Effect of Beta-Sitosterol on Regression of Hypercholesterosis and Atherosclerosis in Chickens.* (24193)

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Many attempts have been made to initiate regression of cholesterol-induced hypercholesterosis and atherosclerosis. Of the substances tested, few were found to be effective (1-6). Beta-sitosterol(7) and di-hydrocholesterol(8) enhanced regression of hypercholesteremia, whereas only beta-sitosterol reduced hepatic cholesterol in the rabbit. Although the chicken undergoes regression of cholesterol-induced atherosclerosis(9,10) few studies have been made to determine whether this process may be facilitated. Beta-sitosterol has been demonstrated to be a most effective agent for preventing cholesterol-induced hypercholesteremia and atherosclerosis in the chicken(11,12). This report is concerned with the effect of beta-sitosterol in diets with and without cholesterol on regression of cholesterol-induced hypercholesterosis and atherosclerosis in the chicken.

Methods. Two hundred one-day-old cockerels,† 50 birds/pen, were fed a broiler ration (7% fat, 24% protein, 3% crude fiber) for an 8-week growth period. Feed and water were allowed *ad lib.* At the end of the 8-week growth period, a random sample of birds was bled to determine normal blood cholesterol level. The cockerels were then placed on an atheroma-inducing diet which contained 2% cholesterol in basal diet. The basal diet was composed of broiler ration + 5% cottonseed oil. At the end of 3 weeks, *i.e.*, the atheroma induction period, a random sample of the population was taken to obtain serum, liver, and aorta (aortic arch, thoracic and abdominal aorta), for analysis. The remaining cockerels were then divided into 4 groups and placed upon the following diets for 8 weeks: A, 1% cholesterol in basal diet; B, 1% cholesterol + 4% sitosterol in basal diet; C, 4% sitosterol in basal diet; D, basal diet only. A representative sample of each group was taken after 2 weeks, and remaining birds were sacrificed at the end of 8 weeks. Serum, liver, and aorta were collected, and the chickens were weighed at each sampling. Liver was removed *in toto* from each chicken, placed in a bottle and quick-frozen. For analysis the liver was thawed and homogenized in a Waring Blendor. Blood was drawn by cardiac puncture, placed in a centrifuge tube, allowed to clot, and centrifuged. Serum was collected and quick-frozen. Adventitial tissue was removed from aortas prior to staining with Sudan IV. Plaque areas were visually estimated and graded. After grading, the aortas were lyophilized. Each tissue was extracted with 1:1 ethyl alcohol-acetone. This extract was used for total and free cholesterol, phospholipids, and total lipids determinations. Total and free cholesterol were determined by the method of Sperry and Webb(13). For total lipid determinations, an aliquot of the alcohol-acetone extract was added to chloroform. This mixture was treated as outlined by Folch(14). Total lipids were determined gravimetrically. Lipid phosphorus was estimated by the method of Fiske and Subba-Row on the residue from the total lipid analysis(15).

Results. The atheroma-inducing diet elicited the characteristic rise in total and free cholesterol of serum as shown in Table I. Associated with this rise of the cholesterol was an increased lipid content of the serum. During the atheroma regression period total and free cholesterol and total lipids in serum of Group A were maintained at levels attained

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TABLE I. Effect of Beta-Sitosterol on Regression of Serum Lipids in the Chicken.

Time	Group	Total chol.*	Free chol.	F/T	Total lipid
Control		106 ± 2.6†	24.4 ± 1	.21	
Induction—3 wk		470 ± 47.9	146.5 ± 16.3	.31	1030 ± 73
Regression—2 wk	A	512 ± 78	118 ± 19	.24	1269 ± 273
	B	151 ± 21	30 ± 5.2	.19	539 ± 50
	C	131 ± 14	28 ± 4.0	.21	476 ± 32
	D	176 ± 36	45 ± 9.5	.25	547 ± 65
8 wk	A	361 ± 53.8	94.3 ± 18.9	.27	659
	B	128.6 ± 5.9	20.1 ± 3.9	.16	392 ± 9.5
	C	123.3 ± 8.8	21.5 ± 2.2	.17	382 ± 28
	D	136.4 ± 11.2	24.0 ± 3.0	.18	332 ± 36

* Data expressed as mg/100 cc serum. † Stand. error of mean.

during the atheroma induction period. During this same period a highly significant decrease in the lipids discussed above was observed in Groups B, C, and D. This drop in lipids occurred to the greatest extent after only 2 weeks on the regression diet; a further decrease was seen at 8 weeks. Although there was no significant difference between Groups B, C, and D, there was a tendency at 2 and at 8 weeks for serum cholesterol to decrease in the following order: Gp C > Gp B > Gp D. Lipid-phosphorus content of serum was not altered in any significant direction.

Total and free cholesterol and total lipid concentration of the liver in Group A continued to rise during the regression period (Table II). Total and free cholesterol and total lipid concentration of Groups B, C, and D were greatly reduced from the levels found for Group A during this period.

During the regression period, total cholesterol of the liver was significantly lower in Group C than in Groups B and D. At 2 and 8 weeks of the regression period there was no significant difference in total cholesterol between Groups B and D. Groups B and C had significantly lower free cholesterol than had Group D at 2 and 8 weeks, whereas at 8 weeks Group C was significantly lower than Group B. Although the most profound drop in hepatic total cholesterol was observed during the first 2 weeks of the regression period, nevertheless there was a further reduction in hepatic cholesterol of Groups B, C, and D during the 2 to 8 week period. The F/T cholesterol ratio of Groups B, C, and D approached unity with reduction of total hepatic cholesterol. There were no significant differences in total lipids of Groups B, C, and D during the regression period. Liver lipid-phosphorus content did not show any definite trends during any phase of the experiment.

Total and free cholesterol content of the aortas of Group A continued to increase significantly from the atheroma induction period through the regression period (Table III). In Groups B, C, and D total and free cholesterol of the aorta was significantly lower than in Group A at the end of this period.

TABLE II. Effect of Beta-Sitosterol on Regression of Liver Lipids in the Chicken.

Time	Group	Total chol.*	Free chol.	F/T	Total lipid	P-values for total chol.	
Induction—3 wk		24.2 ± 1.8†	8.2 ± .60	.33	69 ± 3.3		
Regression—2 wk	A	27.5 ± 1.3	8.9 ± .95	.38	72 ± 9.9		
	B	6.9 ± 2.2	3.6 ± .25	.66	38 ± 2.3	B–C	.3
	C	4.4 ± .29	3.4 ± .12	.81	37 ± 1.6	C–D	.05
	D	6.2 ± .82	4.5 ± .68	.75	38 ± 1.7	D–B	.7
8 wk	A	37.4 ± 3.8	9.4 ± .33	.25	93 ± 15.1		
	B	4.2 ± .19	3.3 ± .10	.79	40 ± 1.1	B–C	.01
	C	3.2 ± .08	2.8 ± .06	.89	39 ± 1.2	C–D	.01
	D	3.9 ± .09	3.5 ± .08	.90	39 ± 2.1	D–B	.2

* Data expressed as mg/g of wet tissue. † Stand. error of mean.

TABLE III. Effect of Beta-Sitosterol on Regression of Aorta Lipids in the Chicken.

Time	Group	Total chol.*	Free chol.	F/T	Total lipid	P-values for total chol.
Induction—3 wk		5.82 ± .32†	3.73 ± .18	.65	198 ± 10.5	
						AI–2 wk AR‡
Regression—2 wk	A	8.08 ± .92	5.34 ± .41	.69	136 ± 17.6	A P = .05
	B	7.70 ± .76	5.90 ± .39	.80	169 ± 11.2	B .05
	C	6.58 ± .51	5.29 ± .39	.81	156 ± 12.9	C .2
	D	5.69 ± .96	4.30 ± .59	.77	179 ± 23.4	D .9
						2 wk AR–8 wk AR
8 wk	A	10.81 ± 1.53	7.10 ± .64	.72	128 ± 10.3	A P = .25
	B	4.84 ± 1.14	3.82 ± .68	.84	130 ± 15	B .05
	C	3.54 ± .36	3.30 ± .41	.89	134 ± 13	C .01
	D	3.96 ± .48	3.51 ± .44	.87	131 ± 8.9	D .1

* Data expressed as mg/g of freeze-dried tissue. † Stand. error of mean.
‡ AI = atheroma induction; AR = atheroma regression.

The groups receiving cholesterol with and without beta-sitosterol, Groups A and B respectively, manifested an increased total and free cholesterol at 2 weeks of atheroma regression. Group A at 8 weeks had a higher total and free cholesterol than at 2 weeks. Group B, on the contrary, showed a significant decrease in total and free cholesterol from the levels observed at 2 weeks. In the groups receiving no cholesterol with and without sitosterol, Groups C and D respectively, no significant changes of total cholesterol at 2 weeks of atheroma regression were observed. At 8 weeks the total cholesterol was significantly lower than at the 2-week period. Total lipid content of all groups showed a tendency to decrease during the atheroma regression period.

Number and degree of atheromas were not greatly altered at 2 weeks of the regression period, with exception of Group C, which did show a decrease (Table IV). The differences were clearly evident at 8 weeks. During the regression phase of the experiment a very slow rate of plaque reversal was observed in Group A, in spite of an increased cholesterol content of the aorta. This decrease in atheromas may be associated with decreases in total lipids. However, decreases in total lipids occurred to the same degree and rate in Groups B, C, and D, and in these groups atheromas were entirely absent. Total cholesterol of the aorta at 8 weeks also was greatly decreased.

Discussion. Cessation of cholesterol feeding with or without sitosterol administration in the chicken resulted in the reduction of hypercholesterosis. Regression also has been observed in other species fed a cholesterol-free diet only(1,2,7,8). Withdrawal of dietary cholesterol results in a negative balance of cholesterol between body tissue and intestinal lumen. The animal body under these conditions may reduce tissue cholesterol by converting cholesterol to bile acids and/or by excreting cholesterol from the intestinal mu-

TABLE IV. Influence of Beta-Sitosterol on Regression of Atheromas in the Chicken.

Time	Group	No. of birds	% distribution of grades 3	2	1	0	% aortas w/atheromas
Induction—3 wk		40	3	17	40	40	60
Regression—2 wk	A	16	6	13	31	50	50
	B	19	5	11	32	52	48
	C	18	0	6	22	72	28
	D	16	19	6	19	56	44
8 wk	A	20	5	5	25	65	35
	B	20	0	0	5	95	5
	C	19	0	0	0	100	0
	D	17	0	0	0	100	0

cosa into the intestinal lumen. However, the rate of regression may not be maximal because excreted cholesterol may be reabsorbed. Indeed, when beta-sitosterol was added to a cholesterol-free diet, the degree of regression was slightly increased. This effect was most evident in liver and was paralleled by similar changes in serum and aorta cholesterol concentrations. Hence, it may be assumed that beta-sitosterol in a cholesterol-free diet enhanced the reduction of a cholesterol-induced hypercholesterosis by inhibiting reabsorption of excreted cholesterol.

Peterson(11,12) and Pollak(16) have reported that addition of sitosterol to a cholesterol-containing diet fed to chickens and rabbits prevented hypercholesteremia. In the present study, beta-sitosterol added to a cholesterol-containing diet caused tissue cholesterol regression in the chicken. Rate of reduction was comparable to that of chickens on a cholesterol-free diet. The mode of action of sitosterol presumably is to prevent absorption of cholesterol. Inhibition of cholesterol absorption may be due to 1) formation of relatively insoluble mixed crystals of cholesterol and sitosterol in the intestinal lumen (16,17), 2) inhibition of a cholesterol esterase which presumably is required for cholesterol absorption(18), or 3) competition for acceptor sites on or within the intestinal mucosa(19,20). Thus, when sitosterol is incorporated into a cholesterol-containing diet, the result, in effect, is a cholesterol-free diet.

Regression of cholesterol and atheromatous involvement of the aorta was obtained by a cholesterol-free diet, with and without beta-sitosterol, or by a cholesterol plus beta-sitosterol diet. A regression of atheromas may occur by suspending cholesterol feeding(9,21, 22). All of the treatments mentioned will lower previously elevated blood cholesterol and other lipids. From these observations it may be concluded that atherosclerosis and lipid infiltration of the aorta is dependent upon concentration of lipids in blood. Thus, blood may be assigned the role of a major donor pool of these lipids to the aorta. Lipids of the aorta are not excluded from the general turnover of body lipids. Indeed, experiments with tritium-labeled cholesterol have shown that there is a turnover of aortic cholesterol, albeit slower than in serum and liver (23). The data presented here support this observation in that the liver and serum cholesterol regressed at a greater rate than did that of the aorta.

Recently Curran and Costello(24) have reported that feeding sitosterols to rabbits for 4 weeks led to development of atherosclerotic plaques in rabbits. However, the data of Beher, *et al.*(7,8) do not support this observation. Shipley, *et al.*(25), have recently reported that continuous administration of beta-sitosterol for a period of 2 years did not produce atheromatous involvement in the rat, rabbit, or dog. The data presented here show that beta-sitosterol when fed with or without cholesterol actually resulted in a decreased sterol level of the liver, blood, and aorta. Indeed, when beta-sitosterol was fed alone, sterol concentration in the liver was significantly lower than in the group fed a sterol-free diet, and the sterol content of the serum and aorta was equal to the group fed a cholesterol-free diet.

Summary and conclusions. 1. A previously elevated cholesterol concentration in the liver, serum, and aorta of the chicken was reduced by administration of beta-sitosterol in presence or absence of dietary cholesterol, or by withdrawal of dietary cholesterol. 2. Tissue cholesterol concentrations observed as a result of these treatments always approached the normal values. 3. When 4% beta-sitosterol was fed in the diet, total sterol content of liver was slightly lower than in birds fed the basal diet only. Further, total sterol content of the serum and aorta tended to be lower than that of birds fed basal diet only. 4. Atheroma regression in the chicken was accomplished by administration of beta-sitosterol in presence of dietary cholesterol. 5. Atheroma regression in the chicken was paralleled by a drop in cholesterol concentration of the blood and liver.

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Unilateral Adenectomy in the Cretin Rat.*† (24194)

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Compensatory enlargement of the remaining organ of a paired organ system generally occurs following unilateral removal of the adrenal(1), kidney(2,3), ovary(4), and testis(5). Factors which alter this response to sub-total removal of organ tissues include age, diet(6,7), sex, time between operation and autopsy, castration(8), hypophysectomy(9, 10,11) and thyroidectomy(9). Prior studies have principally involved the use of adult euthyroid rats. No data, however, have come to our attention in which compensatory enlargement of organs was compared in immature hypothyroid (cretin) rats and euthyroid controls of the same age.

Materials and methods. Inbred Vanderbilt rats, maintained, *ad libitum,* on Purina Fox Chow (meal) and tap water were used. Cretins were obtained by feeding 0.5% thiouracil§ in Purina Fox Chow to pregnant rats and subsequently to them and their litters from the 14th-16th day of gestation to end of experiment. Controls were maintained, with their mothers, on the same regimen without the thiouracil. Only male control and cretin rats whose body weights at 30 days ranged 100 to 109 g and 29 to 36 g, respectively, and female controls and cretins whose body weights were between 83-88 g and 28-35 g, respectively, were utilized. At 30 days of

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