EFFECT OF CARBOHYDRATES AND LIPIDS OF THE DIET ON CHOLESTEROL BIOSYNTHESIS

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Prolonged feeding of rats with a diet balanced as regards lipids and carbohydrates increased incorporation of label from sodium acetate-2-C^{14} and palmitic acid-1-C^{14} into liver cholesterol molecules only if the quota of vegetable oils was excessive. Prolonged addition of sucrose to the diet has no effect on the rate of cholesterol biosynthesis.

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Many investigators [6, 9-11, 16] have observed that cholesterol metabolism (changes in absorption, excretion, and transport of sterols) is dependent on the content and quality of lipids in the diet. However, information on cholesterol biosynthesis is contradictory and has been obtained in the course of comparatively brief experiments.

During the last ten years the role of readily assimilated carbohydrates in the pathogenesis of atherosclerosis has been widely discussed in the scientific literature in connection with the work of Yudkin and others who have demonstrated a positive correlation between the serum cholesterol concentration and the calorific value of simple sugars in the diet [2, 3, 17, 18]. However, this hypothesis has not yet been supported by convincing facts obtained in long-term experiments on animals. Short-term experiments have demonstrated the effect of monoses and starch only on certain aspects of lipid metabolism and, in particular, on the absorption of exogenous cholesterol and on the cholesterol concentration in the blood and internal organs [4, 15].

The object of the present investigation was to study the rate of cholesterol formation in animals kept on semisynthetic diets of equal calorific value containing different quantities of lipids or carbohydrates.

EXPERIMENTAL METHOD

Experiments were carried out on 150 male Wistar albino rats weighing initially 100-130 g. In the daily diet of rats of the control group 20% of the calorific value was provided by protein, 26% by lipids (lard or sunflower oil), and 54% by carbohydrates (corn starch). The lipid content in the diets of the first and second experimental groups was increased to 60%, on account of sunflower oil or lard, respectively. In the diet of the third group, some starch was replaced by sugar (41% of the total calorific value of the diet), and in the diet of group 4 the starch content was increased to 71%, the calorific value remaining unchanged because of a decrease in the lipid content. All diets contained essential vitamins and mixed salts. After 30 days of feeding the animals of each group were divided into two subgroups: one subgroup of the rats of experimental groups 1 and 2 and also of the corresponding control groups received palmitic acid-1-C^{14} (~ 100 μCi) by gastric tube 24 h before sacrifice, while the other subgroups of rats of these same groups received sodium acetate-2-C^{14} (~ 150 μCi, subcutaneously) 2 h before sacrifice. One subgroup of the rats of groups 3 and 4 received glucose-1-6-C^{14} (~ 18 μCi) 24 h before sacrifice, while the other subgroup of the animals of these groups received sodium acetate-2-C^{14} (~ 150 μCi). Incorporation of label into cholesterol and fatty acids of the organs and tissues was investigated. The radioactivity of the samples was determined on a PP-8 apparatus with BFL-T counter. The cholesterol concentration was determined by the method of Sperry and Webb [12], and fatty acids by a method modified by Tret'yakova and

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Grodzenskii [1]. The concentrations of pyruvic, oxaloacetic, and α-ketoglutaric acids in the liver was determined by Käser's method [5], after chromatographic separation of the dinitrophenylhydrazones of these acids. The concentration of acetyl-CoA was estimated from the quantity of acetic acid after hydrolysis of the tissue and subsequent evaporation of volatile organic acids. Preparation of hydroxamates of the resulting organic acids and their fractionation by paper chromatography were carried out as described by Thompson [14]. The accetylhydroxamate was eluted and determined by the method of Stadtman and Barker [13].

EXPERIMENTAL RESULTS AND DISCUSSION

An excess of sunflower oil in the diet caused an increase in the cholesterol concentration in the liver (from 2.8±0.39 to 4.7±0.8 mg/g) and in the blood (from 53±3.8 to 65±3.6 mg%) and a decrease in its concentration in the perinephric and epididymal fat (from 0.57±0.05 to 0.38±0.07 mg/g). The total quantity of cholesterol newly synthesized from sodium acetate-2-C^{14} and isolated from 1 g tissue of 10 investigated organs was increased by 60% compared with control animals. The quantity of label in the liver cholesterol was appreciably increased from $11 \cdot 10^{-4}$ to $18.7 \cdot 10^{-4}$ μCi/g. An increase in the lard content of the rats' diet was not followed by any appreciable change in the content or rate of biosynthesis of cholesterol in the organs, except for the aorta, in which definite acceleration of cholesterol formation was observed (normal $0.57 \cdot 10^{-4}$ μCi/g; experiment $1.1 \cdot 10^{-4}$ μCi/g). In experiments in which palmitic acid-1-C^{14} was given an increase in the content of label in cholesterol was observed in the liver (from $0.71 \pm 0.09 \cdot 10^{-4}$ to $1.3 \pm 14 \cdot 10^{-4}$ μCi/g) and in the blood (from 0.26±0.04 to $0.35 \pm 0.04 \cdot 10^{-4}$ μCi/ml) in the rats of group 1 and by 84 and 35%, respectively, compared with the control. In animals receiving lard in the diet, only a decrease in the blood concentration of radioactive cholesterol was observed, from 0.37±0.1 to $0.16 \pm 0.02 \cdot 10^{-4}$ μCi/ml.

Prolonged administration of sucrose in the rats' diet caused an increase in the blood cholesterol concentration (from 54±1 to 70±8.1 mg%), not only when its synthesis from labeled acetate was not accelerated, but also when it was slightly retarded. An increase in the quota of carbohydrates in the form of corn starch to 71% of the total calorific value of the diet produced no visible deviations in the cholesterol concentration and led to no significant change in the rate of its biosynthesis from labeled sodium acetate or glucose.

It can be concluded from these findings that acceleration of cholesterol synthesis in animals receiving diets of the same calorific value, but unbalanced in their carbohydrates and lipid composition, takes place only if an excess of sunflower oil is given in the diet. It is evident that polyunsaturated fatty acids, some sterols, and tocopherol present in vegetable oils are biologically active substances which promote acceleration of absorption and metabolism of fatty acids. Degradation of these fatty acids under the conditions of alimentary suppression of their biosynthesis by an excess of exogenous fat may lead to the formation of high concentrations of metabolites which are utilized for cholesterol synthesis. In addition, a decrease in the quota of carbohydrates in the experimental diet leads to the creation of an oxaloacetate deficiency in the tissues. Disproportion between the oxaloacetate content and the excess of acetyl-CoA is probably responsible for acceleration of cholesterol synthesis. These hypotheses provide an explanation for the increase in incorporation of label from palmitic acid-1-C^{14} and sodium acetate-2-C^{14} into cholesterol in the organs of the animals of group 1. In addition, the influence of qualitatively different fats on the lipid composition of the cell membranes cannot be ruled out, and this in turn may influence the velocity of metabolic processes. Administration of sucrose to rats in the diet does not accelerate cholesterol biosynthesis, although the rate of assimilation of sucrose and the character of the metabolism of the fructose formed by its hydrolysis ought to provide conditions favoring an increase in the content of some of its precursors [7].

However, the results of additional experiments showed that in rats receiving sucrose the content of pyruvic acid in the liver was increased to 530±72 μmoles, that of oxaloacetic acid to 151±24 μmoles, and that of α-ketoglutaric acid to 218±41 μmoles (compared with control values of 355±51, 86±18, and 106±13 μmoles, respectively), with no change in the acetyl-CoA level. The acetyl-CoA concentration was evidently stabilized by acceleration both of the biosynthesis of fatty acids and of the carboxylation of pyruvic acid into oxaloacetic. Both these hypotheses are confirmed by the experimental data showing acceleration of biosynthesis of fatty acids and an increase in the oxaloacetate content in the liver. The retention of cholesterol in the blood described above is evidently due to disturbance of its transport in the body, because endogenous cholesterol synthesis is unchanged. The increase in the blood concentration of α-lipoproteins demonstrated by Nichols et al. [8] in animals receiving a similar diet possibly accounts for the retention of cholesterol.

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