Hypolipidemic effects of orally administered dextran and cellulose anion exchangers in cockerels and dogs

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ABSTRACT Various cellulose and dextran anion exchangers bind bile salts in vitro under conditions of pH and ionic strength resembling those in the lumen of the small intestine. Of these substances, diethylaminoethyl (DEAE) cellulose, guanidoethyl cellulose, and DEAE Sephadex reduced hypercholesterolemia when added to the diet of cholesterol-fed cockerels. In addition, DEAE Sephadex reduced serum sterols in normocholesterolemic cockerels and dogs, lowered serum phospholipids and triglycerides in cholesterol-fed hypercholesterolemic cockerels and in normocholesterolemic dogs, and increased fecal excretion of bile acids in hypercholesterolemic cockerels.

The data indicate that these insoluble cationic polymers exert their hypocholesterolemic effects by interrupting the enterohepatic circulation of bile acids.

KEY WORDS bile acids · degree of binding · anion exchangers · celluloses · DEAE Sephadex · hypocholesterolemic activity · serum lipids · fecal excretion · enterohepatic circulation · cockerel · dog

Interruption of the enterohepatic circulation of bile acids by decreasing their intestinal reabsorption effectively reduces serum cholesterol levels both in experimental animals and in man. Buchwald (1) has reported that surgical bypass of the ileum decreases serum sterols in rabbits and in man, presumably by interference with absorption of bile acids and sterols. Benzmalecene, a hypocholesterolemic agent in experimental animals and in man (2), also inhibits intestinal transport of bile acids in vitro and in vivo (3). Siperstein, Nichols, and Chaikoff (4) showed that ferric chloride, which precipitates bile salts in vitro, prevents hypercholesterolemia when added to the diet of cholesterol-fed cockerels. Tennent et al. demonstrated (5) the oral hypocholesterolemic effects of cholestyramine, an insoluble, nonabsorbed, quaternary ammonium anion exchange resin that binds bile acids in the intestinal lumen and increases their fecal excretion. In man this resin has produced 20–50% reductions in serum sterols without significant side effects for periods up to 4 yr (6). The data reported here demonstrate oral hypolipidemic effects in normo- and hypercholesterolemic animals of cellulose and dextran anion exchangers that bind bile acids in vitro and enhance their fecal excretion in vivo.

Abbreviations: AE, aminoethyl; DEAE, diethylaminoethyl; TEAE, triethylaminoethyl; GE, guanidoethyl; PAB, *p*-aminobenzyl; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

MATERIALS AND METHODS

Anion Exchangers

Cellulose anion exchangers used in this study were manufactured by Serva Entwicklungslabor, Heidelberg, Germany, and are distributed in the United States by Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, L. I., N. Y. DEAE Sephadex A-25, fine grade, nonbead form, and DEAE dextran were obtained from Pharmacia Fine Chemicals Inc., New Market, N. J. Cholestyramine resin (MK-135) was obtained from Merck, Sharp & Dohme, West Point, Pa.

Lipid Analyses

Serum total sterols were determined by the ferric chloride–sulfuric acid method (7), adapted to the Technicon AutoAnalyzer (Technicon Co., Chauncey, N. Y.). Phospholipids and triglycerides were extracted from serum samples according to the procedure of Folch,

Lees, and Sloane Stanley (8). Triglycerides were determined on 10-ml portions of extract from which phospholipids were removed by shaking with 1 g of Doucil (a zeolite obtained from W. A. Taylor & Company, Baltimore, Md.). After saponification, glycerol was measured by the method of Lambert and Neish (9), adapted to the AutoAnalyzer, and was expressed as equivalent tripalmitin with a monomethylol dimethyl hydantoin standard (10). Phospholipids were determined on portions of extract by the method of Stewart and Hendry (11), also adapted to the AutoAnalyzer. Inorganic phosphorus was multiplied by 25 to give the equivalent amount of phospholipid.

Fecal bile acids were extracted and isolated by the method of Kuron and Tennent (12, 13). Cholic acid derivatives were measured by the method of Irvin, Johnston, and Kopala (14) and dihydroxy bile acids by the method of Mosbach, Kalinsky, Halpern, and Kendall (15).

Statistical significance of the experimental data was determined by Student's "*t*" test; values of $P < 0.05$ were considered significant. Although for some of the reductions in serum sterols demonstrated in these studies values of $P > 0.05$ were obtained, the hypocholesterolemic effects were of such magnitude that the data were included for purposes of comparison. In these cases P values are indicated.

RESULTS

Bile Acid Binding In Vitro

Bile acid-binding capacity of various cationic cellulose and dextran anion exchangers was determined by adding 50 mg of the chloride salt to 5 ml of a 1% solution of bile acid in 0.9% saline, pH 6.2, conditions of pH and ionic strength resembling those in the lumen of the proximal small intestine (16). Preliminary data indicated that solutions of either sodium cholate or sodium taurocholate could be used. Since the unconjugated bile salt was more readily available it was used in all subsequent studies. The mixture was shaken briefly and centrifuged, and a portion of the supernatant fluid was analyzed for cholic acid (Table 1). The data show a broad range of activity for the anion exchangers which does not appear to be related directly to exchange capacity, but which varies with the cationic functional group. Also DEAE Sephadex was more effective than an amount of DEAE cellulose of equal exchange capacity, which indicates that the nature of the polymer skeleton itself is important in determining activity. Cholestyramine (MK-135), a styrene–divinylbenzene copolymer chemically unrelated to cellulose and dextran, decreased cholate by 51% in a similar system.

TABLE 1 BINDING OF CHOLIC ACID IN VITRO BY CELLULOSE AND DEXTRAN ANION EXCHANGERS

Anion Exchanger*	Exchange Capacity	Decrease in Cholate
	meq/g	%
AE cellulose	0.40	5.0
DEAE "	0.68	13.5
TEAE "	0.73	11.0
GE "	0.42	46.0
PAB "	0.34	7.0
ECTEOLA-Cellulose†	0.60	11.0
DEAE Sephadex	4.0	80.6
" cellulose (295 mg)	—‡	69.0

Material, 50 mg, was added to 5 ml of a 1% solution of sodium cholate in 0.9% NaCl. After the mixture had been shaken and centrifuged, cholate was determined in the supernatant fluid.

* AE, aminoethyl; DEAE, diethylaminoethyl; TEAE, triethylaminoethyl; GE, guanidoethyl; PAB, *p*-aminobenzyl.

† Reaction product of cellulose with epichlorohydrin and triethanolamine.

‡ Equivalent in total exchange capacity to 50 mg of DEAE Sephadex.

Effects on Serum Lipids In Vivo

Those derivatives which showed the greatest bile acid-binding activity in vitro—DEAE cellulose, GE cellulose, and DEAE Sephadex—were tested for oral hypocholesterolemic activity in the chicken. MK-135, which is known to reduce serum sterols in this species (5), was included for comparison. 5-Wk old White Leghorn cockerels[1] (Townline Hatcheries, Zeeland, Mich.) were maintained for 4 days on a hypercholesterolemic diet (17), which contained 2% cholesterol, 5% cottonseed oil, and 1% of the material to be tested. The stock diet had no added cholesterol or cottonseed oil. On the 5th day, after an 18 hr fast, the birds were electrocuted and blood samples were taken from the heart for analysis of serum sterols. DEAE Sephadex partially inhibited the rise in serum sterols ($P < 0.01$), while DEAE and GE cellulose were inactive (Table 2). MK-135 was also active in this system ($P < 0.01$). Although there was a possible decrease in weight gain and food intake in the birds treated with MK-135, this was not significant.

During these studies it was found that addition of 2% cholesterol alone to the diet produces a marked elevation of serum sterols in the chicken, and in subsequent experiments cottonseed oil was omitted. In birds fed this cholesterol-supplemented diet, DEAE cellulose reduced hypercholesterolemia 20% ($P < 0.2$) and GE cellulose 32% ($P < 0.05$) (Table 3). DEAE Sephadex and MK-135 lowered serum sterols 64% ($P < 0.01$) and 65% ($P < 0.01$), respectively. None of these materials caused significant decreases in weight gain or food intake.

[1] Obtained 1 day old and reared in brooders on stock diet until 5 wk old.

TABLE 2 EFFECT OF ANION EXCHANGERS ON SERUM STEROLS OF COCKERELS FED CHOLESTEROL AND COTTONSEED OIL

Diet	Serum Sterols	Weight Gain	Food Intake
	mg/100 ml	*g/bird*	
Stock	132 ± 7 (4)*	60	115
Hypercholesterolemic	282 ± 37 (5)	65	124
" + 1% DEAE cellulose	381 ± 80 (5)	57	112
" + 1% GE cellulose	314 ± 45 (4)	66	118
" + 1% DEAE Sephadex	147 ± 27 (5)†	72	122
" + 1% MK-135‡	118 ± 3 (5)†	48	98

Birds were fed diet containing 2% cholesterol and 5% cottonseed oil for 4 days.

* Mean ± SEM. Number in parentheses in this and in subsequent tables is number of birds per group.

† Significantly different from hypercholesterolemic control ($P < 0.01$).

‡ MK-135, cholestyramine resin.

TABLE 3 EFFECT OF ANION EXCHANGERS ON SERUM STEROLS OF COCKERELS FED CHOLESTEROL WITHOUT COTTONSEED OIL

Diet	Serum Sterols	Weight Gain	Food Intake
	mg/100 ml	*g/bird*	
Stock	138 ± 4 (5)*	70	151
Hypercholesterolemic	339 ± 36 (5)	77	154
" + 1% DEAE cellulose	270 ± 30 (6)	71	168
" + 1% GE cellulose	231 ± 16 (6)†	78	164
" + 1% DEAE Sephadex	123 ± 6 (5)‡	84	167
" + 1% MK-135	117 ± 6 (6)‡	66	142

Birds were fed diet containing 2% cholesterol for 4 days.

* Mean ± SEM (No. of birds).

† Significantly different from hypercholesterolemic control ($P < 0.05$).

‡ Significantly different from hypercholesterolemic control ($P < 0.01$).

DEAE cellulose, GE cellulose, and DEAE Sephadex were tested also for hypocholesterolemic activity in normocholesterolemic cockerels in two separate experiments. 5-Wk old cockerels fed a stock diet containing these substances for 4 days were fasted and sacrificed, and blood samples were analyzed as above. The data in Table 4 show that the cellulose anion exchangers were not effective in either study, but DEAE Sephadex reduced serum sterols an average of 19% ($P < 0.01$). Weight gain and food intake were unaffected.

Response of serum sterols of the cholesterol-fed cockerel to various doses of DEAE Sephadex is shown in Fig. 1. At the lowest level tested (0.1%) serum sterols were reduced 24% ($P < 0.2$); at the highest level tested (10%) they were reduced 71% ($P < 0.01$) to a value 39% lower than that in the group fed stock diet. DEAE Sephadex did not significantly influence weight gain or food intake at any level, but did decrease weight gain slightly at 5% and 10% in the diet.

The cholate salt of DEAE Sephadex was prepared and tested in cholesterol-fed cockerels. Control birds receiving 2% cholesterol in their diet had serum sterol levels of 258 ± 26 mg/100 ml, while birds fed 2% cholesterol and 1% DEAE Sephadex complexed with

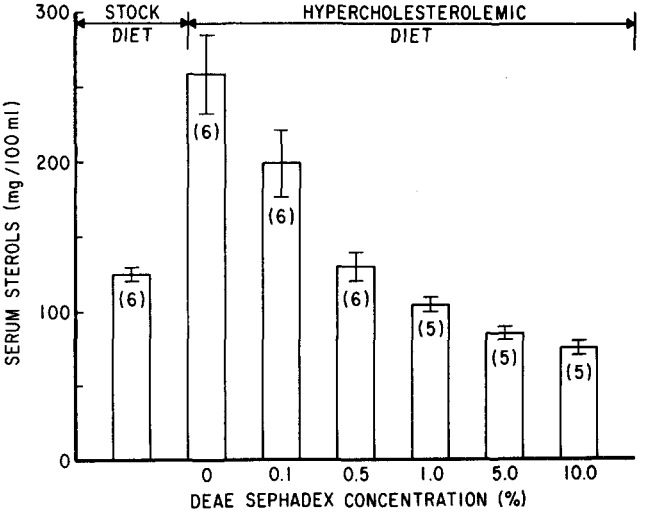

FIG. 1. Effect on serum sterols of DEAE Sephadex in diet of cholesterol-fed cockerels. Birds were fed a hypercholesterolemic diet containing 2% cholesterol for 4 days. Vertical bars represent SEM. Numbers in parentheses are number of birds per group.

TABLE 4 EFFECT OF ANION EXCHANGERS ON SERUM STEROLS OF NORMOCHOLESTEROLEMIC COCKERELS

Diet	Study I*			Study II*		
	Serum Sterols	Weight Gain	Food Intake	Serum Sterols	Weight Gain	Food Intake
	mg/100 ml	*g/bird*		*mg/100 ml*	*g/bird*	
Stock	148 ± 7 (6)†	69	174	136 ± 5 (5)	47	143
" + 1% DEAE cellulose	135 ± 8 (5)	62	158	143 ± 7 (4)	55	137
" + 1% GE cellulose	150 ± 8 (2)	56	159	136 ± 6 (6)	49	148
" + 1% DEAE Sephadex	123 ± 1 (5)‡	60	164	107 ± 5 (6)‡	52	143

* Birds were fed stock diet for 4 days in separate studies I and II.

† Mean ± SEM.

‡ Significantly different from control ($P < 0.01$).

TABLE 5 REVERSAL BY DEAE SEPHADEX OF THE HYPERCHOLESTEROLEMIC EFFECT OF CHOLESTEROL FEEDING IN COCKERELS

Group	Regimen	Serum Sterols	Weight Gain	Food Intake
		mg/100 ml		*g/bird*
1	8 Days stock diet	126 ± 9 (6)*	111	427
2	8 Days + 2% cholesterol	289 ± 32 (6)	113	428
3	4 Days + 2% cholesterol followed by 4 days + 2% cholesterol and 1% DEAE Sephadex	193 ± 37 (6)†	108	460
4	8 Days + 2% cholesterol and 1% DEAE Sephadex	173 ± 19 (5)‡	129	480

Group 3 was fed the hypercholesterolemic diet without DEAE Sephadex for 4 days, then changed to the diet with DEAE Sephadex.
* Mean ± SEM.
† Difference from cholesterol-fed controls is significant at the 90% confidence level.
‡ Significantly different from cholesterol-fed controls ($P < 0.02$).

cholate had levels of 238 ± 18 mg/100 ml. This demonstrates that prior saturation of DEAE Sephadex with a bile acid completely eliminates its hypocholesterolemic activity.

To determine whether or not DEAE Sephadex could reduce preexistent hypercholesterolemia as well as prevent its appearance, we maintained 5-wk old cockerels for 8 days on diets containing 2% cholesterol with or without 1% DEAE Sephadex, while an additional group of birds was fed the cholesterol-containing diet for 4 days and then switched to the same diet plus 1% DEAE Sephadex for 4 days. The data in Table 5 show that hypercholesterolemia was reduced ($P < 0.1$) by DEAE Sephadex feeding.

1% DEAE Sephadex in the diet of cholesterol-fed cockerels maintained serum sterols at levels significantly lower than those found in cholesterol-fed controls over a 4 wk period. Serum sterols of the treated birds averaged about 50% those of the control birds (Fig. 2). Significant reductions of serum triglycerides and phospholipids during the 2nd, 3rd, and 4th wk of treatment are also apparent from the data. Bile acids in fecal samples collected daily during the 4th wk of the study averaged 429 mg/day per group for the control birds and 725 mg/day per group for the treated birds. No significant differences in weight gain were observed in the treated groups, and analysis of blood of the cockerels receiving 1% DEAE Sephadex for 30 days (average dose approximately 900 mg/kg per day) revealed no abnormalities in serum transaminases (SGOT and SGPT), Ca^{++}, Cl^{-}, bicarbonate, or prothrombin time. Gross examination of body tissues showed no evidences of vitamin deficiencies or drug-related organ weight changes; DEAE Sephadex-treated birds appeared to have less lipid deposited in their livers than did cholesterol-fed controls. Microscopic examination of Oil Red O-stained aortas from treated birds revealed no visible fat, whereas slight amounts of fat were present in the media of aortas from controls.

Two Upjohn beagles were fed 250 g of milled Purina

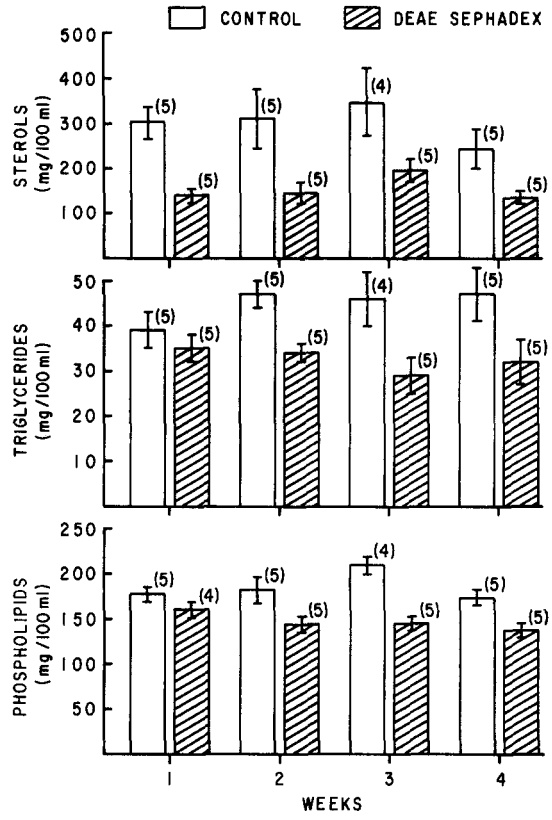

FIG. 2. Effect on serum lipids of DEAE Sephadex in diet of cholesterol-fed cockerels. Birds were fed a hypercholesterolemic diet containing 2% cholesterol for the periods shown. Vertical bars represent SEM. Numbers in parentheses are number of birds per group.

dog chow per day for 1 wk, followed by 250 g of dog chow containing 25 g of DEAE Sephadex per day for 1 wk. Venous blood samples for serum lipid analyses were taken daily after an 18 hr fast. Food consumption was measured. Fig. 3 shows a decrease in serum sterol levels of both dogs during the treatment period. In dog 1 this reached a minimum value of 132 mg/100 ml (26% decrease from the mean control period value of 178 mg/100 ml), and in dog 2 a minimum of 115 mg/

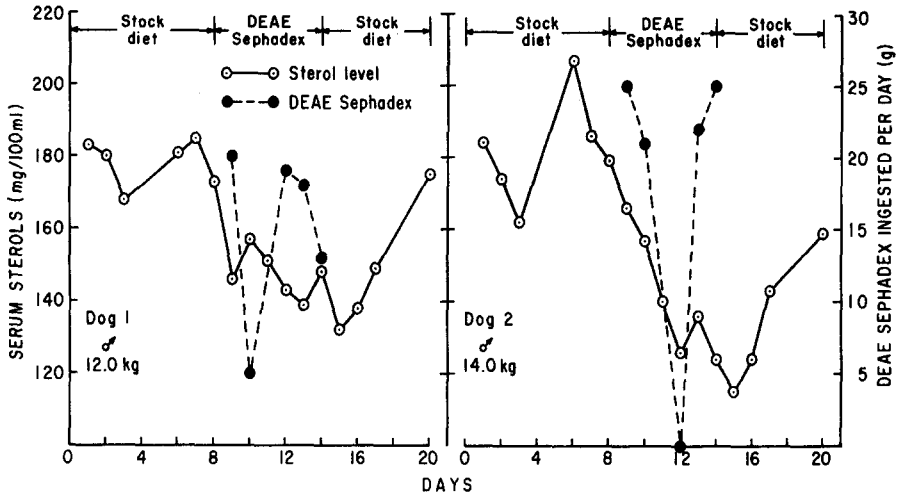

FIG. 3. Effect of DEAE Sephadex on serum sterols of normocholesterolemic beagles. Dogs were fed 250 g of Purina dog chow per day. DEAE Sephadex, 25 g, was added to chow per day during treatment period. Amount of DEAE Sephadex ingested per day is calculated from food consumption measurements.

TABLE 6 EFFECT OF DEAE SEPHADEX ON SERUM LIPIDS OF NORMOCHOLESTEROLEMIC BEAGLES

Day	Diet	DEAE Sephadex Ingested		Serum Sterols		Serum Triglycerides		Serum Phospholipids	
		Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
		g		*mg/100 ml*					
1	Stock			183*	184	40	32	311	337
2	"			180	174	30	23	343	311
3	"			168	162	23	26	330	314
6	"			181	207	35	22	292	313
7	"			185	186	33	27	339	379
8	"			173	179	24	19	314	329
			Average	178	182	31	25	322	331
8	DEAE Sephadex	—	—						
9	" "	20	25	146	166	23	19	280	324
10	" "	5	21	157	157	24	23	274	294
11	" "	—	—	151	140	32	25	265	255
12	" "	19	0	143	126	15	18	262	240
13	" "	18	22	139	136	24	19	353	239
14	" "	13	25	148	124	18	19	190	244
15	" "	—	—	132	115	29	19	230	216
15	Stock								
16	"			138	124	33	30	343	262
17	"			149	143	42	35	217	291
20	"			175	159	48	39	307	316

Dogs were fed 250 g of Purina dog chow containing 25 g of DEAE Sephadex per day.
* Mean of duplicate samples.

100 ml (37% decrease from mean control value of 182 mg/100 ml). Definite, although less pronounced, decreases in serum triglycerides and phospholipids were noted also (Table 6). Since these animals were fed the daily ration ad lib. there was some variation in the quantity of DEAE Sephadex ingested per day. The rapid rise in serum sterols in both animals after their return to stock diet shows the reversibility of the effect of DEAE Sephadex. Weight and prothrombin time were not significantly altered in these animals.

DISCUSSION

Cellulose ion exchangers, cross-linked by hydrogen bonds, and DEAE Sephadex, a chemically modified dextran cross-linked by ether bonds, possess a loose hydrophilic molecular structure that permits free diffusion of even very large molecules such as proteins and nucleic acids through a three-dimensional network of polysaccharide chains. Accessibility of the binding sites of these anion exchangers to large ionic species may be

important in determining their affinity for bile acids, since bile acids are above their critical micelle concentration in the intestinal lumen (18), and hence exist as anionic micelles of particle weight 2000–5000 g/mole or larger (19, 20). Therefore, ion exchangers whose permeability to large ions is decreased by extensive cross-linking should exclude bile acid micelles. That such is the case with polystyrene resins of the MK-135 type is suggested by the fact that the hypocholesterolemic activity of cholestyramine, which correlates with its bile acid-binding capacity in vitro (21), is seriously impaired by divinylbenzene cross-linking greater than 5% (22).

Binding of bile acids in vitro by cellulose and dextran anion exchangers is determined by their cationic functional groups as well as by their basic polymeric structure. GE cellulose and AE cellulose, with approximately equal exchange capacity, differ markedly in their cholate-binding activity (Table 1). On the other hand, DEAE Sephadex, a tertiary amine derivative, displays activity both in vitro and in vivo similar to that of cholestyramine, although the latter compound contains quaternary ammonium functional groups. Thus it appears that a complex combination of physical and chemical properties, rather than any single obvious determinant, imparts hypocholesterolemic activity to these substances.

Hypocholesterolemic activity of DEAE cellulose, GE cellulose, and DEAE Sephadex parallels bile acid-binding activity in vitro. Lack of activity of the cholate salt of DEAE Sephadex and increased fecal excretion of bile acids in cockerels treated with DEAE Sephadex suggest that this compound and the cellulose exchangers enhance excretion of bile acids as insoluble salts of polymeric amines, with concomitant increase in the oxidation of cholesterol to cholic acid in the liver. If the treated animal is unable to compensate for this increase in cholesterol catabolism by an increase in cholesterol biosynthesis, labile cholesterol pools, including serum sterols, will be reduced. It has been proposed previously that cholestyramine acts in this manner (23). In addition, decreased absorption of exogenous and endogenous cholesterol caused by binding bile acids in the intestinal lumen (24) may contribute to the hypocholesterolemic activity of these materials.

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