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Effect of Cholestyramine on Tissue Pools of Cholesterol a Preliminary Report[1] (38546)

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An ideal hypocholesterolemic agent should not only reduce plasma levels of cholesterol, but should also help in the reduction of abnormal tissue cholesterol deposits, specifically those in the arterial intima. Cholestyramine is an excellent hypocholesterolemic agent, deemed particularly useful for treatment of familial hypercholesterolemia (Type II hyperlipoproteinemia). Preliminary evidence obtained in our laboratories raises the possibility that prolonged use of this drug alone may, in some patients, be associated with increment in the tissue cholesterol pools. The hypocholesterolemic action of cholestyramine may well be associated with a reduction in the risk of coronary heart disease, despite a general increment in the tissue cholesterol pools. However, until such proof becomes available, and the relationship between tissue pools of cholesterol and atheromatous lesions is clarified, its use should be monitored closely. The hypocholesterolemic action of cholestyramine may even be enhanced by combining its usage with drugs such as clofibrate, thereby overcoming, at the same time, potentially undesirable effects on tissue cholesterol pools.

Methods. Cholesterol turnover studies were carried out in four ambulant adults with Type II hyperlipoproteinemia (cholesterol >250 mg/100 ml plasma). In three subjects the studies were done twice, once during control and once after 8–10 mo treatment with cholestyramine (12 g/day). In the fourth subject, the turnover studies were done three times; first, control; second, after one year's treatment with clofibrate (2 g/day); and third, after 8 mo treatment with clofibrate and cholestyramine. All subjects ate their usual diet for the studies and maintained their body weight, except the fourth who gained 3 kg during clofibrate treatment but maintained it thereafter.

Cholesterol-4-^{14}C (58 mCi/mmole) and cholesterol-1-2-^{3}H (39 mCi/mmole) were obtained from Amersham/Searle Corporation and tested for radiopurity by thin-layer chromatography (1). Their purity was better than 97% and 95% respectively. Trace amounts of labelled cholesterol dissolved in 1 ml of ethanol and dispersed in 150 ml of physiological saline were given by slow intravenous infusion. Fasting venous blood samples were collected daily for the first 4 days and weekly thereafter for 12 wk for determination of the specific activity of plasma free cholesterol (2). Assuming a two-compartment model, a number of indices of cholesterol metabolism were calculated from the specific activity slopes by the method of Goodman and Noble (3) and of Nestel *et al.* (4). It was assumed that entry as well as exit of cholesterol occurred only in Pool A and none in Pool B (5).

Concentrations of plasma cholesterol (6) and triglycerides (7) were stable during different periods of the study. The plasma volume was assumed to be 4.5% of the body wt (8), and the total cholesterol in plasma pool was obtained by multiplying the concentration with plasma volume.

Results. Effects of cholestyramine (4 g t.i.d.) on plasma lipids are shown in Table I. There was a 15–31% (mean 25%) decrease in plasma cholesterol and a 15–29% (mean 22%) increase in plasma triglycerides on treatment with cholestyramine. The changes were statistically significant at the level of 5%.

In contrast to the reduction in the amount of cholesterol in plasma, there were marked increases in the amounts of tissue cholesterol. The mean increase in the tissue components of Pool A was 120%, and the mean increase

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TABLE I. EFFECT OF CHOLESTYRAMINE ON THE VARIOUS PARAMETERS OF CHOLESTEROL METABOLISM.[a]

Subjects	Sex	Age Yrs	Weight kg	Treatment	Plasma lipids mg/100 ml Mean±SD Cholesterol	Plasma lipids mg/100 ml Mean±SD Triglycerides	α/day	β/day	$-K_{AA}$	$-K_{BB}$	M_A g	M_{AP} g	M_{AX} g	PR_A g/day	$K_B \equiv 0$ K_A	$K_B \equiv 0$ K_{AB}	M_B g	MCF %
1	M	56	87.2	Pre-R_x	366±21	225±30	0.138	0.0147	0.105	0.048	21.52	14.32	7.20	1.033	0.041	0.064	28.53	7.2
		57	87.2	R_x	252±31	259±17	0.154	0.0187	0.114	0.059	33.07	9.06	24.01	1.894	0.048	0.066	37.10	20.9
2	F	50	58.2	Pre-R_x	337±20	146±10	0.099	0.0108	0.079	0.031	20.49	8.82	11.67	0.756	0.032	0.047	31.06	8.5
		51	58.2	R_x	238±28	182±22	0.147	0.0121	0.117	0.041	26.86	6.23	20.63	1.284	0.040	0.077	49.86	20.6
3	F	52	55.8	Pre-R_x	363±15	130±18	0.154	0.0126	0.100	0.066	20.53	9.10	11.40	0.651	0.029	0.071	22.09	7.1
		53	55.8	R_x	308±22	168±25	0.198	0.0165	0.147	0.068	30.03	7.73	22.3	1.609	0.047	0.100	44.16	20.8
4	F	36	62.3	Pre-R_x	439±24	112±9	0.154	0.0096	0.114	0.049	21.89	11.08	10.81	0.722	0.029	0.085	38.19	6.5
		37	65.6	R_x*	395±16	95±7	0.154	0.0108	0.108	0.057	19.88	10.52	9.36	0.628	0.028	0.080	28.00	6.0
		38	65.6	R_x**	296±27	115±12	0.198	0.0144	0.127	0.085	20.77	8.73	12.04	0.776	0.033	0.094	22.97	8.8

[a] Pre-R_x—before treatment. R_x—cholestyramine (12 g./day). R_x*—clofibrate (2 g./day). R_x**—clofibrate + cholestyramine. α—0.69315/t 0.5 (half-life in days of first exponential). β—0.69315/t 0.5 (half-life in days of second exponential). K_{AA}—rate constant for total removal of cholesterol from Pool A. K_{BB}—rate constant for total removal of cholesterol from Pool B. M_A—the size of Pool A. M_{AP}—amount of cholesterol in plasma. M_{AX}—amount of cholesterol in other tissues of Pool A. PR_A—production rate (turnover rate). K_A—rate of excretion from Pool A. K_{AB}—rate of transfer from Pool A to B. M_B—size of Pool B ($S_B \equiv 0$; $K_B \equiv 0$). MCF—fraction of plasma cholesterol cleared per day.

in Pool B was 60%. Daily production rate of endogenous cholesterol was increased in all subjects, with a range of 70–147% and a mean of 96%. This was associated with a 33% increase in the excretion rate from Pool A. The rate of transfer from Pool A to Pool B was increased by 35%, while the rate of transfer from Pool B to Pool A was increased by only 18%.

In the only subject who was given clofibrate in addition to cholestyramine, the increment in the size of Pool B was not seen; however, there was a modest increase in the tissue component of Pool A. Clofibrate also prevented the increase in the production rate caused by cholestyramine (Table I).

Discussion. The use of hypocholesterolemic agents is contingent on the hope that the reduction in plasma levels would also reduce tissue deposits of cholesterol, especially in atheromatous lesions. Studies done in our laboratories showed that hypocholesterolemic action of clofibrate, nicotinic acid, and plant sterols was generally associated with prompt mobilization of tissue cholesterol (9), (10) and this was not obvious in the case of cholestyramine (11). It was also shown by us (10) and by others (12) that prolonged administration of clofibrate causes a substantial decrease in the size of Pool B. Administration of cholestyramine was associated with significant increase in tissue cholesterol pools in each of the three patients examined. These preliminary observations are also supported by our analyses of the data previously published by Goodman and Noble (3). Three out of five subjects given cholestyramine showed significant increases in the size of Pool B. Although there may be some reservations in accepting the absolute values of pool sizes obtained by this method, the direction and the degree of change from control values are in all likelihood valid reflections of tissue cholesterol pools. The drug had been given for 8–9 mo before the second set of determinations were made so that the patients could be considered in reasonable steady state conditions. Moreover, we observed the opposite changes after clofibrate treatment, using the same methods.

The following observations add further support to these observations. Grundy *et al.*, found increases in the size of tuberous and tendon xanthomata in some subjects receiving cholestyramine for hypercholesterolemia (13). Similarly, Moutafis *et al.*, failed to observe any changes in the skin lesions in two hyperlipemic subjects given cholestyramine, despite increased fecal steroid excretions (14).

The cause of this increase in tissue cholesterol pools is not known. Cholestyramine has been known to cause a marked increase in the hepatic (3), (11), (13) as well as extrahepatic synthesis of cholesterol (15), and it is conceivable that the extrahepatic synthesis may be, at least in part, responsible for increase in tissue cholesterol pools.

Although the sites for catabolism for plasma lipoproteins are not known, it is plausible that at least a small fraction is catabolised in tissues included in Pool B. Since cholestyramine causes an increase in the turnover rate of plasma lipoproteins (16), an increase even in this small fraction will result in an increase in tissue deposits of cholesterol, if its transport out of tissues remains constant. In our studies, the increase in rate of transfer from Pool A to Pool B was almost double the increase in the rate of transfer from Pool B to Pool A. Subject 3, who showed the greatest increase in cholesterol, also had the smallest increase in the rate of transfer from Pool B to Pool A.

Data in these studies are insufficient to provide unequivocal information. This report is merely intended to raise a possible issue of concern. Undoubtedly, much more information is needed to clarify the relationships between plasma cholesterol, cholesterol in tissue pools, and cholesterol in atheromatous lesions.

The combined treatment of clofibrate with cholestyramine in the only patient examined by us appeared to prevent increments in tissue cholesterol pools, seen with the cholestyramine treatment alone. The skin lesions, which did not decrease on cholestyramine alone, disappeared quite readily when nicotinic acid was added to the treatment (14). We have previously shown that cholesterol mobilized from tissues is excreted mostly as neutral steroids (10). Treatment with cholestyramine does not significantly change the fecal excretion of neutral steroids (11), (13), but increases only the fecal excre-

tion of bile acids (11), (13). Addition of clofibrate to cholestyramine treatment increases neutral steroid excretion without decreasing bile acid excretion (13).

Increase in plasma triglyceride levels observed by us is in accord with previous observations (13), (17) and perhaps is a manifestation of increase in the turnover of plasma lipoproteins.

Summary. Four Type II Hyperlipoproteinemic subjects were investigated before and after treatment with cholestyramine. Plasma cholesterol was significantly reduced (365 ± 23 vs 273 ± 34 mg/100 ml) and triglycerides significantly increased (149 ± 55 vs 181 ± 59 mg/100 ml) on cholestyramine treatment. The daily turnover of cholesterol, as determined by the method of Goodman and Noble, was nearly doubled by the treatment (0.813 ± 0.11 vs 1.595 ± 0.176 g). Although previous workers have already suggested that cholestyramine does not decrease tissue cholesterol pools, we observed a significant increase in tissue pools in each of the three subjects given cholestyramine alone (10.1 ± 1.4 vs 16.2 ± 6.9 g) for Pool A—excluding plasma; and 27.2 ± 4.6 vs 43.7 ± 6.4 g for Pool B). Treatment for the fourth subject consisted of a combination of cholestyramine and clofibrate. This combination appeared to prevent increases in the size of Pool B and in the size and production rate of Pool A. These preliminary observations suggest that the hypocholesterolemic effect of cholestyramine may be enhanced and its effects on tissue cholesterol prevented by giving it in combination with other agents such as clofibrate.

1. Grundy, S. M., and Ahrens, E. H., Jr., J. Clin. Invest. **45,** 1503 (1966).
2. Horlick, L., Kudchodkar, B. J., and Sodhi, H. S. Circulation **43,** 299 (1971).
3. Goodman, D. S., and Noble, R. P. J. Clin. Invest. **47,** 231 (1968).
4. Nestel, P. J., Whyte, H. M., and Goodman, D. S., J. Clin. Invest. **48,** 982 (1969).
5. Wilson, J. D., J. Clin. Invest. **49,** 655 (1970).
6. Badzio, T., and Boczon, H., Clin. Chim. Acta **13,** 794 (1966).
7. Van Handel, E., and Zilversmit, D. B., J. Lab. Clin. Med. **50,** 152 (1957).
8. Gregersen, M. I., and Rawson, R. A., Physiol. Rev. **39,** 307 (1959).
9. Sodhi, H. S., Kudchodkar, B. J., Endocrinology **88,** A-258 (1971).
10 Sodhi, H. S., Kudchodkar, B. J., and Horlick, L., Atherosclerosis **17,** 1 (1973).
11. Nazir, D. J., Horlick, L., Kudchodkar, B. J., and Sodhi, H. S., Circulation **46,** 95 (1972).
12. Grundy, S. M., Ahrens, E. H., Jr., Salem, G., Schreibman, P. H., and Nestel, P. J., J. Lipid Res. **13,** 551 (1972).
13. Grundy, S. M., Ahrens, E. H., Jr., and Salen, G., J. Lab. Clin. Med. **78,** 94 (1971).
14. Moutafis, C. D., Myant, N. B., Mancini, M., and Oriente, P. Atherosclerosis **14,** 247 (1971).
15. Jansen, G. R., Zanetti, M. E., and Hutchinson, C. F., Arch. Biochem. Biophys. **138,** 433 (1970).
16. Langer, T., Levy, R. I., and Fredrickson, D. S., Circulation **40,** III-14 (1969).
17. Weitzel, A., Estriich, D. L., Splitter, S. D., Pomeroy, V., and Kinsell, L. W., Proc. Soc. Exp. Biol. Med. **130,** 149 (1969).

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