Cholestyramine in Dogs

By G. R. JANSEN and M. E. ZANETTI

The effect of graded levels of cholestyramine on bile acid excretion and plasma cholesterol in dogs has been determined. Resin doses of 1, 3, 6, and 10 Gm./dog/day lowered plasma cholesterol 2, 12, 24, and 37 per cent, respectively. Bile acid excretion gradually increased with increasing resin dose, but was no higher on 10 Gm./day than on 6 Gm./day. The effect of particle size of the resin was also evaluated. Coarse 20–50 mesh resin beads were as effective as 200–400 mesh resin in increasing bile acid excretion and lowering plasma cholesterol. The effectiveness of cholestyramine was not increased by enteric coatings.

It is well known that bile acid excretion is a major pathway for the elimination of the sterol nucleus of cholesterol. Siperstein *et al.* (1) have reported that the increase in plasma cholesterol and aortic atheromata in cholesterol-fed cockerels could be inhibited by feeding ferric chloride, results they attributed to the precipitation of the bile salts in the intestinal tract. Tennent and co-workers (2) have shown similar effects in cholesterol-fed cockerels by feeding cholestyramine,[1] a bile acid binding ion exchange resin. These workers also reported a reduction in plasma cholesterol and an increase in bile acid and neutral sterol excretion in a normocholesterolemic dog treated with 25 Gm. of resin/day. In addition, Bergen *et al.* (3) have reported that cholestyramine ingestion lowers plasma cholesterol of patients suffering from coronary artery disease.

A major problem connected with the clinical use of cholestyramine is the large bulk required. The present report is a description of studies in which factors concerned with the efficiency of the resin and magnitude of the response have been investigated.

METHODS

Twelve adult male beagles, 1 year old and weighing approximately 10 Kg. each, were fed 16.7 Gm. of a synthetic low residue diet/Kg. of body weight/day,[2] an amount just sufficient to maintain body weight. In 6 weeks the average plasma cholesterol value rose from approximately 105 mg./100 ml. to 155 mg./100 ml., at which point it became stabilized. At this time, the dogs were divided into four groups with three dogs/group. They were treated with cholestyramine on a schedule as described for each experiment below. In general, the resin was mixed in the diet and fed for 7 days, during the last 5 of which feces were collected. Experimental periods were preceded and followed by control periods, and a crossover procedure was usually used. Whenever resin was given, the celluflour was omitted from the diet. The dogs were bled weekly and cholesterol determined on oxalated plasma by the method of Abell *et al.* (4). Five-day fecal collections were made with the collection periods separated by feeding carmine in the diet for 2 days. Feces were collected daily, immediately placed in alcohol, and stored in the cold. Using a blender, the fecal collections were homogenized with sufficient 95% ethanol to give a final volume of 1500 ml. in 2-qt. Mason jars. The mixture was allowed to settle overnight, after which fecal bile acids were determined on aliquots of the clear supernatant.[3] In those cases in which resin was present in the feces, 60 ml. of saturated ammonium carbonate was added to each 1500-ml. extract in order to elute the bile acids from the resin. After centrifugation, aliquots of the clear supernatant were evaporated to dryness with a stream of air and an infrared heat lamp and redissolved in 80% ethanol. This procedure volatilized the ammonium carbonate and allowed the bile acids to be retained by the ion exchange resin. The bile acids were isolated by a modification of the ion exchange method of Kuron and Tennent.[3]

For columns, 10-ml. serological pipets, with the mouth pieces removed and roughened glass beads in the bottom, were used. The columns were poured by first filling them half full with 1 *N* acetic acid and then pipeting into each column 4 ml. of a 50% resin suspension,[4] followed immediately with additional 1 *N* acetic acid. The columns were washed successively with 10 ml. of deionized water (CO_2 free), 10 ml. of 50% ethanol, and 10 ml. of 80% ethanol. After application of the fecal extracts, the columns were washed with 20 ml. of 80% ethanol, 10 ml. of 25% ethanol, and 50 ml. of 0.5 *M* ammonium acetate in 5% ethanol. The bile acids were eluted with

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[1] Cholestyramine is the generic name for a quaternary ammonium anion exchange resin in which the basic groups are attached to a styrenedivinylbenzene copolymer skeleton. It is a product of Merck and Co., Inc., Rahway, N. J. The material used in these experiments contained approximately 5% moisture and was in the chloride form. The mesh size of the pharmaceutical grade cholestyramine used was 100% > 100 mesh, 80% > 200 mesh.

[2] Composition of diet in Gm./100 Gm. diet: casein, 29; glucose, 40; Crisco, 20; celluflour, 3; cod liver oil, 2; corn oil, 0.2; bone ash, 2.8; salt mix U.S.P. No. 2, 3; plus a complete vitamin mix.

[3] This is an equilibrium method of extraction and depends for its validity on the distribution of bile acids between solid and liquid phases. Kuron and Tennent [Kuron, G. W., and Tennent, D. M., *Federation Proc.*, **20**, 268(1961)] have shown that this procedure gives excellent recovery of endogenous bile acids (99% compared to exhaustive extraction) by feeding tritiated cholic acid to a dog and extracting, isolating, and counting the fecal bile acids. Recovery of cholic acid added to extracts of dog feces was 97% based on the amount added. By equilibrating C^{14}-labeled deoxycholic and cholic acid with extracts of dog feces (8 mg. deoxycholic or 4 mg. cholic acid/Gm. feces) and analyzing the supernatant for radioactivity with a Tri-carb liquid scintillation counter, the authors have obtained recoveries of 101 ± 1% and 93 ± 2%, respectively, using this equilibrium method.

[4] The resin used was AG1-X2 (100–200 mesh) obtained from Calbiochem. After fines were removed, the resin was converted to the acetate form and stored as a 50% suspension in 1 *N* acetic acid.

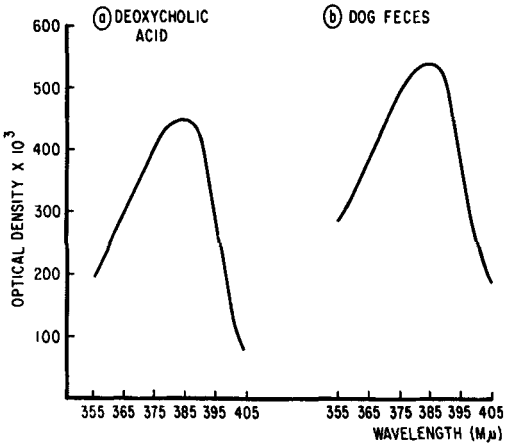

Fig. 1.—Absorption spectra in 65% H_2SO_4 for (*a*) 50 mcg. of deoxycholic acid/5 ml. 65% H_2SO_4, (*b*) column purified bile acids from ethanolic extract dog feces, calculated to contain 50 mcg. of dihydroxy bile acids/5 ml. 65% H_2SO_4. Absorption spectra obtained with a Cary model 11 recording spectrophotometer.

0.20 *M* of ammonium acetate in 50% ethanol and the eluates collected in 25-ml. volumetric flasks. Dihydroxy bile acids were determined by the method of Mosbach *et al.* (5) and cholic acid by a Kuron and Tennent modification[5] of the method of Irvin *et al.* (6). For all spectrophotometry, a Beckman DU spectrophotometer was used. The method of Mosbach *et al.* (5) determines the dihydroxy bile acids, deoxycholic acid, and chenodeoxycholic acids without discrimination, but lithocholic acid will not be detected. The purification of the fecal dihydroxy bile acid fraction is illustrated by Fig. 1 in which its absorption spectra is compared with a deoxycholic acid standard.[6] Nonspecific background is corrected for by subtracting the linear component as determined by averaging the absorption at 365 and 405 mμ from the peak absorption at 385 mμ (7).

Using these methods, column recoveries of 99.7 ± 1.0% and 99.3 ± 1.0% for deoxycholic and cholic acid, respectively, were obtained.

RESULTS

Effect of Graded Doses of Cholestyramine.—In the first experiment after a control week in which no resin was given, the four groups of dogs were given daily for 7 days, 1, 3, 6, and 10 Gm. of cholestyramine, respectively, followed by a second control week. The dogs were bled on the seventh, fourteenth, and twenty-first day. As shown in Table I, the percentage reductions in plasma cholesterol for 1, 3, 6, and 10 Gm. of resin averaged 0, 13, 24, and 35%, respectively. This experiment was repeated approximately 4 months later with the groups crossed over, and the plasma cholesterol values also are shown in Table I. Depressions of 4, 11, 24, and 39%, respectively, were obtained, results similar to those obtained in the first experiment.

The excretion of bile acids observed during these two experiments is summarized in Table II. The bile acid figures shown are the total of dihydroxy bile acids (deoxycholic and chenodeoxycholic acids) and cholic acid. Fecal bile acid excretion is greatly increased by cholestyramine resin, with as little as 1 Gm./day causing an increase from 81 to 226 mg./day in one experiment and from 73 to 247 mg./day in the other. Considering the average of experiments *A* and *B*, bile acid excretion gradually increased as the resin dose was raised to 6 Gm. resin/day, at which point there was approximately a sixfold increase in daily bile acid excretion as compared to controls. The approximate threefold increase in bile acid excretion caused by 1 Gm. resin/day was paralleled by a threefold increase in concentration of bile acid in the feces. However, the further doubling that took place when the resin dose was increased from 1 to 6 Gm. only increased the concentration of bile acids in the feces in experiment *A*. In experiment *B*, the increase was obscured by an increase in fecal weights. Bile acid excretion was no higher during the ingestion of 10 Gm. than during that of 6 Gm. of resin/day. There were no consistent and significant changes in the ratio of dihydroxy to trihydroxy bile acids as a result of resin treatment. In most cases, dihydroxy acids represented approximately 85% of the total. The higher resin doses of 6 and 10 Gm./day appeared to be associated with an increase in the proportion of cholic acid. Several of the dogs on these higher doses excreted more cholic than dihydroxy bile acid. However, this was quite variable, and more data are required.

Fecal neutral sterols were estimated by applying the method of Abell *et al.* (4) to feces and checked with the Sperry-Webb digitonide procedure (8). The results suggested that there was approximately a 75% increase in neutral sterols at the highest dose tested and could help to explain why this dose gave the largest reduction in plasma cholesterol. However, these results should only be interpreted as suggestive because of the specificity problem in applying the digitonide and Lieberman-Burchard procedures to fecal extracts. Hyun *et al.* (9) showed that cholestyramine can reduce the intestinal absorption of cholesterol-4-C^{14}.

Effect of Mesh Size.—It has been shown in *in vitro* experiments with anion exchange resins such as cholestryamine that the rate of uptake of anions is an inverse function of particle size (10). The effect of particle size *in vivo* was evaluated in a 4-week experiment with the first and fourth weeks serving as control periods. During the second and third weeks, 20–50 mesh,[7] 100–200 mesh,[7] 200–400 mesh,[7] and pharmaceutical grade cholestyramine[8] were added to the diets as shown in Table III so that 2.5 Gm. resin/day was ingested by each animal. The values for fecal bile acids are shown in Table III and the plasma cholesterol changes in Table IV. There were no differences among the groups in bile acid excretion or in plasma cholesterol. Surprisingly, the coarse 20–50 mesh resin was as effective as the 200–400 mesh or the pharmaceutical grade cholestyramine in increasing bile acid excretion and in lowering plasma cholesterol. The average reduction in plasma cholesterol in this experiment was 11%.

[5] This modification is as follows: after the dried aliquots of the column eluates are reacted with 2 ml. of the H_2SO_4 reagent of Irvin *et al.* (6), 3 ml. of 1:1 acetic acid–ethyl acetate is added. After shaking the tubes, the O.D. at 690 mμ is determined. This procedure removes turbidity.

[6] Obtained from Nutritional Biochemicals Co.

[7] The resins used were the corresponding Dow 1-X2 resins obtained from Calbiochem as AG1-X2 resins.

[8] 100% > 100 mesh, 80% > 200 mesh.

TABLE I.—EFFECT OF CHOLESTYRAMINE DOSAGE ON PLASMA CHOLESTEROL

Resin Dose, Gm./Dog/Day	Dog No.	Plasma Cholesterol, mg./100 ml. Pretreatment	Treatment[a]	Post treatment[b]	% Decrease
		Experiment *A*			
1	2116	119	123	123	
	1173	162	156	154	
	2175	129	129	127	
	Av.	137	136	135	0
3	2170	132	116	133	
	2172	110	88	93	
	2177	164	149	136	
	Av.	135	118	121	13
6	2167	142	96	123	
	2169	300	236	238	
	2174	158	123	141	
	Av.	200	152	167	24
10	2117	146	88	142	
	2168	136	90	122	
	2171	149	105	139	
	Av.	144	94	134	35
		Experiment *B*			
1	2167	129	114	126	
	2169	242	235	227	
	2174	149	133	149	
	Av.	173	161	167	4
3	2116	138	127	140	
	2168	137	113	136	
	2171	164	147	159	
	Av.	146	129	145	11
6	2117	118	108	120	
	2173	180	107	183	
	2175	131	109	128	
	Av.	143	108	144	24
10	2170	145	97	133	
	2172	104	53	91	
	2177	151	92	141	
	Av.	133	81	122	39

[a] After 1 week of resin treatment. [b] One week after discontinuation of resin treatment.

TABLE II.—EFFECT OF CHOLESTYRAMINE ON BILE ACID EXCRETION

Resin Dose, Gm./Dog/Day	Control Wk. mg. Bile Acids/Day[a]	Control Wk. mg. Bile Acids/Gm. Feces Dry Wt.	Experimental Wk. mg. Bile Acids/Day	Experimental Wk. mg. Bile Acids/Gm. Feces Dry Wt.
		Experiment *A*		
1	81 ± 25[b]	4.1 ± 0.6	226 ± 6	15.2 ± 1.8
3	47 ± 8	2.7 ± 0.6	283 ± 31	15.9 ± 4.2
6	54 ± 6	3.0 ± 0.3	446 ± 61	24.8 ± 6.0
10	58 ± 10	2.9 ± 0.3	335 ± 52	18.0 ± 4.6
		Experiment *B*		
1	73 ± 9	4.8 ± 0.6	247 ± 32	20.2 ± 3.7
3	77 ± 18	5.6 ± 0.8	364 ± 63	22.9 ± 3.4
6	118 ± 27	7.2 ± 1.1	556 ± 69	24.5 ± 3.6
10	78 ± 18	5.3 ± 1.1	509 ± 75	15.5 ± 1.0

[a] Bile acids = sum of deoxycholic, chenodeoxycholic, and cholic acids excreted per dog/day. [b] Standard error.

TABLE III.—EFFECT OF RESIN MESH SIZE ON BILE ACID EXCRETION[a]

Group	Wk.	1	2	3	4
1	Treatment: mg. bile acid/day[b]	No resin 88 ± 10[d]	No resin 105 ± 10	20–50 Mesh 442 ± 52	No resin 70 ± 10
2	Treatment: mg. bile acid/day	No resin 103 ± 26	100–200 Mesh 352 ± 8	200–400 Mesh 345 ± 52	No resin 79 ± 2
3	Treatment: mg. bile acid/day	No resin 135 ± 68	200–400 Mesh 371 ± 72	Cholestyramine[c] 408 ± 57	No resin 59 ± 10
4	Treatment: mg. bile acid/day	No resin 80 ± 16	Cholestyramine[c] 357 ± 33	100–200 Mesh 442 ± 69	No resin 47 ± 7

[a] Resin dose, 2.5 Gm./dog/day. [b] Sum of deoxycholic, chenodeoxycholic, and cholic acids excreted per dog/day. [c] The mesh size of the pharmaceutical grade resin is 100% > 100 mesh, 80% > 200 mesh. [d] Standard error.

TABLE IV.—EFFECT OF RESIN MESH SIZE ON PLASMA CHOLESTEROL[a]

Group	Wk. 1 Treatment	Wk. 1 Plasma Cholesterol, mg./100 ml.	Wk. 2 Treatment	Wk. 2 Plasma Cholesterol, mg./100 ml.	Wk. 3 Treatment	Wk. 3 Plasma Cholesterol, mg./100 ml.	Wk. 4 Treatment	Wk. 4 Plasma Cholesterol, mg./100 ml.
1	No resin	147	No resin	159	20–50	138	No resin	144
		169		185	mesh	162		170
		131		138		125		140
	Av.	149	Av.	161	Av.	142	Av.	151
2	No resin	151	100–200	140	200–400	130	No resin	144
		113	mesh	100	mesh	96		116
		157		167		143		157
	Av.	140	Av.	136	Av.	123	Av.	139
3	No resin	150	200–400	137	Cholestyr-	132	No resin	146
		251		224	amine[b]	215		254
		141		144		137		159
	Av.	181	Av.	168	Av.	161	Av.	186
4	No resin	128	Cholestyr-	133	100–200	144	No resin	151
		162	amine[b]	127	mesh	126		170
		167		150		145		143
	Av.	152	Av.	137	Av.	138	Av.	155

[a] Resin dose, 2.5 Gm./dog/day. [b] The mesh size of the pharmaceutical grade cholestyramine resin is 100% > 100 mesh, 80% > 200 mesh.

TABLE V.—EFFECT OF ENTERIC-COATED CHOLESTYRAMINE ON PLASMA CHOLESTEROL[a]

Group	Wk. 1 Control Plasma Cholesterol, mg./100 ml.	Wk. 2 Cholestyramine Coating[b]	Wk. 2 Cholestyramine Plasma Cholesterol, mg./100 ml.	Wk. 3 Control Plasma Cholesterol, mg./100 ml.	Wk. 4 Cholestyramine Coating	Wk. 4 Cholestyramine Plasma Cholesterol, mg./100 ml.	Wk. 5 Control Plasma Cholesterol, mg./100 ml.
1	118	Uncoated	105	119	B	135	129
	168		156	182		160	168
	126		116	131		117	127
	Av. 137		Av. 126	Av. 144		Av. 137	Av. 141
2	137	A	123	129	C	138	134
	106		99	112		99	109
	138		116	135		129	147
	Av. 127		Av. 113	Av. 125		Av. 122	Av. 130
3	128	B	96	116	Uncoated	109	111
	206		203	227		224	243
	142		123	141		117	129
	Av. 159		Av. 141	Av. 161		Av. 150	Av. 161
4	138	C	125	138	A	136	139
	137		122	131		127	137
	150		155	152		139	157
	Av. 142		Av. 134	Av. 140		Av. 134	Av. 144

[a] Resin dose, 2.0 Gm./dog/day. [b] Stabilities of coatings in 0.1 *N* HCl: coating A > 34 min., B > 80 min., C > 164 min.

Effect of Enteric Coating of Cholestyramine.—Since cholestyramine is a quaternary ammonium anion exchange resin, it would be expected to absorb many anions other than bile salts during its passage through the intestinal tract. Because of this competition, the efficiency of the resin in binding bile acids is relatively low. In the dog experiment reported by Tennent and co-workers (2), in which 25 Gm./day resin doses were employed, the daily bile acid excretion was only about 1.5% of the capacity of the resin. In the experiments reported in Table II, the efficiencies for 3 and 6 Gm. resin/day were 6 and 5% of capacity, respectively, also quite low. If the resin could be protected until it reaches the vicinity of the bile duct at the approximate time of gall bladder contraction, it seems possible that the efficiency of the resin would be increased. To test this hypothesis, the effectiveness of enteric-coated cholestyramine tablets was evaluated. The coatings were of cellulose acetate phthalate and methylcellulose and were applied to 0.5 Gm. compressed tablets of pharmaceutical grade cholestyramine. The stabilities in 0.1 *N* HCl of the three coatings evaluated were (*a*) >34 min., (*b*) >80 min., and (*c*) >164 min., respectively. All disintegrated rapidly in dilute NaOH. These tablets or uncoated control tablets were given to dogs 15 min. before each daily meal for two 7-day periods separated by a control week. The daily dose was four tablets (2 Gm. of cholestyramine)/dog, and the plasma cholesterol changes are shown in Table V. There were no significant differences among the four groups. The average decrease in plasma cholesterol in this experiment was 7% with 11 out of 12 dogs showing a reduction the first week and 10 out of 12 the second. Fecal bile acids were measured and no differences among the groups observed.

DISCUSSION

It seems likely that, as suggested by Tennent and co-workers (2), the cholesterol lowering property of cholestyramine is primarily a result of binding of bile acids in the intestinal tract. With daily resin doses of 1, 3, 6, and 10 Gm., reductions in plasma cholesterol of 2, 12, 24, and 37%, respectively, were obtained. However, it is of interest that the threefold increase in bile acid excretion caused by 1 Gm. resin/day did not significantly lower plasma cholesterol. Increasing the dose to 6 Gm./day gave a further doubling in fecal bile acid output and caused a 24% lowering in plasma cholesterol. Also, the bile acid excretion did not further increase in going from 6 to 10 Gm./day. The greater cholesterol drop at the 10-Gm. dose may be due in part to increased neutral sterol excretion. However, one might expect this to be an indirect effect as a result of binding bile acids, as suggested for ferric chloride by Siperstein and co-workers (1), and the higher resin dose apparently did not further increase total bile acid excretion.

A possible explanation for the larger plasma cholesterol reduction and apparent increased neutral sterol excretion by dogs receiving the highest resin dose is that they result in part as a result of the binding of fatty acid anions released by lipolysis of glycerides in the intestinal lumen. This along with a decreased bile acid concentration might further decrease cholesterol absorption. Alternatively, it has been shown that some bile acids are hypercholesterolemic and some hypocholesterolemic (11). Perhaps the action of cholestyramine is in some measure a result of a change in the pattern of bile acids absorbed and transported to the liver or the timing of the interaction between bile acids and resin. Another possibility not excluded is that at the high resin dose there is an increase in the proportion of conjugated bile acids, lithocholic acid, or other nondetected bile acids excreted. More data are required to clarify these points.

At first, it seemed somewhat surprising that no difference in effectiveness was observed between the resins of small and large particle size. However, since the binding of anions is relatively nonspecific and only about 5% of the resin's capacity is used to bind bile acids, it would appear that the finer resin besides binding bile acids faster is also inactivated at a faster rate.

The decrease in plasma cholesterol in the experiments with the enteric-coated tablets was consistent with the dose-response curve found earlier, indicating that the coatings dissolved and also that the compressed resin tablets disintegrated satisfactorily in the intestinal tract. The lack of improvement in resin efficiency in this experiment, although disappointing, does not indicate that conditions for more effective bile acid binding might not be found in future experimentation. Perhaps if enteric-coated tablets of the proper stability were combined with use of an effective chologogic substance—for example, cholecystokinin—the efficiency of the resin could be considerably improved. A limitation in the use of cholestyramine to lower plasma cholesterol in humans is the large bulk required, and therefore any improvement in efficiency that would lead to smaller effective doses would be desirable.[9]

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