A STUDY OF VARIATIONS IN TOTAL AMINO ACIDS OF RAT LIVER AS INFLUENCED BY DIETARY DEFICIENCIES OF PYRIDOXINE, BIOTIN AND FOLIC ACID

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In recent communications from this laboratory (Nadkarni and Sreenivasan, 1957 *a*, *b*, *c*) the effects of pyridoxine, biotin and folic acid on the metabolism of serine were discussed. Results are reported here on the amino acid composition of the total liver proteins of rats fed on normal and deficient diets. It was considered that such data incidental to the main programme of work would be of importance in view of the increasing recognition of vitamin-amino acid interrelationships.

Growth arrest in a deficiency of B vitamins implies their influence in the synthesis of cellular proteins. In a reverse manner, it is well known that animals reared on a high protein diet exhibit an increased requirement for B vitamins. Furthermore, it is well recognised that a proper balance between calories and proteins is of great importance. B vitamins, in many instances, act as regulatory substances in the production as well as utilization of energy from foods; they are important components of enzyme systems serving to metabolise carbohydrates, fats and proteins for various physiological processes. It is therefore easy to appreciate that the efficiency of protein utilization could be modified by dietary levels of B vitamins (Sure and Dichek, 1941; Sure, 1950, 1954; Kleiber and Jukes, 1942; Laurence *et al.*, 1944; Bosshardt *et al.*, 1950; Charkey *et al.*, 1950; Richardson *et al.*, 1951; James and Abbott, 1952; Nelson and Evans, 1953).

There are reports specifically implicating pyridoxine, biotin and folic acid in the metabolism of proteins and amino acids. Thus, it is known that the requirement for vitamin B_6 is influenced by qualitative and quantitative make-up of the proteins in a diet (Cerecedo and Foy, 1944; Page and Gingras, 1946, 1947; Anderson *et al.*, 1951; Snell, 1953; Beaton *et al.*, 1950, 1953 *a*, 1953 *b*). The known enzymatic functions of vitamin B_6 also suggests its important role in protein metabolism (Snell, 1953). Fasting levels of urea and non-protein nitrogen increase with protein levels in vitamin B_6-deficient animals (Hawkins *et al.*, 1946). Production of antibodies in the system is also apparently related to pyridoxine (Axelrod, 1953). Besides, vitamin B_6 deprivation causes impairment in globulin (Stoerk, *et al.*, 1947) and hæmoglobin synthesis (Mckibben *et al.*, 1942; Cartwrite *et al.*, 1944; Reid *et al.*, 1945) and in the synthesis of the protein moieties of certain enzymes (Ershoff, 1951; Lichstein, 1956). The suggestion has been made (Christensen *et al.*, 1952, 1953) that, in the transfer of amino acids into the cell interior (Vanslyke and Meyer, 1913–14; Luck, 1928; Hamilton, 1945), B vitamins and especially vitamin B_6 may take part.

Evidence correlating biotin with protein metabolism is comparatively scanty. The vitamin is apparently related to the fundamental process of growth as the biotin content of embryonic tissue and tumourous tissue is very high (West and Woglom, 1941). In induced biotin deficiency, there is observed not only typical symptoms of dermatitis but also granulocytopœnia, leukepœnia and anæmia (Daft *et al.*, 1942). Biotin influences the metabolism of aspartic acid and the deamination of certain amino acids (Gothoskar and Sreenivasan, 1953 *a*, 1953 *b*). It has been shown that in a deficiency of biotin there is decreased incorporation of carbon dioxide into the purine bases isolated from visceral nucleic acids (McLeod and Lardy, 1949). Gothoskar, Rege and Sreenivasan (1954) have observed a depressing effect of biotin on the synthesis of both DNA and RNA in micro-organisms. The relationship of nucleic acids to protein synthesis (Brachet, 1941; Gale, 1955) would implicate biotin indirectly in protein metabolism. The suggestion has been apparently made that biotin may function in certain metabolic processes only indirectly by aiding the synthesis of the protein moieties of the enzyme systems involved (Winzler *et al.*, 1944; Blanchard *et al.*, 1950).

Special significance is conferred on folic acid in protein utilization, because this vitamin is involved in transmethylations, nucleotide synthesis and the metabolism of glycine, serine, threonine, cystine, methionine, tryptophan, histidine and possibly other amino acids (Sreenivasan, 1955). Besides, its close metabolic relationship to vitamin B_{12}, whose importance in general and oxidative metabolism is well known, may be expected to emphasize further its influence on protein metabolism.

While, the general effects of the different B vitamins on amino acid metabolism have been extensively reported, there is no information concerning changes in tissue protein composition resulting from their deficiencies. The present studies were, therefore, undertaken on the amino acid composition of the liver protein of normal and of vitamin B_6, biotin, and folic acid-deficient rats.

Experimental and Results

The animals were the same as in the experiments reported earlier on vitamin B_6, biotin and folic acid deficiencies (Nadkarni and Sreenivasan, 1957 *a*, *b*, *c*).

Determination of liver amino acids.—The homogenised liver of the normal (control) or of the vitamin-deficient rat was suspended in 15 ml. of 6 N hydrochloric acid in a sealed tube and hydrolysed for 10 hours at 15 lb. steam pressure and cooled. Tissue material equivalent to 200 mg. dry weight was taken in each sealed tube. 2·0 ml. of 2·5 M sodium acetate solution was added to the hydrolysate and pH was adjusted to 4·5. The hydrolysate was made upto 50 ml. volume and filtered. A portion of the filtrate was taken with the ethyl ether to remove lipid material and pH of the aliquot was brought to 6·8 with dilute NaOH and the volume was adjusted again. This hydrolysate was used for assay of all amino acids other than tryptophan.

For the estimation of tryptophan, tissue material was hydrolysed enzymically by the method recommended by Wooley and Sebrell (1945). Homogenised liver equivalent to 200 mg. dry weight was taken in a 100 ml. conical flask, to which was added 25 ml. of 0·1 N sulphuric acid and 10 mg. of pepsin (B.D.H.). The flasks were incubated overnight at 37° C., 3·0 g. of $K_2HPO_4 \cdot 12\,H_2O$ were added and the pH brought to 8·4. After adjustment of pH, 50 mg. trypsin (B.D.H.) were added and the flasks were incubated for three days at 37° C. The pH was adjusted again to 6·8 and the volume was brought to 100 ml. Solutions containing 10 times the amount of enzyme preparations were simultaneously digested and these, when assayed, were treated as blanks. The amount of tryptophan calculated to be present in the enzymes used for the digestion was then deducted from the total values for tryptophan found in the assayed materials.

All assays were carried out by specific microbiological procedures (Table I) with uniform assay media of Henderson and Snell (1948), using a Cannon Dispensor-Titrator assembly (Henderson, Brickson and Snell, 1948).

The results for the different groups of animals in pyridoxine-, biotin- and folic acid-deficiency are given in Tables II, III and IV respectively. The values represent averages of four determinations from the homogenates of livers from four animals in each case. Results expressed as *dl*-forms are expected to be half for *l*-forms.

TABLE I

Organisms used for amino acid assays

Amino acid	Range of Assay µg.	Assay Organism	Reference
dl-methionine ..	0–3·2	*Lactobacillus fermenti*	Barton-Wright and Curtis. 1948
Glycine ..	0–2·0	*Leuconostoc mestenteroides* P–60	Dunn *et al.*, 1949
dl-serine ..	0–4·0	*Lactobacillus casei*	Alexander *et al.*, 1953
l-tyrosine ..	0–2·0	*Lactobacillus arabinosus* 17–5	Barton-Wright, 1946
dl-β-phenylalanine	0–4·0	*Leuconostoc mesenteroides* P–60	Barton-Wright, 1946
dl-isoleucine ..	0–4·0	*Lactobacillus arabinosus* 17–5	Schweigert *et al.*, 1944
dl-leucine ..	0–2·4	,,	,,
dl-valine ..	0–4·8	,,	,,
l-lysine ..	0–16	*L. mesenteroides* P–60	Barton-Wright, 1946
l-histidine ..	0–1·0	,,	,,
dl-aspartic acid	0–4·0	,,	Stokes and Gunness, 1945
dl-threonine ..	0–2·0	*Streptococcus fæcalis*	Stokes *et al.*, 1945
dl-tryptophan ..	0–2·4	*L. arabinosus* 17–5	Wooley and Sebrell, 1945

TABLE II

Effect of vitamin B_6 *deficiency on liver amino acids*

Amino acid	Vitamin B_6-deficient	Vitamin B_6-fed
	Per cent dry wt.	
dl-methionine ..	1·06±0·21	1·89±0·20
Glycine ..	2·33±0·16	3·78±0·20
dl-serine ..	5·31±0·15	6·38±0·15
l-tyrosine ..	1·64±0·22	2·48±0·21
dl-β-phenylalanine	4·46±0·30	6·68±0·22
dl-isoleucine ..	4·89±0·40	7·22±0·16
dl-leucine ..	4·24±0·30	5·91±0·15
dl-valine ..	4·11±0·31	7·52±0·32
l-lysine ..	11·62±0·60	13·74±0·30
l-histidine ..	1·30±0·11	2·36±0·15
dl-aspartic acid	4·67±0·20	6·61±0·30
dl-threonine ..	2·35±0·10	3·29±0·30
dl-tryptophan ..	0·74±0·02	1·19±0·04

TABLE III

Amino acid levels in biotin-deficient and biotin-fed rats

Amino acid	Biotin-deficient	Biotin-fed
	Per cent. dry weight	
dl-methionine ..	1·82±0·10	1·84±0·11
Glycine ..	3·92±0·20	3·96±0·18
dl-serine ..	6·59±0·11	6·75±0·12
l-tyrosine ..	2·43±0·10	2·50±0·10
dl-β-phenylalanine	6·68±0·41	7·65±0·21
dl-isoleucine ..	7·65±0·50	7·83±0·20
dl-leucine ..	5·22±0·15	5·93±0·20
dl-valine ..	8·44±0·20	9·23±0·16
l-lysine ..	15·54±0·11	15·82±0·12
l-histidine ..	2·16±0·05	2·17±0·15
dl-aspartic acid	6·48±0·21	6·46±0·22
dl-threonine ..	3·12±0·31	3·50±0·13
dl-tryptophan ..	1·00±0·01	1·14±0·02

TABLE IV

Amino acid levels in folic acid-deficient and folic acid-fed rat

Amino acid	Folic acid-deficient	Folic acid-fed
	Per cent. dry weight	
dl-methionine ..	1·22±0·16	1·50±0·11
Glycine ..	2·41±0·25	3·15±0·13
dl-serine ..	5·08±0·15	6·25±0·15
l-tyrosine ..	2·08±0·16	2·39±0·20
dl-β-phenylalanine	5·35±0·30	6·36±0·26
dl-isoleucine ..	6·59±0·34	6·54±0·22
dl-leucine ..	6·07±0·30	6·56±0·20
dl-valine ..	7·02±0·31	7·73±0·32
l-lysine ..	12·37±0·13	12·6 ±0·59
l-histidine ..	1·43±0·15	2·14±0·16
dl-aspartic acid	4·65±0·25	6·21±0·36
dl-threonine ..	2·97±0·21	3·60±0·30
dl-tryptophan ..	0·99±0·02	1·25±0·04

Data on liver weight, and total liver nitrogen and liver vitamins are summarised in Table V. The methods used for determination of liver nitrogen, liver pyridoxine, liver biotin and liver folic acid are outlined in earlier communications (Nadkarni and Sreenivasan, 1957 *a*, *b*, *c*).

TABLE V

Data on liver weight, total liver nitrogen and liver vitamin

Group	Liver weight per 100 g. body weight g.	Liver nitrogen		Liver vitamin μg./g. fresh wt.
		Per cent. dry weight	Per cent. fresh weight	
$+B_6$ ..	$3 \cdot 37 \pm 0 \cdot 4$	$13 \cdot 64 \pm 0 \cdot 9$	$3 \cdot 91 \pm 0 \cdot 14$	$6 \cdot 91 \pm 0 \cdot 01$
$-B_6$ ..	$4 \cdot 58 \pm 0 \cdot 4$	$11 \cdot 72 \pm 1 \cdot 4$	$3 \cdot 43 \pm 0 \cdot 24$	$4 \cdot 10 \pm 0 \cdot 02$
+Biotin ..	$2 \cdot 8 \pm 0 \cdot 3$	$13 \cdot 56 \pm 0 \cdot 8$	$3 \cdot 89 \pm 0 \cdot 03$	$0 \cdot 242 \pm 0 \cdot 002$
−Biotin ..	$5 \cdot 6 \pm 0 \cdot 4$	$11 \cdot 32 \pm 1 \cdot 0$	$3 \cdot 33 \pm 0 \cdot 20$	$0 \cdot 081 \pm 0 \cdot 002$
+Folic acid ..	$3 \cdot 56 \pm 0 \cdot 2$	$12 \cdot 84 \pm 0 \cdot 4$	$3 \cdot 71 \pm 0 \cdot 20$	$5 \cdot 75 \pm 0 \cdot 03$
−Folic acid ..	$3 \cdot 80 \pm 0 \cdot 1$	$11 \cdot 01 \pm 1 \cdot 3$	$3 \cdot 45 \pm 0 \cdot 10$	$2 \cdot 87 \pm 0 \cdot 04$

DISCUSSION

A comparison of the data on the amino acid composition of livers of rats from the three normal groups reveals the close constancy in the values.

In a deficiency of vitamin B_6 there is a sure and appreciable decrease in the values for all amino acids. This is also reflected in the decreased liver total proteins. However, there is an increased liver weight per unit weight suggesting that total liver protein is not correspondingly affected. The pronounced effect of a vitamin B_6 deficiency on liver amino acid levels is in conformity with observations reviewed earlier.

Biotin deficiency apparently does not influence the composition of the liver amino acids assayed although there is a decrease in liver proteins though not in total proteins in whole liver. This latter observation may have a bearing on the effect of biotin on nucleotide metabolism (McLeod and Lardy, 1949; Gothoskar, Rege and Sreenivasan, 1954). Brachet (1941) and Gale (1955) had pointed out the relationship of protein synthesis to nucleic acids.

As with vitamin B_6, the influence of folic acid on liver amino acids is more widespread. This is especially so with glycine, serine, histidine and threonine. Rege and Sreenivasan (1954) had shown the influence of folic acid on nucleotide synthesis and hence the general effect of folic acid deficiency on liver proteins may also be due to its controlling influence on nucleotide metabolism.

Summary

A marked decrease in the amino acid composition of rat liver proteins in vitamin B_6 and folic acid deficiency but not in biotin deficiency is reported.

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