lipogenesis (Black and Bratzler, '52; Knoebel and Black, '52; Ling and Chow, '52) which possibly results from increased choline synthesis (Arnstein, '55; Henry and Kon, '56). Similar increases in fat deposition have been observed on supplementing low quality protein diets with other lipotropic factors, methionine and choline (Shils, De Giovanni and Stewart, '55). In the present study, therefore, it was of interest to examine the composition of the weight gained by the casein-fed animals when the intake of the B vitamins was raised to optimal.

The data obtained from the analysis of the viscera of the casein-fed animals are presented in table 2. The results obtained from a pool of the different organs compare favorably with those obtained from individual determinations.

In all organs examined, an increase in tissue weight was evident on the low-vitamin diet. This effect was marked in the lung tissue and was least in the spleen. A striking increase in the nitrogen content of the pooled viscera and in individual tissues, with the high-vitamin diet, is, however, indicative of a more efficient feed utilization. However, in all these tissues a concomittant rise in lipids was also observed.

The growth data obtained when the dietary protein level was raised to 18% showed trends analogous to those observed with the 10% protein diets. Here, however, the growth rates with casein and egg albumin diets at optimal vitamin intake almost overlapped during the initial 5 weeks and significant differences due to protein type were apparent only at the end of the 6th week. Thus the average gains at the end of the 6-week experimental period with sub-optimal and optimal intakes of B vitamins were respectively 74 and 110 gm with casein diets and 95 and 126 gm with egg albumin diets.

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TABLE 2

Effect of the level of dietary B vitamins on the visceral composition[1] of rats fed a 10% casein diet

(Experiment 1)

Tissue	Fresh organ weight		Tissue constituents			
	B vitamin supplement		Total nitrogen		Total lipids	
			B vitamin supplement		B vitamin supplement	
	Sub-optimal level	Optimal level	Sub-optimal level	Optimal level	Sub-optimal level	Optimal level
	gm	*gm*	*mg/gm fresh weight*		*mg/gm fresh weight*	
Heart	0.58 ± 0.04	0.55 ± 0.03	24.6 ± 1.5	32.2 ± 1.0	61.4 ± 5.1	67.6 ± 1.6
Lung	1.33 ± 0.05	0.99 ± 0.06	16.1 ± 0.7	23.7 ± 1.1	49.9 ± 4.1	83.1 ± 1.7
Liver	5.55 ± 0.58	5.35 ± 0.29	26.3 ± 1.4	37.4 ± 0.6	69.1 ± 2.2	78.0 ± 8.0
Spleen	0.38 ± 0.03	0.47 ± 0.03	24.6 ± 0.4	30.6 ± 0.4	70.9 ± 2.7	84.7 ± 2.8
Kidney	1.41 ± 0.05	1.17 ± 0.06	26.3 ± 1.5	35.8 ± 1.3	70.6 ± 2.8	83.1 ± 2.2
Adrenal	0.041 ± 0.006	0.031 ± 0.005	26.9 ± 0.6	34.9 ± 1.7	—	—
Muscle			25.1 ± 2.1	29.9 ± 0.8	39.3 ± 3.7	41.5 ± 3.6
Pooled viscera[2]			24.1 ± 0.6	31.9 ± 0.6	64.8 ± 2.5	79.2 ± 3.1

[1] Figures represent, in each case, the mean value from at least 5 replicates ± standard error of the mean.
[2] Pooled viscera included equal parts by weight of heart, lung, liver, spleen and kidney.

TABLE 3

Effects of the level of dietary B vitamins on certain liver and plasma constituents of rats fed diets with casein or egg albumin at the 18% level

(Experiment 1)

Protein	B vitamin supplement[1]	Liver			Plasma nitrogen	
		Total nitrogen	Non-protein nitrogen	Total lipids	Total	Non-protein
		mg/gm fresh weight			*mg/100 ml*	
Casein	Sub-optimal (8)	22.2 ± 1.2[2]	2.1 ± 0.3	101.0 ± 4.3	880 ± 11	81.2 ± 3.7
Casein	Optimal (7)	27.6 ± 1.6	1.7 ± 0.3	92.6 ± 3.4	1068 ± 22	49.1 ± 4.3
Egg albumin	Sub-optimal (8)	24.1 ± 0.9	1.9 ± 0.4	96.3 ± 5.1	962 ± 31	63.3 ± 5.1
Egg albumin	Optimal (7)	25.2 ± 1.5	1.8 ± 0.2	90.1 ± 3.2	1035 ± 26	42.9 ± 3.0

[1] Figures within parentheses represent the number of animals used in each experiment.
[2] Mean value ± standard error of mean.

Data obtained from liver and plasma analyses in these animals (table 3) reflect the composition of the growth. An increase in plasma protein concentration and a decrease in its non-protein nitrogen content resulted from a higher intake of B vitamins with either casein or egg albumin diets. These effects are suggestive of improved utilization of dietary amino acids. A similar trend could be seen in the data for liver nitrogen. There was also a striking modification of the effect of the B vitamins on liver fat content observed with the groups fed the 10% casein diet. On the other hand, in rats fed egg albumin there was no appreciable change in liver composition due to B vitamins.

Utilization of wheat gluten, egg albumin and mixed protein dietaries

Considerable improvement in the growth rate of rats fed the wheat gluten diet was noted as a result of supplementation with the higher level of the B vitamins. A partial to complete replacement of the wheat protein by egg albumin caused a corresponding decrease in growth response to the vitamins. The growth data and liver contents of nitrogen, lipids and sulfhydryl compounds are presented in table 4. There was no significant change in liver lipid content with increased intake of B vitamins but there was definite improvement in liver protein-nitrogen, especially with the wheat gluten diet.

The data on liver contents of vitamins and cofactors are presented in table 5. Although the increased retention of the vitamins and cofactors in the liver tissue with higher intake of the B vitamins was to be expected, it was observed that even an improvement in the average quality of dietary protein increased the retention of these growth factors except in the case of CoA which showed a reverse trend. The dependence of liver levels of certain vitamins on dietary protein was also demonstrated by Sarett and Perlzweig ('43), who observed that rats receiving a diet low in protein were unable to incorporate both nicotinic acid and riboflavin into the liver, even when a liberal intake of B vitamins was provided. Guggenheim, Halevy, Neumann and Usieli ('56) reported that low-protein and protein-free diets lower the liver contents of citrovorum factor (CF) and folic acid (PGA) and depress the ability of the tissue to convert PGA into CF in rats. The decrease in liver CoA as a result of improvement in the quality of dietary protein could apparently have been due to alterations in liver level of vitamin B_{12}. An inverse relationship between the CoA content of the liver and the vitamin B_{12} status of the organism has, thus, been demonstrated in chicks (Boxer, Ott and Shonk, '53) and in rats (Boxer, Shonk, Gilfillan, Emerson and Oginsky, '55; Wong and Schweigert, '56). The difference due to dietary protein quality levelled off at

TABLE 4

Effects of the level of dietary B vitamins on the liver composition of rats fed diets derived from wheat and egg albumin

(Experiment 2)

Source of protein	Level of B vitamins	Weight gain in 8 weeks	Liver: Total nitrogen	Liver: Non-protein nitrogen	Liver: Total lipids
		gm	*mg/gm fresh weight*		
Wheat flour and wheat gluten	Sub-optimal	90.2 ± 4.3[1] (10)[2]	19.6 ± 2.0	2.3 ± 0.2	74.6 ± 4.3
(18% protein)	Optimal	128.9 ± 6.6 (8)	29.9 ± 1.3	2.1 ± 0.3	69.9 ± 3.1
Wheat flour and egg albumin	Sub-optimal	152.8 ± 5.2 (10)	30.1 ± 2.3	2.1 ± 0.2	77.8 ± 5.1
(18% protein)	Optimal	167.0 ± 3.4 (9)	35.3 ± 1.9	3.1 ± 0.3	79.0 ± 2.2
Egg albumin	Sub-optimal	170.6 ± 4.8 (9)	34.6 ± 3.8	3.0 ± 0.6	79.2 ± 3.7
(18% protein)	Optimal	180.2 ± 7.2 (8)	35.6 ± 1.4	3.2 ± 0.2	81.1 ± 4.0

[1] Mean value ± standard error of mean.
[2] Figures within parentheses indicate the number of animals in each group.

TABLE 5

Liver contents[1] of riboflavin, pyridine nucleotides, coenzyme A, folic acid and vitamin B_{12} in rats fed diets derived from wheat and egg albumin
(Experiment 2)

Diet description		Riboflavin		Pyridine nucleotides		Co A	PGA	Vitamin B_{12}
Source of protein	Level of B vitamins	Total	FAD	PN	PNH			
		μg/gm	μg/gm	μg/gm	μg/gm	units/gm	μg/gm	mμ/gm
Wheat flour and wheat gluten	Sub-optimal	20.5 ± 1.2[2]	14.8 ± 0.8	559 ± 35	170 ± 18	213 ± 12	1.43 ± 0.32	28.9 ± 4.3
	Optimal	27.1 ± 1.0	24.7 ± 1.1	883 ± 21	313 ± 21	243 ± 11	1.87 ± 0.30	37.3 ± 3.8
Wheat flour and egg albumin	Sub-optimal	27.5 ± 2.7	22.7 ± 4.5	816 ± 59	311 ± 27	161 ± 10	1.71 ± 0.38	52.5 ± 4.9
	Optimal	30.8 ± 1.2	26.2 ± 1.5	946 ± 61	261 ± 14	213 ± 17	2.32 ± 0.28	69.9 ± 7.3
Egg albumin	Sub-optimal	26.9 ± 2.1	24.8 ± 3.1	745 ± 20	253 ± 10	145 ± 9	1.70 ± 0.21	81.9 ± 3.2
	Optimal	28.5 ± 1.3	28.0 ± 1.4	845 ± 63	261 ± 9	196 ± 13	2.04 ± 0.36	105.7 ± 12.1

[1] All values are expressed on fresh weight of the tissue.
[2] Mean value derived from at least 7 independent determinations ± standard error of mean.

the higher intake of the B vitamins. Under the latter conditions there was a relatively greater increase in FAD than in free riboflavin and also in PNH in comparison with PN.

Although the lower level of vitamins used in these experiments is sub-optimal, gross deficiency symptoms of any of the B vitamins were not observed. That the vitamins at the lower level were limiting was indicated by the improved growth obtained with the use of adequate protein and higher levels of B vitamins.

There seems to exist an inverse relationship between protein quality and the effectiveness with which B vitamins can be used at higher levels. With good quality or high-protein diets, the weight gains due to B vitamins are, perhaps, largely protein. On the other hand, the effects on growth of improved levels of B vitamins with inadequate protein dietaries may in part arise from increased lipogenesis. The improvements in protein utilization due to B vitamins may also be linked to the increased retention of the vitamins in the form of their cofactors with the use of high vitamin diets.

SUMMARY

Sub-optimal and optimal concentrations of 7 B vitamins (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, folic acid and vitamin B_{12}) have been used in purified diets in two series of experiments with the albino rat, one with casein and egg albumin, and the second, with wheat gluten, egg albumin and a mixture of the two serving as protein sources at levels of 10 or 18%.

With diets containing 10 or 18 % of casein and egg albumin, an increase in the levels of the B vitamins reduced the differences in growth rate due to protein quality. An examination of the viscera of animals fed the 10% casein diet revealed that the enhanced growth was the result of increased synthesis of both protein and lipid, although individual organs had decreased in weight.

With an 18% wheat gluten diet, a partial to total replacement of the protein by egg albumin resulted in progressive dimunition of growth promotion by the higher levels of the B vitamins.

On the egg albumin or mixed protein diets at the 18% level, B vitamins at the higher level increased plasma and liver protein and promoted increased retention of vitamins and cofactors in the liver tissue.

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