DEFINITION OF ENDOGENOUS AMINO-ACID IMBALANCE IN CHOLINE-PROTEIN DEFICIENCY

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In rats with choline-protein deficiency the free serine and phosphoethanolamine content in the liver tissue arises approximately three times, while that of free aspartic and glutamic acids rises twice and 1.5 times respectively. Methionine disappears practically completely from the liver tissue. Choline-protein deficiency may be defined as a state of endogenous amino-acid imbalance.

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One of the earliest and most characteristic manifestations of choline-protein deficiency is fatty infiltration of the liver, leading to the development of cirrhotic changes, and in some cases, of malignant tumors of the liver [10, 11, 13]. The mechanism of the pathological changes in the liver arising as a result of protein and choline deficiency have so far received comparatively little study. Only very recently have results been obtained concerning the enzymologic characteristics of this type of pathology [2-4]. These states are marked by considerable changes in the activity of several enzymes of lipid and protein metabolism, notably by inhibition of activity of fat-mobilizing lipases and transaminases in liver tissue [4].

Since choline-protein deficiency from one standpoint may be regarded as a severe deficiency of labile methyl groups, of which methione is one of the principal sources, it may be postulated that these states are characterized by marked disturbances in the relative proportions of free amino acids in the tissues and, in particular in the liver tissue, i.e., that they are a sign of endogenous amino-acid imbalance [5-7].

Accordingly, determination of free amino acids and intermediate products of choline biosynthesis could be especially interesting from this point of view.

In the present investigation changes in the content of free amino acids and other ninhydrin-positive compounds in liver tissues of rats with choline-protein deficiency were studied.

EXPERIMENTAL METHOD

Experiments were performed on male Wistar rats weighing initially 50-80 g. To reproduce an alimentary model of choline-protein deficiency, a diet was given consisting of 5% choline-free casein, 8% lard, 83.5% starch, 3% of a salt mixture, and 0.5% cystine. Control animals received the same diet, but containing 20% casein, and in addition they received 15 mg choline chloride daily. Both control and experimental rats received all necessary vitamins daily. Free amino acids and a series of ninhydrin-positive compounds in protein-free extracts of the liver and in the blood serum were determined by iron-exchange chromatography, using an automatic analyzer (Beckman model 120-B [16]).

To obtain protein-free extracts of rat liver, the tissue was ground in a mortar with liquid nitrogen, and the proteins were precipitated with 1% picric acid, which was then removed by an iron-exchange resin (Dowex 2 × 10). To introduce the corresponding correction into the content of free amino acids in the liver on account of the free amino acids of the blood left in the liver, this volume of blood was determined, using a method based on the color reaction of hemoglobin with benzidine in the presence of hydrogen peroxide [9]. The volume of residual blood in the liver under our experimental conditions was 8-15%. The total quantity of fat extracted by the method of Folch and co-workers [14], followed by evaporation in vacuo, was determined gravimetrically. Twenty animals were used in the experiments, of which nine were controls. The experimental results were subjected to statistical analysis.

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TABLE 1. Content of Free Amino Acids and Other Ninhydrin-Positive Compounds in Liver of Rats with Choline-Protein Deficiency (in μmoles/g fresh defatted tissue, 60-90 days after start of experiment), M ± m

Compounds	Control	Experiments	P
Serine	0,88±0,11	2,95±0,29	<0,001
Phosphoethanolamine	0,70±0,12	2,15±0,49	>0,002<0,01
Aspartic acid	0,87±0,07	1,85±0,18	<0,001
Glutamic acid	3,16±0,44	4,74±0,45	>0,02<0,05
Threonine	0,41±0,03	0,62±0,10	>0,05<0,1
Alanine	2,67±0,26	3,1±0,76	>0,5
Glycine	2,00±0,21	2,2±0,24	>0,5
Proline	0,19±0,013	0,27±0,03	>0,5
Valine	0,27±0,03	0,23±0,02	>0,25<0,5
Isoleucine	0,20±0,011	0,19±0,013	>0,5
Leucine	0,31±0,02	0,33±0,03	>0,5
Tyrosine	0,10±0,01	0,13±0,01	>0,1<0,25
Phenylalanine	0,10±0,008	0,11±0,01	0,5
Urea	3,00±0,53	2,38±0,66	0,5
Methionine	0,05±0,005	Traces	—

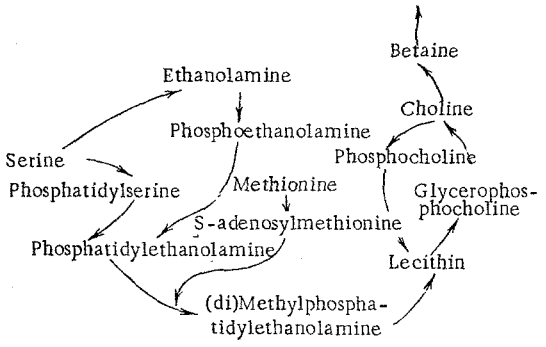

Fig. 1. Scheme of choline biosynthesis.

EXPERIMENTAL RESULTS

The results of these experiments showed that administration of a diet deficient in choline and protein led to the rapid development of fatty infiltration of the liver, reaching a maximum after 2-3 months (the total fat content in individual animals at this time had reached 20-30% of the fresh tissue weight).

Fractionation of the liver lipids by T. N. Ptitsyna and M. M. Levachev revealed a sharp increase in the content of triglycerides, and of free and bound cholesterol, together with a very considerable decrease in the level of phospholipids, especially lecithins [8]. A significant increase was observed in the total content of amino acids, mainly on account of compounds participating in choline synthesis, the concentration of which was increased about three times (content of free serine in the liver tissue 335%, of phosphoethanolamine 307% of the normal values), and also as a result of an increase in the content of monoaminodicarboxylic acids (content of free aspartic acid 207%, of glutamic acid 150%), occupying a central position in amino-acid metabolism [1].

Free methionine disappeared practically completely from the liver tissue (Table 1). So far as changes in the content of other amino acids are concerned, they were not significant (Table 1).

The accumulation of free serine, the biological precursor of ethanolamine and choline in the phosphatides of the animal body, in the liver tissue is in harmony with results indicating depression of synthesis of phospholipids in the liver of animals with choline-protein deficiency [2, 12]. The increase in concentration of free phosphoethanolamine—a product of the splitting of phosphatidylethanolamine—also probably indicates slowing of the natural pathway of methylation of phosphatidylethanolamine into phosphatidylmonomethylethanolamine and lecithin. Accumulation of serine and phosphoethanolamine under the conditions of a severe deficiency of labile methyl groups may be regarded as an adaptive process aimed at securing the largest possible number of these groups (formation of a special form of serine-phosphoethanolamine trap; Fig. 1).

It may be postulated that this mechanism bears some relationship to the problem of carcinogenesis, for during the action of certain other agents producing carcinoma of the liver, a considerable increase in the content of free phosphoethanolamine is observed in the liver tissue of rats [15].

The accumulation of free aspartic and glutamic acids in the liver may evidently be connected to some extent with the considerable inhibition of glutamate-alanine (C. E. 2, 6, 1, 2) and glutamate-aspartate (C. E. 2, 6, 1, 1) transaminases of the liver in choline-protein deficiency, as previous investigations have shown [3, 4].

The changes discovered in the content of free amino acids and ninhydrin-positive compounds in the liver tissue thus suggest that the disturbances of lipid metabolism, expressed by development of fatty infiltration of the liver tissue, are connected to some extent with a disturbance of amino-acid metabolism. Choline-protein deficiency may likewise be regarded as a state of endogenous amino-acid imbalance.

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