UTILIZATION OF CARBOHYDRATES IN LIPOGENESIS IN EXPERIMENTAL PROTEIN DEFICIENCY

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Experiments on rats receiving casein in the diet in an amount equivalent to 8% of its calorific value for 30 days showed an increased rate of synthesis of fatty acids in the liver. However, glucose metabolites were utilized to a lesser degree, and sodium acetate to a greater degree in lipogenesis under these circumstances than when a normal protein level was combined with a high carbohydrate content. This suggests the active resynthesis of fatty acids from their breakdown products. The increase in the content of pyruvic acid and of radioactive label in it from glucose, and the decrease in the acetyl CoA and oxaloacetic acid level in the liver indicate slowing of the conversions of pyruvic acid, which could limit the utilization of carbohydrates in fatty acid synthesis.

Protein deprivation causes a disturbance of lipid metabolism in which the rate of biosynthesis of fatty acids and cholesterol is increased in the liver and other organs [1, 9, 14], while the content and rate of formation of phospholipids and lipoproteins, responsible for transport of the lipid components, are reduced [6]. It has also been found that fatty degeneration of the liver is associated with protein deficiency in the diet, accompanied by an excess of energy-yielding material, notably carbohydrates [12].

In the investigation described below the degree of utilization of metabolic products of glucose in the biosynthesis of fatty acids was studied in vivo under conditions of protein deficiency, for it has been shown that a high carbohydrate level in the diet, together with a normal protein content, increases the rate of lipogenesis [2]. Another object of the investigation was to determine the quantity of certain components of lipid, carbohydrate, and intermediate metabolism.

EXPERIMENTAL METHOD

Experiments were carried out on 90 male Wistar rats weighing initially 120-150 g, and fed for 30 days ad lib. with semisynthetic isocalorific diets with the necessary content of vitamins and salts. Of the total calorific value of the diet received by the experimental rats, 8% was contributed by casein, 74% by corn starch, and 18% by lard. The animals of control group 1 received the same quantity of carbohydrates (71%) as the experimental animals, but a normal protein content, while the rats of control group 2 received a diet with the carbohydrate level reduced to the accepted physiological value (56%), but a normal content of protein (24%) and fat (20%). In the animals of all three groups the metabolism of 1-6C^{14}-glucose (100 μCi was injected subcutaneously into each animal, and decapitation was carried out after 2 and 24 h) and of sodium 2-C^{14}-acetate (150 μCi was injected subcutaneously, the animals were sacrificed after 2 h) was investigated. The content of pyruvic and oxaloacetic acids in the liver was determined by Käser's method [7], cholesterol by the method of Sperry and Webb [10], fatty acids by the method of Folch et al. [5], and acetyl CoA was estimated from the quantity of acetic acid formed after hydrolysis of the tissue, removal of the organic acid by steam distillation, and their conversion into the hydroxamates and determination by

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TABLE 1. Content of Lipids in Rat Liver (in mg/g fresh liver tissue) and Radioactivity of Fatty Acids and Cholesterol Isolated from 1 g Tissue (in 10^{-4} μCi)

Index	Experimental rats	Control rats	
		group 1	group 2
Content of fatty acids	50±4*	38,5±1,7	29,2±6
Content of cholesterol	2,44±0,12*	2,06±0,15	2,02±0,2
Radioactivity of fatty acids from 1-6C^{14}-glucose	1,48±0,11*	2,63±0,34	1,0±0,07
Radioactivity of fatty acids from 2-C^{14}-acetate	114±8*	24,5±2,4	15,0±1,2
Radioactivity of cholesterol from 1-6C^{14}-glucose	0,59±0,04	0,68±0,02	0,74±0,05
Radioactivity of cholesterol from 2-C^{14}-acetate	2,5±0,3	2,62±0,8	2,8±0,4

* $P \leq 0.05$.

TABLE 2. Content of Keto-Acids and Acetyl CoA in Rat Liver (in μmoles/g fresh tissue) and Their Radioactivity (in 10^{-4} μCi from 1-6C^{14}-glucose)

Index	Experimental rats	Control rats	
		group 1	group 2
Content of pyruvic acid	470±31*	284±27	340±51
Content of oxaloacetic acid	62±13	119±12*	86±18
Content of acetyl CoA (in μmoles acetic acid)	80±20*	155±21	180±35
Radioactivity of pyruvic acid	1,27±0,21	0,9±0,1	0,86±0,13
Radioactivity of oxaloacetic acid	0,27±0,03	0,34±0,04	0,26±0,04

* $P \leq 0.05$.

the method of Stadtman and Barker [11]; activity of glucose-6-phosphate dehydrogenase was determined by the method of Löhr and Waller [8].

EXPERIMENTAL RESULTS AND DISCUSSION

The criterion of development of experimental protein deficiency in these experiments was a decrease in the protein content in the liver (245±20 mg/g in the experimental series and 315±22 mg/g in the control) and an increase in the content of lipids (87±4 mg/g and 59±2 mg/g, respectively). The maximum increase in the content of fatty acids and cholesterol was observed in the experimental rats. As Table 1 shows, incorporation of C^{14}-glucose into fatty acids took place at a faster rate in protein deficiency. However, the content of labeled glucose metabolites in the fatty acids was somewhat lower in the experimental rats than in animals receiving a diet with a normal protein content and increased carbohydrate content. Conversely, a greater increase in the rate of biosynthesis of fatty acids from sodium 2-C^{14}-acetate was observed in the liver of the animals with protein deficiency. The rate of incorporation of C^{14}-glucose and sodium acetate into cholesterol was the same in the animals of all groups (Table 1). It follows from Table 2 that the content of pyruvic acid in the liver of the experimental rats was increased, while that of oxaloacetic acid was reduced (in the latter case compared with control group 1). Accumulation of radioactivity in the composition of pyruvic acid was observed in the liver of the experimental rats, accompanied by a slight decrease of its level in oxaloacetic acid. The content of acetyl CoA was very low compared with animals of both control groups. The difference found in the pyruvic acid level in the experimental and control rats compelled the determination of the intensity of carbohydrate utilization in the pentose cycle. This was tested in relation to activity of glucose-6-phosphate dehydrogenase, and a slight decrease in the activity of this enzyme was observed in the experimental animals (control 10.1 μmoles NADP · H_2/min/g protein; experiment 8.05 μmoles NADP · H_2/min/g protein).

The results indicate an increased rate of fatty acid synthesis in the liver with an increase in the quota of carbohydrates in the diet both with a deficiency and a normal quantity of protein in the diet. However, the results of these experiments relative to fatty acid synthesis from metabolites of labeled glucose indicate that their utilization in lipogenesis is reduced in the presence of protein deficiency. This result can be attributed to a decrease in the rate of conversion of pyruvic acid in this particular type of experimental protein deficiency. Confirmation of this view is given by the results indicating an increase in the pyruvic acid content and an increase in the intensity of its labeling from C^{14}-glucose, together with a decrease in the level of acetyl CoA and of oxaloacetic acid. Data in the literature likewise point to an increase in the blood pyruvic acid level in Kwashiorkor [13], which some workers associate with vitamin B_{12} deficiency [3], but others with a disturbance of oxidative processes in the Krebs cycle [4].

In conclusion, it will be noted that with an increase in the carbohydrate quota in the diet, whether the protein content was deficient or normal, similar disturbances of lipid metabolism took place, with an increase in the rate of fatty acid synthesis from metabolites of C^{14}-glucose; the differences were quantitative in character. Meanwhile, biosynthesis of fatty acids from sodium acetate took place much more rapidly in protein deficiency, suggesting activation of the resynthesis of fatty acids from their breakdown products. Protein deficiency has a marked effect on carbohydrate metabolism, the changes in which differ qualitatively from those observed in rats kept on a diet with a normal protein content. Characteristic features of carbohydrate and intermediate metabolism in protein deficiency are an increase in the pyruvic acid content in the liver, accumulation of label from radioactive glucose in the pyruvic acid, a decrease in the content of oxaloacetic acid and acetyl CoA, and a decrease in glucose-6-phosphate dehydrogenase activity. These results are evidence of considerable changes in the manifestation of the insulin effect, which invariably arises when the carbohydrate content in the diet is raised, in the presence of protein deficiency.

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