Diet and Cholesteremia. IV. Effects of Carbohydrate and Nicotinic Acid.* (25322)

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Serum cholesterol concentrations of rats fed a hypercholesteremic diet with sucrose as carbohydrate, are reported to exceed those of comparable animals fed a similar diet with starch as the carbohydrate(1). Also, in preliminary study, the substitution of wheat flour for sucrose at a level of 29.5% in a hypercholesteremic diet caused some reduction in serum cholesterol concentration in the rat. Since wheat gluten in the diet alleviated hypercholesteremia(2), it was not clear whether reduction in hypercholesteremia from feeding wheat flour was attributable to the type of carbohydrate, to the wheat gluten or to other factors. Effects of some other carbohydrates, of higher levels of wheat flour and of sorbitol in the diet were therefore studied. High doses of nicotinic acid reduced hypercholesteremia in human patients(3,4) and rabbits(5); so effects of high dietary levels of this vitamin and of feeding inositol-hexanicotinate† on serum cholesterol concentration in rats have also been studied.

Methods. Male weanling rats of Sprague-Dawley, Holtzman and Rolfsmeyer strains weighing 50-55 g were used. There were 6 rats in each group and the animals were fed *ad lib.* The basal diet contained hydrogenated coconut oil, 25%; cholesterol, 1%; cholic acid, 0.5%; salts, 4%; and casein, 25%, except in Exp. 1 (Table I) in which casein was fed at 40% level. All dietary changes were made at the expense of sucrose. Nicotinic acid and inositol-hexanicotinate were mixed with the diet. Vitamins were supplied in adequate amounts as described previously(6). At end of 3 weeks food was withdrawn for 15-18 hours, then blood was removed from each rat. Food consumption for each group was measured for entire experimental period. Serum cholesterol concentrations of individual rats were measured by the method of Henly(7). Oxidized pyridine nucleotides in blood(8) and both reduced(9) and oxidized(10) pyridine nucleotides in livers of animals ingesting high doses of nicotinic acid were estimated. Experimental procedures have been described (6).

Results. Substitution of either high extraction wheat flour or ground wheat for sucrose at a level of 29.5% in the diet, caused some reduction in serum cholesterol concentration (Table I, Exp. 1). When the carbohydrate was 44.5% of diet (Table I, Exp. 2), substitution of extracted wheat flour or ground wheat for sucrose again caused reduction in hypercholesteremia; the reduction with ground wheat being marked and significant. Dextrin (gelatinized corn starch), substituted for sucrose as the carbohydrate, did not affect serum cholesterol concentration. Substitution of 30% of lactose for sucrose in the diet, on the other hand, caused a more marked hypercholesteremia.

Since lactose is not likely to be consumed at such high levels, the effect of lower levels of lactose in the diet on serum cholesterol concentration was studied. The results are shown in Table I, Exp. 3. Diets containing 5-20% of lactose caused increases in serum cholesterol concentration; however, increasing the lactose level above 5% did not further elevate serum cholesterol concentration. Further evidence of hypercholesteremic effect of lactose was obtained when both lactose and wheat gluten were included in the diet. The fall in serum cholesterol concentration caused by supplemental amounts of wheat gluten was partially prevented by including 10% of lactose.

Changes in serum lipid phosphorus concen-

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† Inositol-hexanicotinate was kindly supplied by Philadelphia Ampoule Labs., Philadelphia, Pa.

TABLE I. Effects of Different Carbohydrates, Sorbitol, Nicotinic Acid and Inositol Hexanicotinate on the Serum Cholesterol Concentration in the Rat.

Exp. No.	Dietary alterations	Avg daily food intake/rat, g	Avg wt gain (3 wk), g	Serum: Total cholesterol, mg %	Serum: Lipid P, mg %	Liver: Total lipids, %	Liver: Total chol., %
1	29.5% sucrose	9.7	109 ± 4*	655 ± 76*	5.8		
	29.5% wheat flour (high extraction)	9.4	109 ± 4	519 ± 61	5.5		
	29.5% ground wheat	10.5	125 ± 2	547 ± 46	6.4		
2	44.5% sucrose	8.8	98 ± 5	511 ± 64	5.4		
	44.5% wheat flour (high extraction)	11.2	133 ± 5	434 ±102	6.2		
	44.5% ground wheat	10.9	133 ± 5	326 ± 65	5.4		
	44.5% dextrin	9.9	110 ± 3	502 ± 79	6.1		
	30% lactose + 14.5% sucrose	8.1	85 ± 1	1050 ± 79	10.7		
3	Sucrose	9.2	100 ± 5	539 ± 31	6.7		
	5% lactose	8.5	91 ± 3	687 ± 80	7.0		
	10% "	9.3	98 ± 4	747 ± 88	7.1		
	15% "	8.6	90 ± 3	695 ± 37	7.1		
	20% "	8.8	91 ± 3	665 ±121	7.3		
	10% lactose + 10% gluten	9.0	114 ± 4	408 ± 38	6.4		
	10% wheat gluten	9.8	114 ± 5	312 ± 30	4.9		
4	Sucrose	9.7	95 ± 4	652 ± 58	7.7	16.5	6.51
	10% lactose (Tech)	9.2	95 ± 3	733 ± 75	7.3	16.3	6.96
	20% " "	8.7	92 ± 2	912 ± 86	9.1	19.0	7.22
	10% " (U.S.P.)	9.5	94 ± 2	922 ± 62	9.1	16.7	7.09
	20% " "	8.1	88 ± 2	1067 ± 62	10.9	17.7	7.67
5	Sucrose	8.7	109 ± 2	698 ± 60			
	Glucose (A.C.S.)	8.5	107 ± 3	652 ± 53			
	" monohydrate	9.9	111 ± 2	571 ± 58			
6	25% casein	8.9	101 ± 3	539 ± 46	5.7		
	Idem + 10% sorbitol	7.8	87 ± 3	804 ±101	8.2		
	" + .5% nicotinic acid	8.6	97 ± 3	819 ±100	7.9		
	" + .57% inositol-hexanicotinate	9.7	103 ± 2	655 ±123	7.2		

* S. E. M.

tration paralleled changes in serum cholesterol concentration.

Failure to observe elevation of serum cholesterol concentration when lactose content was increased from 5% to 20%, and the observation that serum cholesterol concentrations of rats fed 30% of lactose in previous experiment were much higher (Table I, Exp. 2), suggested that source and purity of lactose might be the cause of the discrepancy. To test this, the effects of 2 grades of lactose, of sucrose and of glucose were compared.

Substitution of lactose for sucrose (Table I, Exp. 4) enhanced hypercholesteremia; however, the effect depended upon quality and purity as well as upon quantity of lactose in the diet. Ingestion of lactose also caused slightly higher deposition of cholesterol in the liver.

When sucrose was replaced with glucose in the diet (Table I, Exp. 5) there was no significant change in serum cholesterol concentration. However, feeding glucose-monohydrate‡ in place of sucrose caused a lowering of hypercholesteremia and a stimulation of food intake.

Table I, Exp. 6 shows that feeding 10% of sorbitol, 0.5% of nicotinic acid or 0.57% of inositol-hexanicotinate in the diet caused a rise in serum cholesterol concentration. Increasing the dietary level of nicotinic acid (Table II) also caused hypercholesteremia

‡ Cerelose, Corn Products Refining Co., N. Y.

TABLE II. Effect of High Doses of Nicotinic Acid on the Lipid and Pyridine Nucleotide Concentrations in the Blood and Liver of Rats.

Level of nicotinic acid in diet	Avg daily food intake, g	Avg wt gain (3 wk), g	Serum		Blood PN, γ/ml	Liver			
			Total chol., mg %	Lipid P, mg %		Total lipids, %	Total chol., %	PNH, γ/g	PN, γ/g
Control	9.7	95 ± 4	652 ± 58*	7.7	50 ± 1*	16.5	6.51	278	644
.5% N.A.	8.7	88 ± 3	748 ± 48	7.2	71 ± 2	16.0	6.23	270	660
1.0% "	9.0	90 ± 4	749 ±111	7.4	76 ± 6	17.9	6.93	286	708
2.5% "	8.5	83 ± 3	879 ± 59	7.4	73 ± 3	16.7	6.12	236	661

* S. E. M.
PN = Oxidized pyridine nucleotide. PNH = Reduced pyridine nucleotide.

under these conditions. Ingestion of high doses of nicotinic acid had no effect on liver lipids or on liver pyridine nucleotides. Oxidized pyridine nucleotides in blood, however, showed an appreciable and significant elevation over values for control animals consuming an adequate amount of nicotinic acid. No toxic effects of high doses of nicotinic acid were observed, except that animals receiving 2.5% level of nicotinic acid showed a slight growth depression.

Discussion. Reduction in serum cholesterol concentration when wheat flour was substituted for sucrose, is most likely due to cholesterol-lowering activity of wheat gluten(11). Failure of dextrin to reduce hypercholesteremia argues against an effect from the change in type of dietary carbohydrate.

Lowering of serum cholesterol values when starch is substituted for sucrose(1) may occur only when a severe hypercholesteremic regimen is employed. The effect of starch is attributed to its enhancement of cholic acid excretion but no such enhancement was observed by Matschiner *et al.*(12). Fillios *et al.*(13) using a more moderate dietary regimen found that cholesterol-lowering effect of starch disappeared after 3 weeks, while Adams *et al.* (14) found no cholesterol-lowering due to starch.

Cholesterol-lowering effect of glucose monohydrate is probably not the result of a change in kind of carbohydrate *per se*. It is more likely a result of stimulation of food intake and therefore of higher protein intake(11) or may be due to some impurity in this product. Substitution of anhydrous glucose for sucrose caused no such reduction (Exp. 5). Also a significant rise in serum cholesterol accompanying glucose feeding has been reported (14).

Higher liver and serum cholesterol values when lactose was fed, may be the result of increased intestinal absorption of cholesterol (15) or an effect of galactose on cholesterol metabolism. Sorbitol, reported to increase absorption of certain nutrients, also caused elevation in serum cholesterol concentration.

The different effects of different grades of a particular carbohydrate suggest that purity or moisture content of a carbohydrate may

influence its effect on cholesteremia. This could explain plasma cholesterol-lowering observed with glucose monohydrate in chicks and rabbits(16).

The effect of high doses of nicotinic acid on serum cholesterol concentration in the rat differ from effects observed in man(3,4) and rabbits(5). Besides the possibility of species difference, nicotinic acid was administered as a single dose in experiments on man, whereas it was mixed in the diet in the present study. The suggestion(3) that serum cholesterol-lowering effect of high doses of nicotinic acid may be due to increased oxidative activity in the body leading to formation of "oxycholesterols," is not supported by the observation that feeding high doses of nicotinic acid caused increases in both oxidized pyridine nucleotide and cholesterol concentrations of blood. Merrill and Lamley-Stone(5) suggested that cholesterol-lowering effect of nicotinic acid may be due to its detoxication products. Schön(17) suggested that nicotinic acid depressed synthesis of cholesterol by bringing about a relative lack of coenzyme A. If this mechanism is operative, then little effect of nicotinic acid could be expected in our experiments since cholesterol feeding itself causes a marked decrease in cholesterol synthesis. Further, niacin deficiency does not have any effect on serum cholesterol concentration of mice fed a diet with or without added cholesterol(18).

Summary. When rats were fed a diet containing 25% of hydrogenated coconut oil, 1% of cholesterol and 0.5% cholic acid, substitution of wheat flour for sucrose caused a reduction in serum cholesterol concentration. Diets containing sucrose, dextrin and anhydrous glucose produced the same degree of hypercholesteremia. When sucrose was replaced in part with lactose or sorbitol hypercholesteremia was enhanced. Nicotinic acid at high levels and inositol-hexanicotinate at 0.57% level in the diet failed to lower serum cholesterol concentration. High doses of nicotinic acid had no effect on pyridine nucleotide content of the liver, but raised the oxidized pyridine nucleotide content of blood significantly.

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