Effect of Colestipol (U-26,597A) on Experimental Atherosclerosis in Rabbits[1] (36984)

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Inhibition of cholesterol absorption has been shown to decrease significantly the severity of induced atherosclerosis in rabbits. Sitosterol (1, 2) and several amides of linoleic acid (3–5) have been effective inhibitors of atherosclerosis in cholesterol-fed rabbits, and cholestyramine (6) has been found to be antiatherogenic in rabbits fed a semisynthetic, cholesterol-free, atherogenic regimen (7, 8).

Parkinson, Gundersen and Nelson (9) have recently reported that colestipol (U-26, 597A), a high-molecular-weight, insoluble copolymer of tetraethylenepentamine and epichlorhydrin, exhibits hypocholesteremic activity when fed to man, dogs or cockerels. The mechanism of action of colestipol involves inhibition of cholesterol absorption. This report describes experiments in which the effect of colestipol on cholesterol-induced atherosclerosis in rabbits was tested.

Materials and Methods. Male, Dutch belted rabbits (1.8–2.0 kg) were used in all experiments. All rabbits were fed a diet of rabbit chow augmented with 2% cholesterol and 6% corn oil. The diet of the test group was further augmented with 1% colestipol. The control and colestipol groups were designated C and U, respectively. After 8 wk the rabbits were weighed, bled by venipuncture, and killed; and their livers and aortas were removed. The total cholesterol content of serum and liver was determined by the method of Mann (10). Liver cholesterol determinations were carried out on 1 g portions of liver that had been saponified in 15% alc KOH. Aortas were graded visually for atherosclerotic lesions, using a 0–4 scale, according to the scheme of Duff and McMillan (11). Aortas were graded without knowledge of which group they were from. After excision, aortas were randomized before grading. Aortas of each group were pooled and the lipid was extracted with chloroform–methanol, 2:1 (12). Free and total cholesterol levels of the aortas were determined by the method of Sperry and Webb (13).

Aortic cholesteryl esters were obtained by thin-layer chromatography of the aortic lipid extract, using petroleum ether–ether–acetic acid, 75:24:1. Cholesteryl ester fatty acids (CEFA) and total aortic fatty acids (TAFA) were converted to methyl esters, and the fatty acid spectra of these fractions were determined by gas–liquid chromatography (glc). The glc was carried out on a column of 15% ethylene glycol succinate on 100–200 mesh gas chrom P. An argon ionization detector was used. The column was standardized for retention times and quantitated at 160° and 20 psi of argon gas, using purified standards (Supelco, Inc., Bellefonte, PA).

In the postcholesterol feeding experiments, a large group of rabbits was fed the atherogenic diet for 2 mo. At this time the rabbits were bled and separated into four groups of equal average cholesterol levels. One group (C1) was killed at this time and served as the initial control, the other three groups were placed on diets consisting of rabbit chow (C2), chow plus 5% corn oil (O) and chow plus corn oil and 1% colestipol (P). After 2 mo on these diets, the animals were weighed, bled and killed; serum and liver cholesterol levels were determined; and average atheromata were assessed.

Results and Discussion. The results of three experiments are presented in Table I. At the

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TABLE I. Influence of Colestipol on Experimental Atherosclerosis in Rabbits.[a]

Group[b]	Survival ratio	Wt gain (g)	Liver wt (g)	Cholesterol		Atheromata	
				Serum (mg/100 ml)	Liver (g/100 g)	Arch	Thoracic
Expt 1							
U	10/10	374	135.5	1758 ± 219[c]	2.34 ± 0.21[d]	1.0	0.6
C	10/10	334	129.7	1864 ± 381	3.25 ± 0.27	1.4	1.0
Expt 2							
U	10/10	226	114.1	1764 ± 299	11.83 ± 0.62	1.5	1.3
C	9/10	198	118.1	2232 ± 245	10.49 ± 0.78	2.1	1.4
Expt 3							
U	10/11	114	106.1	1660 ± 307	10.43 ± 0.73	1.7	1.1
C	10/11	34	110.8	1608 ± 314	11.54 ± 0.95	2.2	1.6

[a] Rabbits were fed 2% cholesterol and 6% corn oil with or without 1% colestipol for 8 wk.
[b] U = diet containing 1% colestipol, C = atherogenic regimen.
[c] Standard error.
[d] $p < .02$.

level of 1% of the diet, colestipol did not significantly reduce the serum or liver cholesterol levels of rabbits fed 2% cholesterol, but there was a marked reduction in the severity of atheromata. The lack of effect on cholesterol levels may be due to the ratio of colestipol to cholesterol used in these experiments. Parkinson, Gundersen and Nelson (9) have demonstrated a dose-related hypocholesteremic response with colestipol. Using another inhibitor of cholesterol absorption, sitosterol, Pollak (1) showed that its effect became more marked as the ratio of sitosterol to cholesterol in the diet rose. Heptinstall and Porter (2) obtained an antiatherogenic effect using a sitosterol–cholesterol ratio of 3:1.

It is thus evident that the hypocholesteremic effect of substances such as sitosterol or colestipol is dose related. The dose response is also dependent on the level of cholesterol in the diet. In our experiments the dietary level of colestipol was half that of cholesterol, which may explain the absence of a striking hypocholesteremic effect in group U. Averaged for the three experiments, serum cholesterol levels in group U were 1727 mg/100 ml, compared to 1890 mg/ml in group C. The data suggest an effect of colestipol which is mediated earlier than its effect on serum cholesterol levels. Whether colestipol affects

TABLE II. Distribution of Atheromata in Rabbits Fed Cholesterol (2%) and Corn Oil (6%) With or Without Colestipol (1%) (Summary of 3 Experiments).

Grade	Colestipol (30/31)[a]		Control (29/31)	
	Arch	Thoracic	Arch	Thoracic
4.0	0	0	0	0
3.5	1	1	0	0
3.0	3	1	5	0
2.5	3	0	2	0
2.0	4	4	9	6
1.5	3	4	9	9
1.0	6	5	2	11
0.5	5	7	2	3
0.0	5	8	0	0
Av	1.36 ± 0.21[b,c]	0.97 ± 0.12[c]	188 ± 0.13	1.31 ± 0.09

[a] Survival ratio.
[b] Standard error.
[c] $p < .05$.

TABLE III. Aortic Cholesteryl Ester (CEFA) and Total Aortic Fatty Acids (TAFA) (% Composition).

	CEFA		TAFA	
Fatty acid	Control	Colestipol	Control	Colestipol
14:0	1.5	1.2	1.5	1.8
16:0	37.6	38.5	31.0	29.9
16:1	1.1	1.4	2.2	2.8
18:0	14.8	14.2	7.3	7.2
18:1	37.7	36.6	26.9	27.3
18:2	7.5	7.9	27.7	27.3
18:1/18:2	5.02	4.63	0.97	1.00

some specific lipoprotein fraction can be answered by further experimentation.

The distribution of atheromata for the three experiments is shown in Table II. The differences between the control and colestipol-fed groups are significant at the 5% level. It is also significant that an appreciable number of the colestipol-fed rabbits exhibited no atheromata. In experiments with sitosterol, Heptinstall and Porter (2) observed aortas free of atheromata in rabbits fed sitosterol-cholesterol in a ratio of 3:1. Peterson, Nichols and Shneour (14) found no atheromata in cockerels fed cholesterol (1%) and soy sterols (1.3%) for 10 wk. Colestipol, even when fed at a level lower than that of cholesterol, also completely inhibited atherosclerosis in 17% of the rabbits.

The average aortic CEFA and TAFA are given in Table III. The ratio of oleic (18:1) to linoleic (18:2) acids in CEFA was somewhat lower in the aortas of rabbits fed colestipol. The 18:1/18:2 ratio in the TAFA was similar for groups C and U. In work with another compound (DH-581) which inhibited atherosclerosis in rabbits, we also found 18:1/18:2 ratios lower in aortic CEFA of treated rabbits (15). In man, the 18:1/18:2 ratio of aortic CEFA rises with age and increasing severity of lesions (16, 17).

In the aortas of man or of experimental animals, the ratio of free to ester cholesterol (FC/EC) falls with increasing severity of atherosclerosis (18). The FC/EC ratio in aortas of normal rabbits varies between 10 and 20 (18). In Expts 2 and 3, the FC/EC ratios for groups C and U were 3.3 and 4.0, and 0.7 and 2.6, respectively. The actual cholesterol levels (mg/100 g) were: Expt 2: U, 334; C, 482; Expt 3: U, 302; C, 527.

The results of the postcholesterol experiment are given in Table IV. The initial control group (C1) was group C in Expt 1 (Table I). In the postcholesterol feeding phase, serum cholesterol levels were significantly reduced ($p < .001$) in group C2, O and P. Liver cholesterol levels were significantly reduced ($p < .05$) only in groups C2 and P.

The atheromata of the groups returned to a diet of rabbit chow (C2) were considerably more severe than those observed in the initial control (C1). Addition of corn oil to the diet inhibited the exacerbation of the lesions. We have consistently observed this effect (19). Addition of colestipol to the diet after establishment of lesions did not affect progression of the lesions.

Beher, Anthony and Baker (10) made a similar observation in rabbits fed 2.5% sitosterol for 4 mo after establishment of atherosclerotic lesions. In their experiments, athero-

TABLE IV. Influence of Colestipol (1%) on Preestablished Atheromata in Rabbits.[a]

	Survival	Wt gain (g)		Liver wt	Liver cholesterol	Serum cholesterol (mg/100 ml)		Atheromata	
Group	ratio	8 wk	16 wk	(g)	(g/100 g)	8 wk	16 wk	Arch	Thoracic
C1	10/10	334	—	129.7	3.25 ± 0.27[b]	1864 ± 381	—	1.4	1.0
C2	10/10	275	41	114.4	2.08 ± 0.46	1864 ± 379	175 ± 56	2.4	1.5
O	10/10	312	67	125.6	3.49 ± 0.59	1864 ± 395	172 ± 34	1.8	1.1
P	10/10	264	162	122.2	2.02 ± 0.43	1864 ± 328	122 ± 21	2.2	1.4

[a] All rabbits were fed 2% cholesterol, 6% corn oil for 8 wk. Rabbits in Group C1 were autopsied at 8 wk. Group C2 was fed chow for weeks 9–16; Group O, chow plus 5% corn oil; Group P, chow plus corn oil plus 1% colestipol.

[b] Standard error.

mata in the initial control and in rabbits fed corn oil or corn oil plus sitosterol after establishment of lesions were 2.7, 2.6 and 3.0 respectively.

Summary. Colestipol, a high-molecular-weight polymer which binds bile acids, exerts a hypocholesteremic effect in several species. The addition of 1% colestipol to an atherogenic regimen (2% cholesterol, 6% corn oil) did not significantly affect serum or liver cholesterol levels of rabbits but did cause a significant reduction ($p < .05$) in atheromata in both the aortic arch and thoracic aorta. An appreciable number of rabbits fed 1% colestipol were free of atheromata.

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