Uptake of Radiosulphate by Mucopolysaccharides of Aorta in Cholesterol Fed Cockerels. (25007)

K. KOWALEWSKI

McEachern Cancer Research Laboratory and Dept. of Surgery, University of Alberta, Edmonton, Alberta

Several hypotheses have been proposed to explain the entrance of lipids into the arterial wall in the process of atherogenesis. Virchow suggested that the deposition of fat is secondary to the pathological alteration of connective tissue cells and ground substance of the vascular wall(1). This old idea seems to have gained importance in recent years. Faber(2) noted that alteration in the histochemical staining of arterial mucopolysaccharides is the earliest demonstrable feature of atherosclerotic lesions. He found that an increased output of carbohydrate-sulphuric acid esters plays a significant role in deposition of cholesterol in the vascular wall. Altschuler and Angevine(3) suggested that a process of ionic exchange between circulating cations and partially depolymerized chondroitin sulphuric acid may play a role in deposition and fixation of lipids. It is, therefore, apparent that depolymerization of mesodermal connective tissue ground substance is involved in the pathogenesis of atherosclerosis. This hypothesis is supported by more recent experimental work on cholesterol fed animals. Not only was hypercholesterolemia in rabbits associated with elevated serum hexosamine levels, but also increased metachromatic and P.A.S. positive materials were observed in and subjacent to atheromatous lesions(4). The above alterations of connective tissue ground substance also may be investigated by S^{35} uptake technic. It has been shown that radioactive sulphate may be incorporated into the aorta of animals and that the extent of uptake closely parallels intensity of staining for acid mucopolysaccharides. It was assumed that the principal substance incorporating S^{35} in the aorta was chondroitin sulphate(5,6). A recent report(7) on S^{35} uptake in the aorta of cholesterol-fed rabbits seems to confirm previous findings based on histochemical methods. Katz and Stamler(1), however, question the relevancy of rabbit cholesterol-induced aortic lesions for understanding the pathogenesis of human disease. They consider the chick as a more suitable experimental animal and give arguments in favor of this statement. Their advice, therefore, has been followed in selection of material for this work. The purpose of the present experiment was to study the effect of cholesterol feeding on uptake of S^{35} by the aortic wall mucopolysaccharides in cockerels.

Materials and methods. Cockerels of White Rock-Broiler strain used for this study were obtained from a commercial hatchery when 8 days old and immediately confined to thermostatic brooders. The birds were kept in groups of 8 to 10 per brooder and received routine chicken food with tap water as desired. When the birds were 2 weeks old cholesterol (2%) and cottonseed oil (5%) were added to the diet and this treatment continued during an 8 week period. Isotope was administered 24 hours before birds were killed. Radiosulphate (S^{35} in H_2SO_4, Atomic Energy, Canada) was injected subcutaneously in dosage of 1 mc/kg of bird's body weight. The dose was dissolved in 5 ml of distilled water together with 40 mg of sodium sulphate(8,9). The cockerels were anesthetized, killed by decapitation and the blood collected. Serum and livers were used for determination of lipids. From each bird, aorta and femur were carefully dissected for isotope study. Bone was studied to compare S^{35} uptake of the aorta with that of a tissue (bone) having specifically high sulphate-fixing capacity. Aortas were studied for S^{35} radioactive mucopolysaccharides following the method described previously(10,11). The bones were dissolved by the nitric-perchloric acid wet-digestion procedure described previously(12). The final precipitate of barium sulphate obtained after processing the tissues was transferred to planchettes and counting carried out using a thin mica window Tracerlab G-M tube. A Tracer-

TABLE I. Serum and Liver Lipids in Cholesterol Fed Cockerels, after 8 Weeks of Feeding.

	Control diet	Cholesterol diet
No. of birds	16	16
Serum total cholesterol, mg/100 ml	138 ± 6* (93–173)†	1143 ± 106 (507–1716)
Serum phospholipids, mg/100 ml	208 ± 5 (175–247)	380 ± 23 (250–527)
Serum, C/P‡	.67 ± .03 (.40–.82)	2.8 ± .12 (2.0–3.7)
Liver total cholesterol, mg/100 g	302 ± 15 (178–366)	10161 ± 428 (7681–13122)
Liver phospholipids, mg/100 g	2092 ± 73 (1440–2317)	3350 ± 54 (3040–3765)
Liver, C/P	.15 ± .1 (.08–.26)	3.0 ± .1 (2.3–3.5)

* Mean ± S.E.
† Range.
‡ Total $\frac{\text{cholesterol}}{\text{phospholipids}}$ ratio.

lab Superscaler S.C. 18A was used for reading. Results were expressed in counts per minute (c.p.m.). Total cholesterol was determined following the method of Abell *et al.* (13). For the study of lipid phosphate the samples were extracted with alcohol-ether and analysis carried out by the method of Zilversmit and Davis(14). Total cholesterol/phospholipid ratio (the C/P ratio) was obtained by calculation.

Results. Before the beginning of cholesterol diet body weights averaged 220 g and 232 g in control and cholesterol fed groups of birds respectively. At the end of experiment respective weights were 1680 g and 1570 g. Table I records data on determination of lipids in serum and livers of cockerels. Both total cholesterol and phospholipids are increased in cholesterol fed birds. Significant rise of C/P ratio, which is considered as a decisive factor in atherogenesis of cockerels(15) also may be noted. Table II presents the results of measuring of S^{35} uptake by the fraction of aortic tissue considered to contain sulphated mucopolysaccharides. Total radioactivity of femora of the same birds is also recorded. Significant difference between the 2 groups of cockerels is apparent as far as aortic S^{35} uptake is concerned. There is no marked difference in S^{35} uptake by the bones of these birds.

TABLE II. Uptake of S^{35} by Bone and Radioactivity of S^{35} Sulphated Mucopolysaccharides of Aortas (S.M.) in Cockerels.

	Control diet	Cholesterol diet
No. of birds	16	16
S^{35} in bone, c.p.m./100 mg dry wt	942 ± 43* (720–1305)†	987 ± 47 (713–1276)
S^{35} in S.M. fraction of aorta, c.p.m./100 mg dry wt	762 ± 45 (537–999)	1612 ± 106 (1162–2362)

* Mean ± S.E.
† Range.

Discussion. Increased S^{35} uptake by the sulphated mucopolysaccharides of the aortic wall in cholesterol fed cockerels was found in this experiment. This finding is in agreement with previous work on alteration of vascular wall ground substance associated with clinical and experimental atherosis. The fact that cholesterol feeding does not influence S^{35} uptake by bones is significant. It means that the changes in lipid metabolism produced under described experimental conditions do not necessarily result in increase of the S^{35} uptake by all tissues with sulphate-binding capacity.

Summary. Cockerels were fed during an 8 week period with cholesterol (2%) and cottonseed oil (5%) enriched diet. Parallel to increase of serum and liver lipids and C/P ratio, there was a significant rise of S^{35} uptake by the sulphated mucopolysaccharides of the aorta.

Technical assistance of M. Solony and J. Michiels is gratefully acknowledged.

1. Katz, L. N., Stamler, J., *Experimental Atherosclerosis,* Ch. C. Thomas, Publ., 1953, Springfield, Ill.,
2. Faber, M., *Arch. Path.,* 1942, v48, 342.
3. Altschuler, C. H., Angevine, D. M., *Am. J. Path.,* 1951, v27, 141.
4. Schwartz, C. J., Peters, J. A., Day, A. J., *Austral. J. Exp. Biol. and Med. Sci.,* 1958, v36, 109.
5. Odeblad, E., Boström, H., *Acta Chem. Scand.,* 1953, v7, 233.
6. Buck, R. C., *J. Histochem. and Cytochem.,* 1955, v3, 435.
7. Buck, R. C., Heagy, F. C., *Can. J. Bioch. and Physiol.,* 1958, v36, 63.
8. Kowalewski, K., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 432.
9. ———, *Endocrinology,* 1958, v62, 493.

10. Kowalewski, K., Williams, H. T. G., *Can. J. Bioch. and Physiol.*, 1958, v36, 847.
11. Kowalewski, K., Strutz, W., *Acta Endocrinologica*, 1959, v31, 107.
12. Osborne, J. C., Kowalewski, K., *Surg. Gyn. and Obst.*, 1956, v38, 103.
13. Abell, L. L., Levy, B. B., Brodie, B. B., Kendell, F. E., *J. Biol. Chem.*, 1952, v195, 357.
14. Zilversmit, D. B., Davis, A. K., *J. Lab. and Clin. Med.*, 1950, v35, 155.
15. Stamler, J., Katz, L. N., *Am. J. Physiol.*, 1950, v163, 752.

Received April 21, 1959. P.S.E.B.M., 1959, v101.

Comparative Effects of Bile Acids on Intestinal Absorption of Cholesterol.* (25008)

GEORGE V. VAHOUNY, H. M. GREGORIAN AND C. R. TREADWELL
Dept. of Biochemistry, George Washington University School of Medicine, Washington, D.C.

Schoenheimer and co-workers reported the first critical experiments on facilitatory effect of bile salts on cholesterol absorption in various animals(1-3). Using serum or liver cholesterol levels as indicators of quantitative differences between bile acids, these workers found that glycocholate had the greatest effect in enhancing cholesterol absorption, while cholic acid and desoxycholic acid were less effective(2,3). Member *et al.*(4) reported that cholate or glycocholate fed with cholesterol to rabbits increased blood and aorta cholesterol levels, while dehydrocholate, hyodesoxycholate, and desoxycholate were ineffective. In chronic feeding experiments with rats, Swell and co-workers(5) found that blood cholesterol levels were elevated by inclusion of cholate or taurocholate in high fat-high cholesterol diets; desoxycholate was less effective, while dehydrocholate was without noticeable effect. Correlation of these data with *in vitro* studies on comparative effects of different bile salts on cholesterol esterase activity(6) led to the suggestion that the observed effects of dietary bile salts were due to their requirement as cofactors for cholesterol esterase activity. Another explanation is that due to hydrotropic properties of these substances(7), dispersion of cholesterol and its tranfer into the mucosa is increased. Recently, Swell and coworkers(8) demonstrated that bile salts are involved in passage of cholesterol from the intestinal lumen into the mucosa. Further, these authors suggested that a specific type of bile salt was involved, since dehydrocholate was ineffective in promoting transfer of cholesterol into the intestinal mucosa, while taurocholate increased both mucosal and lymph cholesterol levels(8). Other workers have suggested that accumulation of cholesterol in blood and liver when bile salts are fed is related to depressed rate of cholesterol catabolism or excretion(9,10). The purpose of the present investigation was to determine directly, using changes in lymph cholesterol levels, the comparative effects of various bile salts on intestinal absorption of cholesterol in the rat.

Methods and materials. Male rats of Carworth Farms strain with thoracic duct lymph fistula were prepared and handled as described previously(11). Following a fasting period of 18-24 hours, the animals were given 3 ml of aqueous test emulsion intragastrically (11). Control and test emulsions contained 50 mg of albumin, 50 mg of cholesterol and 288 mg of oleic acid/3 ml of 0.9% saline. In addition, the experimental emulsions contained 4 molar equivalents of one of 7 different bile acids/mole of cholesterol fed. Commercial samples of lithocholic acid, deoxycholic acid, dehydrocholic acid, sodium taurocholate and sodium glycocholate (Nutritional Biochemicals) were employed. In addition, highly purified samples of cholanic acid,† cholic acid‡ and synthetic

* This work supported by grants from Life Insurance Medical Research Fund, and Nat. Heart Inst.

† Mann Research Lab., M.P. 163.5°C; sp. rot., + 21°.

‡ We are indebted to Dr. M. Korzenovsky, Eli Lilly and Co., for sample of purified cholic acid.