Effect of Dietary Carbohydrate on Experimentally Induced Hypercholesteremia and Hyperbetalipoproteinemia in Rats.* (22250)

OSCAR W. PORTMAN,† ELEANOR Y. LAWRY, AND DOROTHY BRUNO.
(Introduced by F. J. Stare.)
Department of Nutrition, Harvard School of Public Health, Boston, Mass.

The biliary excretion of bile acids by rats is increased when corn starch is used as the dietary carbohydrate in lieu of sucrose(1). A major portion of 4-C^{14} cholesterol administered to the rat or to man is excreted as labeled bile acids in the bile(2,3), and feeding of high levels of cholesterol to rats results in an increased biliary excretion of bile acids(1). Feeding of bile acids with cholesterol to rats results in a marked hypercholesteremia and hyperbetalipoproteinemia(4). There is still some question(5,6), however, as to the principal mechanisms involved. Any factor influencing bile acid excretion might be reflecting variations in conversion of cholesterol to bile acids. The effects of feeding various carbohydrates on experimentally induced hypercholesteremia and hyperbetalipoproteinemia in the rat were, therefore, investigated.

Methods. Rats were male albinos weighing 250 g (range: 240-260 g) and were received from Charles River Laboratories. The animals were individually caged and given water and diet *ad libitum*. There was essentially equal caloric intake in experimental groups and mean weight gains were identical (about 2 g/day). The basal diet consisted of casein 20%, corn oil 8%, carbohydrate 56%, cholesterol‡ 5%, celluflour 5%, salts(7) 4%, vitamins and trace nutrients as previously described(8). In some diets 1.5% cholic acid§ was included. Diets will be designated in the text and Tables according to type of carbohydrate and presence of cholic acid. Rats were fed experimental diets for 28 days. Serum total cholesterol was determined by the method of Abell *et al.*(9); the serum beta-lipoproteins were determined by the method of Gofman *et al.*(10). Liver total cholesterol values were determined by modification of the method of Abell *et al.*(9).

Results. Effect of starch and sucrose feeding on cholate induced hypercholesteremia and hyperbetalipoproteinemia. The results of analyses for serum total cholesterol and beta-lipoprotein concentrations and liver total cholesterol concentrations are indicated in (Exp. 1) Table I. Rats of the starch-cholic groups had lower serum total cholesterol values (mean = 257 mg %) than the sucrose-cholic group (mean = 378 mg %) ($p < 0.01$). The liver total cholesterol concentration was strikingly elevated in both groups but to about the same extent. Approximately two-thirds of the beta-lipoproteins in all the animal patterns had migration rates between S_f 20 and S_f 60 with maximum concentration at approximately S_f 35 and negligible amounts below S_f 12.‖ The mean serum concentration of S_f 20-100 molecules of the starch-cholic group was 216 mg % compared to a mean value of 413 mg % for the sucrose-cholic group ($p < 0.01$). Probably these differences between the 2 dietary groups were related to the previously demonstrated(1) lesser excretion of bile acids by rats fed diets containing sucrose.

Effect of sucrose, fructose, and glucose feeding on cholate induced hypercholesteremia. Serum total cholesterol and serum beta-lipoprotein responses obtained after feeding cholesterol and cholic acid diets containing sucrose, fructose, and glucose for 28 days are

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‡ Cholesterol was furnished by Armour & Co.

§ Cholic acid was furnished by Mr. de Haen, Miles-Ames Research Div., Elkhart, Ind.

‖ Determination of quantity of lipoproteins was based on refractive indices established on pooled human sera.

TABLE I. Effect of Dietary Carbohydrate on Serum and Liver Cholesterol Concentrations and Serum Beta-Lipoprotein Concentrations in Rats.

Exp.	Diet*	No. of animals	Serum cholesterol, mg %	Liver cholesterol, mg %	Serum β-lipoproteins (mg %) S_f 0–11	12–20	20–100	100–400
1	Cholate-sucrose	19	378 ± 23 †	8560	20	67	413 ± 49†	128
	-starch	19	257 ± 12	8420	14	48	216 ± 13	8
2	Cholate-sucrose	10	414 ± 36		23	82	521	60
	-glucose	10	361 ± 29		22	95	417	20
	-fructose	10	411 ± 20		16	56	504	90
3	Sucrose	10	124 ± 4.5					
	Starch	10	106 ± 3.9					
4	Cholate-sucrose	10	380 ± 35					
	-sucrose sulfa‡	8	348 ± 28					
	-starch	8	270 ± 25					
	-starch sulfa	9	361 ± 23					

* Rats were fed for 28 days on diets described in text. Diets contained 5% cholesterol. Presence of cholic acid and type of carbohydrate are indicated in "Diet" column.

† All values are means ± stand. errors of the means.

‡ Sulfasuxidine was added at 0.2% of the diet where "sulfa" is indicated in the "Diet" column.

indicated in (Exp. 2) Table I. Serum cholesterol was elevated in each group. Rats fed glucose had somewhat lower serum cholesterol and beta-lipoprotein levels, although the difference was not of high order of statistical significance. The lower serum cholesterol levels in rats fed a glucose containing diet compared to those fed the sucrose diet corresponded with the previous finding that there was a slightly greater 24-hour biliary excretion of bile acids in the former group(1).

Serum cholesterol response when cholesterol was fed without cholic acid. Serum total cholesterol values obtained after feeding sucrose and starch containing diets with cholesterol but without cholic acid for 28 days are indicated in (Exp. 3) Table I. The sucrose group had a mean value of 124 mg % compared to 106 mg % for the starch group ($p < 0.01$). This finding probably indicates that the starch group has a lower serum cholesterol value due to its greater excretion of intrinsically derived bile acids(1).

Effect of adding sulfasuxidine to cholate containing diets. Variations in intestinal flora could explain the different effects of sucrose and starch on cholesterol-cholate induced hypercholesteremia. Therefore, 4 series of rats were fed the cholesterol and cholate containing diet. Two series were given starch as the dietary carbohydrate, and 2 were given sucrose. One of the starch groups and one of the sucrose groups were fed 0.2% of diet as sulfasuxidine. The cholate-sucrose, cholate-sucrose-sulfa, and cholate-starch-sulfa groups had similar mean serum cholesterol values, whereas the cholate-starch group had a lower ($p = 0.01$) mean serum cholesterol value (Exp. 4) Table I. It would thus seem that the gastrointestinal flora in animals fed a diet containing corn starch might contain some organisms which were influencing the metabolism of cholesterol or its catabolic end product, cholic acid. Norman(11) has shown that administration of chemotherapeutic agents to rats destroys gastrointestinal organisms responsible for hydrolysis of conjugated bile acids.

Thus, in cases where a greater biliary excretion of bile acids (as with feeding of corn starch) had been previously observed(1) a lesser degree of hypercholesteremia was observed when cholesterol or cholesterol and cholic acid were added to the diet. There is an inferred causal relationship between lower excretion of bile acids and increased cholesteremia, although the mechanisms involved and their relationship to dietary carbohydrate have not been established.

Summary. Male albino rats were fed diets

for 28 days in which the type of carbohydrate was varied and serum cholesterol and beta-lipoproteins were determined. 1. Rats fed diets containing sucrose as the carbohydrate and with added cholesterol and cholic acid had higher serum cholesterol and serum beta-lipoprotein concentrations than did control rats fed diets with corn starch substituted for sucrose. Liver cholesterol concentrations were not significantly different. 2. Feeding of sucrose, glucose, and fructose as the dietary carbohydrate had essentially equal effects on cholesterol-cholic acid induced hypercholesteremia. 3. Rats which were fed cholesterol containing diets without cholic acid and with corn starch as the carbohydrate had somewhat lower serum cholesterol values than did the controls which were fed sucrose. 4. When sulfasuxidine was added to sucrose containing diets, there was no change in serum cholesterol values; however, addition of sulfasuxidine to starch containing diets resulted in elevation of serum cholesterol values to the level of that in rats fed sucrose.

1. Portman, O. W., Mann, G. V., and Wysocki, A. P., *Arch. Biochem.*, 1955, v59, 224.

2. Siperstein, M. D., and Chaikoff, I. L., *Fed. Proc.*, 1955, v14, 760.

3. Siperstein, M. D., and Murray, A. W., *J. Clin. Invest.*, 1955, v34, 1449.

4. Page, I. H., and Brown, H. B., *Circulation*, 1952, v6, 681.

5. Byers, S. O., Biggs, M. W., and Gunning, B., *J. Exp. Med.*, 1953, v97, 511.

6. Frantz, I. D., and Hinkelman, B. T., *ibid.*, 1955, v101, 225.

7. Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, v138, 459.

8. Portman, O. W., and Mann, G. V., *ibid.*, 1955, v213, 733.

9. Abell, L. L., Levy, B. B., Brodie, B. B., and Kendall, F. E., *ibid.*, 1950, v187, 97.

10. Gofman, J. W., Lindgren, F. T., and Elliott, H., *ibid.*, 1949, v179, 973.

11. Norman, A., *Acta physiol. scand.*, 1955, v33, 99.

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Isolation of Partially Purified Human Plasmin (Fibrinolysin). (22251)

JACOB B. FISHMAN AND DANIEL L. KLINE.* (Introduced by C. N. H. Long.)

Department of Physiology, Yale Medical School, New Haven, Conn.

Although many methods for the preparation of plasmin from blood have been described, the utilization of highly purified plasminogen as a starting material appeared to offer the possibility of providing much purer plasmin than any previously reported.

This paper describes the preparation of plasmin from purified plasminogen, and the alcoholic fractionation of the resulting solution to obtain a plasmin product which is stable at low temperature and, in terms of fibrinolytic activity, is far more potent than any previously described fibrinolysin.

Materials and methods. Plasminogen. Lyophilized powder prepared by the method developed in this laboratory(1) from human plasma fraction III†. *Streptokinase* (SK). Commercial Varidase (Lederle).‡ Lots Nos. 7-1089-165A and 7-1089-142A were used. These contained 4,700 and 3,000 SK units per mg, respectively. *Fibrinogen.* Bovine Fraction I (Armour) was reprecipitated three times by the method of Laki(2) and was lyophilized. The critical assays were rechecked with fibrinogen which was reprecipitated seven times to eliminate the possibility that contaminating bo-

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