Influence of Fatty Acids and Sterols on Atherosclerosis in the Avian Abdominal Aorta.* (26238)

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We have previously demonstrated that dietary corn oil retards the avian aortic atherosclerosis which develops both in presence and absence of dietary cholesterol(1,2). The present study was undertaken to (a) ascertain whether the fatty acids or the sterols in corn oil provided the observed protection and to (b) elucidate the importance of circulating and aortic cholesterol levels in relation to atherogenesis.

Experimental. Day-old Leghorn cockerels were fed a standard ration for 17 days, then were divided into 4 groups of 20 and given the experimental diets for 6 months. The basal ration contained in percent: soybean oil meal (50% protein) 50.0; alfalfa meal 2.5; dicalcium phosphate 2.5; corn oil 2.0; limestone with trace minerals (Mico concentrate, Limestone Corp. of America, Newton, N. J.) 1.0; salt 0.5; choline Cl 0.2; DL-methionine 0.2; B vitamin mix 0.1; A, D and E mix 0.1 (for composition of both mixtures see Fisher and Johnson(3)); antioxidant (Santoquin, Monsanto Chemical Co., St. Louis, Mo.) 0.02; and glucose to 100. The experimental design included the following: 1) control group receiving the unsupplemented ration, 2) a group receiving 2% cholesterol and an additional 5% corn oil, 3) a group getting 2% cholesterol and 0.05% corn oil sterol (approximately equivalent to sterol content of 5% corn oil) and 4) a group receiving 2% cholesterol plus 3% fatty acids (containing 72.9% linoleic acid which is approximately equivalent to the amount of linoleic acid in 5% corn oil). All supplements replaced equal amounts, by weight, of glucose. The 2% corn oil was included in the basal ration to facilitate absorption of the dietary cholesterol in the group of birds fed the sterol fraction of corn oil.

Plasma cholesterol was determined intermittently during the study. At the end of the 6-month experimental period all birds were killed, their aortas removed, visually graded, and the abdominal section of each aorta analyzed for total fat, cholesterol and polyunsaturated fatty acids by methods previously described(1). The aortic scoring system was designed primarily to evaluate degree of lumen encroachment by proliferative lesions, and secondarily to evaluate gross fatty infiltration, hardness and roughness of the intimal surface. No detailed analyses were carried out on the thoracic segments of the aorta since these showed negligible involvement for all groups.

Twice during the study, 24 hour collections were made of the excreta. These were dried, twice extracted for 24 hours on a shaker with chloroform-methanol(2:1), and cholesterol (sterol) determined on the filtered extracts by the method of Zlatkis *et al.*(4).

Results and discussion. The pertinent observations are given in Table I. Although the differences among groups are comparatively small, it appears that the group receiving the fatty acids equivalent to 5% corn oil had the greatest atherosclerotic involvement as judged by frequency of high scores, average score and average abdominal aortic weight. Conversely, the group receiving the sterols equivalent to 5% corn oil appears to have the least severe involvement. In this connection Beveridge *et al.*(5) working with the hypocholesterolemic effect of corn oil in humans, found at least part of the active principle to be present in the sterol fraction. In a similar vein, Howard and Gresham(6) have interpreted their results to mean that essential unsaturated fatty acids may facilitate deposition of cholesterol in tissues and production of atherosclerosis.

The group which received the fatty acid fraction also had the highest amount of aortic and plasma cholesterol, but it is note-

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TABLE I. Effect of Corn Oil and Fractions on Cholesterol-Induced Avian Atherosclerosis.

Measurements	Dietary groups			
	Control	2% cholesterol plus 5% corn oil	2% cholesterol plus Corn oil sterol	2% cholesterol plus Fatty acids
		Abdominal aorta		
Score	1.9 ±.18*	1.9 ±.09	1.8 ±.06	2.0 ±.08
Frequence of high scores†	6/18	6/18	5/19	10/20
Wt (mg)	64.9 ±3.2	65.6 ±3.7	67.7 ±2.6	73.0 ±2.7
Cholesterol (% of dry defatted tissue)	1.39 ± .07	2.19 ± .26	2.04 ± .21	2.52 ± .22
Fat (mg/g dry tissue)	130.1	85.4	89.4	113.3
Dienoic acid (% of fat)	16.7	22.2	15.1	23.9
Monoenoic acid (% of fat)	16.1	31.9	25.9	6.6
Saturated acid (% of fat)	60.9	37.9	51.6	63.9
		Plasma		
Cholesterol (mg %)	203 ± 16	464 ± 41	381 ± 40	478 ± 45
		Excreta		
Fat (% of dry matter)	4.3	12.4	10.2	11.6
Cholesterol (mg/g dry defatted excreta)	5.8	61.0	63.4	60.9
Cholesterol (% of fat)	13.5	49.4	62.1	52.3

* Mean ± stand. error.

† For this comparison, scores over 2.0, avg for the group showing greatest involvement, were used.

worthy that neither the aortic or plasma cholesterol levels among the other 3 groups bore consistent relationship to severity of atherosclerosis.

The polyunsaturated fatty acid analyses of abdominal aortic fat show the highest dienoic acid content for the groups that had received high dietary linoleic acid in the 5% corn oil- or the 3% fatty acid supplement. The high cholesterol content of aortic fat in the group receiving the fatty acids thus is not related to any essential fatty acid deficiency(7) since it is associated with the highest level of dienoic acid.

While dienoic acid content of the aortic fat reflects dietary intake, the monoenoic and saturated fatty acids for the corn oil *vs.* the fatty acid supplemented group show considerable variation. In the corn oil-fed birds the increase in dienoic acid clearly occurred at the expense of the saturated fatty acids, whereas in the fatty acid-supplemented animals the increase in dienoic acid was at the expense of the monoenoic acid only.

Attention is drawn to the much reduced deposition of total fat in the abdominal aorta in birds receiving corn oil sterol (as such, or with the whole corn oil supplement) as compared to control birds or those receiving the fatty acid supplement. The significance of this observation in relationship to atherogenesis must await further experimentation.

The sterol excretion data in Table I demonstrate that dietary cholesterol was equally well absorbed in the 3 cholesterol-supplemented groups. Thus, lower blood and aortic cholesterol levels as well as lower severity of atherosclerosis in the animals fed the sterol-supplemented diet cannot be explained on the basis of interference with cholesterol absorption.

Summary and conclusion. Male chickens were fed for 6 months on rations containing (a) no supplement, (b) cholesterol plus corn oil, (c) cholesterol plus corn oil-sterol and (d) cholesterol plus fatty acids rich in linoleic acid. Atherosclerotic severity tended to be greatest in the group given fatty acids and least on the sterol-containing diet. Since sterol excretion indicated a similar rate of cholesterol absorption on the cholesterol-supplemented diets, it appears that the principle in corn oil which retards avian aortic atherogenesis is present in the sterol fraction.

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Effect of Insulin on Serum Proteins in Scurvy.* (26239)

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It has been reported(1) that in scorbutic guinea pigs a_2 and β_2 globulins are diminished; a_1-globulin is increased and no change takes place in β_1 and γ-globulins. Insulin is involved in biosynthesis of proteins (2) and hypoinsulinism has been observed in scurvy (3,4). The derangement of serum proteins in scorbutic guinea pigs might have been due to diminished insulin formation. It was, therefore, of interest to study the effect of prolonged injection of small doses of insulin on the protein pattern of serum in guinea pigs during the progress of scurvy. The carbohydrate and lipid containing proteins of normal, scorbutic and insulin-treated scorbutic guinea pigs were also determined.

Methods. Forty-eight male guinea pigs each weighing between 250 and 300 g were divided into groups of 3 animals, one normal, one scorbutic and one insulin-treated scorbutic. All the animals were fed a scorbutogenic diet(5). The amount of diet consumed per day by the scorbutic member of a group was fed to the other 2 members of the group on the subsequent day. The 'normal' animals were fed 5 mg ascorbic acid per animal per day. All the animals received 2 drops of a concentrate of Vit. A and D twice a week. From the 10th day on each animal of the insulin treated group was given a daily subcutaneous injection of protamine zinc insulin (Lilly) in a dose increasing from 0.1 unit per 100 g body weight to 0.3 unit over the period of experiment. On the 24th day of experiment all the animals were sacrificed after an over-night fast, blood collected from neck veins, and clear serum analyzed for different protein fractions(6), glyco- and mucoproteins (7) and lipoproteins(8,9). Swahn(10) had shown that uptake of Sudan Black B by whole serum on filter paper was correlated with total lipid content of serum. Total area of the plotted curve was, therefore, equivalent to total serum lipid content of sample used for electrophoresis. The a and β-lipoproteins and the "o" fraction were expressed, on the basis of their respective areas, as percentages of the total area and hence of total lipid.

Results. Serum albumin and a_2 and β_2 globulins which diminished and a_1 globulin which increased in scurvy were brought back to approximately normal level after prolonged insulin treatment of the scorbutic animal. Serum glycoprotein and mucoprotein fractions which increased in scurvy remained unaffected after insulin treatment. Serum a-lipoprotein and "o" fractions considerably diminished and β-lipoprotein fraction significantly increased in the scorbutic condition. Insulin treatment did not produce marked effect on these fractions. The results are given in Table I.

Discussion. The alterations in serum protein patterns of scorbutic guinea pigs could

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