Effect of Cholestyramine on Cholesterol Metabolism in Young Adult Swine. (31018)

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Cholestyramine lowers plasma cholesterol levels in man(1,2), chick(1,3), and dog(4), but not in the rat(3,5). However, it sequesters bile acids and increases their fecal excretion in all species tested. Cholesterol biosynthesis by the liver is only moderately increased in the chick during cholestyramine administration(3), but is increased 10- to 15-fold in the rat(3,5). Thus, the effectiveness of cholestyramine in lowering plasma cholesterol appears to depend on: (a) a decrease in the bile acids returned to the liver *via* the enterohepatic circulation, (b) a concomitant increase in hepatic oxidation of cholesterol to bile acids, and (c) little or no increase in compensatory synthesis of cholesterol by the liver.

This report describes the effects of dietary cholestyramine on plasma cholesterol levels, fecal bile acid excretion and cholesterol biosynthesis by the liver in the pig.

Materials and methods. Five Hampshire-Yorkshire cross bred SPF baby pigs* from one litter were obtained by hysterectomy and raised to four weeks of age in special facilities(6) on a commercial baby pig formula product.† They were then placed in individual floor pens in an air-conditioned room (24°C, 50% relative humidity) and fed the starter-grower basal diet, shown in Table I, *ad libitum* for 5 weeks.

At 9 weeks of age, 2 female pigs were selected as controls while the remaining 2 females and one male were fed the same basal diet with cholestyramine added. Cr_2O_3 (0.3%) was added to all diets as a fecal indicator. The cholestyramine treated group received 2% cholestyramine in the diet for 4 weeks, then 4% cholestyramine for an additional 4 weeks. At the end of the study, the animals were sacrificed by stunning and exsanguination.

* Pure-For-Sure, Flora, Ind.

† SPF-lac, Borden Co., New York.

Representative fresh fecal samples were collected over a 3-day period each week. They were stored in the frozen state, pooled and thoroughly mixed before assaying. Cr_2O_3 was determined by the procedure of Hill *et al* (7). Calculations of fecal excretion were based on relative Cr_2O_3 levels in the diet and feces. Fat and cholesterol were extracted using the procedure of Williams(8). Fat was determined gravimetrically and cholesterol-like materials determined by the Lieberman-Burchard reaction using the reagents of Abell *et al*(9). Bile acids were determined using ion exchange and gas chromatography as reported by Harkins *et al*(10).

Non-fasting blood samples were obtained weekly, at the same time of day, from the

TABLE I. Composition of Basal Diet.

Ingredients	%	Proximate analysis	%
Pablum® Mixed Cereal[1]	48.0	Protein	18.0
		Fat	20.0
Pablum® High Protein Cereal[1]	16.0	Carbohydrate[8]	48.6
		Ash	4.5
Non-fat dry milk[2]	13.0	Fiber	1.6
Fat[3]	20.0	Moisture	7.3
Glucose, anhydrous[4]	1.55		
Non-nutritive fiber[5]	1.0		100.0
Trace mineral mixture[6]	.05		
Vitamin mixture[7]	.4		
	100.0		

[1] Mead Johnson & Co., Evansville, Ind.
[2] Dairy Craft, Producer's Creamery, Cabool, Mo.
[3] Mixture of 55% lard, 25% coconut oil and 20% corn oil.
[4] Cerelose, Corn Products Co., Pekin, Ill.
[5] Cellulose fiber, General Biochemicals, Chagrin Falls, Ohio.
[6] Furnished 6 mg Zn, 2 mg Co, 50 mg Mn and 13 mg Cu/100 g of diet.
[7] Furnished 444 I.U. vit A, 55 I.U. vit D_2, 1 mg d-α-tocopherol acetate, 25 mg ascorbic acid, 0.5 mg thiamine hydrochloride, 0.5 mg riboflavin, 4 mg niacin, 3 mg calcium pantothenate, 0.3 mg pyridoxine hydrochloride, 0.05 mg folic acid, 330 mg choline bitartrate, 0.005 mg vit B_{12} and 0.014 mg biotin/100 g of diet.
[8] Estimated by difference.

anterior vena cava using the procedure described by Hoerlein *et al*(11). Hematocrits were determined by procedure of Van Allen (12), plasma cholesterol by the method of Abell *et al*(9), and total lipids by the technique of Sunderman and Sunderman (13). At the end of the 8-week study, the animals were sacrificed and organ weights determined. The aortas were removed for cholesterol determination(9) and samples of liver taken for analyses of solids and fat (14), vitamin A(15), cholesterol(9) and for the *in vitro* metabolic studies.

Liver homogenates were promptly prepared for measurement of *in vitro* incorporation of C^{14}-acetate into sterols and fatty acids using the procedures previously described(16) with the following modifications. The incubation flasks contained 2 μc (1 μmole) sodium acetate-1-C^{14} and no added succinate. In addition, $C^{14}O_2$ was collected during the incubation using 2 N NaOH as the trapping agent. For $C^{14}O_2$ determination, 1 ml of the base solution was transferred to a counting vial and diluted with 15 ml of a scintillator solution containing 7 g of 2,5 diphenyloxazole (PPO), 0.5 g 1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene (dimethyl POPOP), 100 g naphthalene, 40 g Cab-O-Sil M5‡ per liter, in dioxane. Sterol digitonides were dissolved in glacial acetic acid; an aliquot was taken for cholesterol determination(9) and 1 ml transferred to a counting vial and diluted with 15 ml of a scintillator solution containing 3 g PPO, 0.5 g dimethyl POPOP, and 35 ml absolute methanol per liter, in toluene. The fatty acid residue was weighed and dissolved in a scintillator solution containing 3 g PPO and 0.5 g dimethyl POPOP per liter of toluene. C^{14} was determined using an automatic Tri-Carb liquid scintillation spectrometer Model 314E.§

Results and discussion. Weight gain and food efficiency. Feeding cholestyramine for 8 weeks had no apparent effect on weight gain, food intake, or food efficiency, (Table II). During this period, the control pigs gained an average of 41.7 kg, whereas those receiving cholestyramine gained 45.6 kg. The one male pig in this group gained 54.5 kg, whereas the 2 females gained 41.1 kg, similar to that of the female control pigs. The higher food consumption of the male pig in the cholestyramine fed group also increased the average food intake for this group (87.4 kg) above that of the controls (78.4 kg). However, cholestyramine had no effect on food efficiency; the controls gained 120 g/1000 cal and the cholestyramine fed pigs gained 121 g/1000 cal.

TABLE II. Effect of Cholestyramine on Weight Gain, Food Efficiency, Organ Weights, Liver Composition, and Aorta Cholesterol Levels of Young Adult Swine.

	Control diet	Control diet plus cholestyramine
No. of animals	2	3
Selection wt, kg	27.7	29.7
8 week wt gain, kg	41.7	45.6
Total food consumed, kg	78.4	87.4
Caloric efficiency, g gain/1000 calories	120	121
Organ weights		
Final body wt, kg	69.2	76.4
Liver, g	925.5	1122.7
g/kg body wt	13.4	14.6
Spleen, g	91.0	90.7
g/kg body wt	1.3	1.2
Heart, g	253.0	284.0
g/kg body wt	3.7	3.8
Kidneys, g	173.5	210.7
g/kg body wt	2.5	2.8
Adrenals, mg	2635.0	3270.0
mg/kg body wt	38.0	43.9
Liver composition		
% solids	30.1	29.6
% fat	4.2	4.5
Cholesterol, mg/g	3.2	2.8
Vitamin A, I.U./g	479	310
" " I.U./ liver	441,882	344,601
Aorta cholesterol, mg/g	1.4	1.3

The controls averaged 44% and in the cholestyramine fed pigs 40%.

Organ weights and liver composition. The liver, spleen, heart, kidney, and adrenal weights are summarized in Table II. No marked differences were observed between the two groups.

Cholestyramine feeding also had no effect on the fat and total solids content of the liver (Table II). The liver cholesterol levels

‡ Cabot Corp., Boston, Mass.

§ Packard Instrument Co., La Grange, Ill.

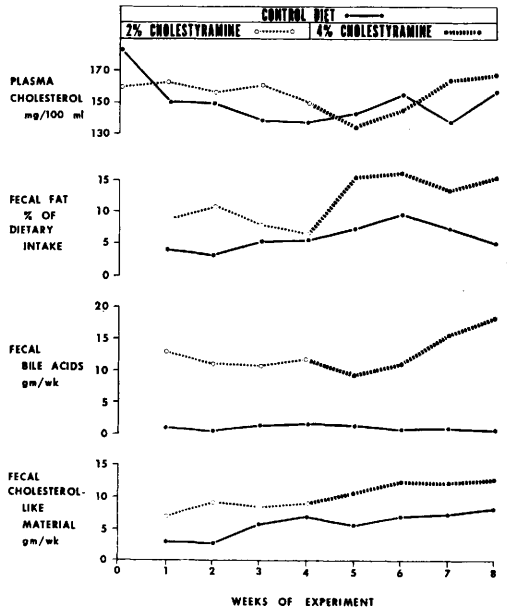

FIG. 1. Effect of cholestyramine on plasma cholesterol, fecal fat, fecal bile acids and fecal cholesterol-like materials in young adult swine.

of the cholestyramine fed group (average 2.8 mg/g) were slightly lower than those found in the control group (average 3.2 mg/g). The difference between these groups was statistically significant ($P<.02$), but the small magnitude of this difference and the few animals per group limit the interpretation of the data. Liver vitamin A levels were significantly lower in the cholestyramine fed pigs; those receiving cholestyramine had 310 I.U./g while the controls had 479 I.U./g ($P<.01$). Studies in the rat have also shown that excess cholestyramine in the diet decreases absorption of vit. A, resulting in lower liver vit. A levels(17).

Aorta cholesterol levels were not affected by cholestyramine feeding. These values were 1.4 and 1.3 mg/g for the control and treated groups, respectively.

Plasma lipids. Feeding 2% cholestyramine for 4 weeks had no effect on plasma cholesterol levels, as shown in Fig. 1. The plasma cholesterol levels of the control group (non-fasted) varied from about 140 to 150 mg/100 ml during this period, whereas values for the cholestyramine fed group varied from 150 to 160 mg/100 ml.

After 4 weeks, the level of cholestyramine in the diet was increased to 4%. One week later, the plasma cholesterol level of this group was only 133 mg/100 ml, but during the remaining 3 weeks on this regimen, cholesterol levels slowly increased and were essentially the same as those of the controls—156 mg/100 ml for the control group and 166 mg/100 ml for the cholestyramine treated group. These pigs received about 70 g of cholestyramine per day or about 1.1 g/kg of body weight/day; in man, only 10 to 15 g of cholestyramine, or 0.15 to 0.20 g/kg/day, effectively lowers plasma cholesterol levels.

Total plasma lipids were not affected by feeding cholestyramine.

Fecal excretion. Fecal fat excretion was increased slightly by 2% cholestyramine in the diet, and further increased by 4% cholestyramine (Fig. 1). About 4% to 9% of the fat intake was found in the feces of the control group, whereas 7% to 11% was found when the pigs were receiving 2% cholestyramine, and 13% to 16% when the pigs were receiving 4% cholestyramine. These findings are quite similar to those reported for the rat(18).

Cholestyramine apparently had little effect on the overall absorption of the major nutrients in the diet. This was determined from the dry weight of stool and its Cr_2O_3 content as compared with the level of Cr_2O_3 in the diet. The feces accounted for 5 to 6% of the weight of the diet in the control pigs and 6 to 7% in the cholestyramine fed pigs.

Data on bile acid excretion (Fig. 1) showed a large increase in the cholestyramine fed pigs. The control pigs excreted from 0.4 to 1.6 g of bile acids per week, whereas the others excreted from 10.8 to 13.0 g/wk when they were receiving 2% cholestyramine and from 9.1 to 18.0 g/wk when they were receiving 4% cholestyramine. The level of cholesterol-like material (Lieberman-Burchard reactive) in the feces was consistently higher in the pigs receiving cholestyramine than in the control pigs (Fig. 1). During the last week of the experiment, the treated pigs excreted over 12 g/wk as compared to 8 g/wk in the controls.

In vitro *liver lipid synthesis.* The rates of oxidation of C^{14} acetate and synthesis of

TABLE III. Effect of Cholestyramine on the *in vitro* Metabolism of Acetate C^{14} by Liver Homogenates.*

	Control diet		Control diet plus cholestyramine	
No. of pigs	2		3	
CO_2 expired, % of substrate	56 50		54 54 59	
Mean		53		56
Total fatty acids, cpm/mg fatty acids	624 418		778 752 652	
Mean		521		727
Sterol digitonides, cpm/mg cholesterol	8220 4973		133,200 135,450 110,700	
Mean		6596		126,450

* The individual values shown for each animal are the averages of duplicate analyses.

sterols and fatty acids from this substrate by liver homogenates were measured when the pigs were sacrificed. Rates of oxidation of C^{14} labeled acetate to $C^{14}O_2$ were similar in both groups (Table III). The specific activity of fatty acids extracted from the reaction mixture following saponification was only slightly higher in the cholestyramine fed group (727 cpm/mg), than in the controls (521 cpm/mg).

Incorporation of the labeled C^{14} acetate into digitonin precipitable sterols by the liver homogenates was greatly increased in the cholestyramine fed animals. The specific activity of the sterol digitonides was 126,450 cpm/mg of cholesterol in the cholestyramine treated pigs, as compared with 6,596 cpm/mg in the controls. This showed a highly significant ($P<.01$) 19-fold increase in the rate of sterol synthesis in the pigs receiving cholestyramine.

Since intestinal bile acids were sequestered and excreted in rather large amounts, the lack of effect of cholestyramine on plasma cholesterol levels in the pig apparently was due to the capacity of the liver of this species to increase sterol synthesis sufficiently to compensate for the loss of bile acids.

Summary. Nine-week-old swine were fed the bile acid sequestrant, cholestyramine, at 2% and 4% of the diet for successive 4-week periods. Cholestyramine increased bile acid excretion about 10-fold, but the rate of liver biosynthesis of cholesterol was 19 times higher in the cholestyramine fed pigs than in the control pigs. This marked increase in cholesterol synthesis was apparently sufficient to prevent a decrease in plasma cholesterol levels. Feeding 4% cholestyramine (approximately 70 g/day) had no effect on growth, food efficiency, organ weights, plasma lipids and aorta cholesterol. Liver cholesterol levels were decreased slightly, liver vitamin A stores were decreased about one-third, and excretion of fecal fat and cholesterol-like (Lieberman-Burchard reactive) materials were increased in the cholestyramine fed pigs.

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Skeletal Response to Exogenous Ascorbic Acid by Vitamins D_3 Deficient Chicks.* (31019)

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Possible metabolic relationships in bone tissue between ascorbic acid and vitamin D have not been an area of extensive research interest. Despite the paucity of information regarding such possibilities some recent work suggests that the two vitamins may have certain affiliations. Skeletal uptake and release of Ca^{45} was markedly enhanced in vit. D_3 deficient chicks which were progeny of ascorbic acid-fed forebears(1). The addition of this water soluble vitamin to rachitogenic diets(2) also indicated an alteration in other skeletal responses. Principal changes included influences on energy metabolism and mineral release during *in vitro* incubation of the bone tissue. Evidence of a highly correlated association between skeletal energy utilization and demineralization was also exhibited by animals given ascorbic acid(2). The present study concerns the skeletal response to ascorbic acid which was administered subcutaneously in advanced stages of vit. D_3 deficiency.

Methods and materials. One-day-old Leghorn chicks were placed on 2 dietary treatments consisting of a complete diet(3) or this same ration without supplementary vit. D_3. The animals were given feed and water free-choice throughout the experiment. They were reared in electrically-heated batteries in a room with incandescent lights. After 26 days of dietary treatment, the vit. D_3 deficient group was divided into 2 groups of 10 animals each. These animals and 10 others which had received the complete diet were injected subcutaneously for 2 consecutive days with solutions as shown in Table I. Ascorbic acid was administered in 0.9% saline solution at a level of 5 mg per 100 g body weight per day. The concentration of this vitamin in the saline solution was controlled so that no individual received more than 1.5 ml per day and all were given a comparable volume per unit of body weight.

Twenty-four hours following the last injection the animals were sacrificed, the right tibia removed immediately, cleaned of adhering soft tissue and placed in cold 0.9% saline solution. After cooling, the bone was split lengthwise and portions of the compact bone, free of marrow, were harvested and placed in 2.8 ml of cold media (pH 7.4) containing the following in μmoles: Tris (hydroxymethyl) aninomethane 20, $MgSO_4$ 15, glucose 10, NaCl 155 and ADP (adenosine diphosphate) 10. Hexokinase was added at 0.5 mg per flask. Incubation was conducted in the Dubnoff metabolic shaker at 37°C for 3 hours using air as the gas phase.

Chemical determinations of the media included glucose(4), lactic acid(5), hydroxyproline(6), calcium(7), phosphate(8). Noncollagenous nitrogen (NCN)(9) was determined on the incubated bone samples. Similar bone tissue from the tibia was fat-extracted for 16 hours with pentane, dried to a constant weight at 105°C, ashed overnight

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