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Corn Sterols and Avian Atherosclerosis.* (28382)

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Peterson *et al.*(1,2) have reported that relatively high levels of dietary soybean sterols (1.3%) prevented hypercholesterolemia and atherosclerosis in cholesterol-fed chicks, and lowered blood cholesterol in man. They suggested that the soy sterols interfered with cholesterol absorption. Beveridge *et al.*(3) showed that a considerable part of the hypocholesterolemic activity of corn oil was associated with its sterol fraction, and Fisher *et al.*(4) reported that the beneficial effects from feeding unsaturated plant fats to chickens were attributable primarily to their sterol fraction rather than to the unsaturated fatty acid fraction. Reiner *et al.*(5), in reporting on hypercholesterolemic sterols derived from marine algae, suggested that appreciable amounts of sterol might be absorbed and thus act in a manner other than by interference with absorption of cholesterol alone. This view is supported by the finding of Fisher *et al.*(4) that the feeding of a small quantity of corn sterols does not increase cholesterol excretion of chickens.

The present work was undertaken to study the effect of prolonged ingestion of a low level of corn oil sterols (0.05% of diet) in counteracting the atherogenicity of a diet containing 20% whole egg powder. We also checked (a) whether the atherogenic properties of egg powder were due to its fat content, and (b) whether lasting effects might remain in chickens given a brief, early exposure (3 months out of 20) to an atherogenic egg powder diet.

Experimental. Day-old Leghorn cockerels, divided into 6 groups of 30 birds each, were used for this study and maintained throughout a 20-month experimental period in temperature-regulated rooms. For the first 12 weeks, 2 groups were given the control ration supplemented with 20% egg powder, which supplied 0.5% cholesterol. At the end of this time the egg powder diet of one group was replaced with the control ration for the remainder of the experimental period. The other 4 groups were respectively given the following diets throughout the experimental period: (a) a control ration (for composition see Fisher *et al.*(4)), essentially free from cholesterol and containing a small amount of fat derived from soybean meal; (b) control ration supplemented with 2% corn oil and 0.05% corn sterols;† (the former was added to facilitate sterol absorption, should it occur) (c) control ration supplemented with 10% egg oil,‡ which was equivalent to the fat supplied by 20% whole egg powder and (d) control ration supplemented with both 20% whole egg powder and 0.05% corn sterols. The measurements made on the experimental animals by methods previously described(6,7) included blood pressure, blood clotting time, blood and aortic cholesterol, aortic hydroxyproline, and scoring of the

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‡ Egg oil was generously supplied by Henningsen Inc., New York.

TABLE I. Blood and Aorta Measurements of Cockerels Given Various Diets for 20 Months.

Measurements	Control	Experimental groups: Corn oil, 2% Corn oil sterol, .05%	Whole egg powder, 20%	Egg oil, 10%*	Whole egg powder, 20% Corn oil sterol, .05%	Egg powder for 12 wk†
Plasma cholesterol (mg/100 ml)						
3 mo	117 ± 5§	120 ± 8	278 ± 20	343 ± 18	246 ± 23	263 ± 20
12-20 mo‡	67 ± 4	64 ± 5	197 ± 13	304 ± 33	151 ± 20	78 ± 3
Abdominal aortic lipid (% of dry tissue)						
Total fat, 20 mo	12.0 ± 1.5	7.2 ± 1.2	12.3 ± 1.9	12.8 ± 2.3	10.4 ± 1.3	10.2 ± 2.1
Cholesterol, 12-20 mo‡	1.2 ± .1	1.2 ± .1	3.5 ± .4	2.6 ± .3	2.9 ± .4	1.4 ± .2
Abdominal atherosclerosis						
Score, 12-20 mo‡	2.5 ± .1	2.5 ± .1	3.0 ± .1	2.8 ± .1	2.5 ± .1	2.2 ± .1
Weight-area ratio, 20 mo (mg fresh tissue/cm²)	50.2 ± 2.4	49.4 ± 2.5	53.3 ± 3.0	55.0 ± 3.8	49.2 ± 3.7	43.9 ± 1.0

* Supplied the same amount of egg fat as 20% whole egg powder. Both supplied approximately 0.5% cholesterol.
† Followed by unsupplemented control ration for remainder of experimental period.
‡ Represents combined average of values determined at 12 and 20 months; each value represents 20 ± 2 birds.
§ Average value with standard error.

aortas. A 'morphometric' measurement, consisting of the ratio of weight to surface area of the abdominal aorta, was also included. Birds were sacrificed for aortic analysis at 12 and at 20 months.

Forty-eight hour excreta collections were carried out at 12 weeks for a total sterol analysis to determine whether the dietary plant sterols had interfered with cholesterol absorption and therefore possibly caused an increased excretion. These determinations were also carried out by methods previously described(4).

Results and discussion. There were no effects of dietary variables on blood pressure, blood clotting time, aortic hydroxyproline or on any of the measurements made on the thoracic portion of the aorta. These values were therefore omitted from the tables of results. Since there were only minor differences between values measured at 12 and at 20 months, these were averaged for presentation in Table I.

Plasma cholesterol. There were significant differences in plasma cholesterol levels among treatments (Table I). The groups given either whole egg powder or its equivalent in egg oil (both of which contributed approximately 0.5% cholesterol), had much higher cholesterol levels than the controls ($P < 0.01$). The birds given corn sterol had similar blood cholsterol levels as those not receiving sterol. The groups that had received the egg powder diet for the first 12 weeks showed a high plasma cholesterol level at that time, but reverted to a cholesterol level similar to that of the controls by 1 year of age. The 12- and 20-month cholesterol averages are generally considerably lower than the 3-month values. Although previous observations from this and other laboratories have suggested an adaptation, in blood cholesterol level, to prolonged ingestion of cholesterol(8,9,10), this does not adequately explain the present findings, since the level of the control groups not ingesting cholesterol was also lowered.

Aortic lipid. The total fat values for the abdominal segment of the aorta showed an interesting difference among dietary treatments (Table I). Both of the groups given

TABLE II. Lipid Excretion on Various Diets.

Diets	Total lipid (% dry excreta)	Total sterol* (% dry excreta)	Total sterol* (% excreta lipid)
Control	2.68	.53	19.8
Corn oil, 2%			
Corn oil sterol, .05%	4.20	.82	19.5
Whole egg powder, 20%	7.15	1.12	15.6
Egg oil, 10%†	7.38	1.34	18.2
Whole egg powder, 20%			
Corn oil sterol, .05%	6.94	1.23	17.7

* Measured by the ferric chloride Zak reaction(11) which gives the same color density for corn sterols as for cholesterol.

† Supplied the same amount of egg fat as 20% whole egg powder. Both supplied approximately 0.5% cholesterol.

corn oil sterols had a lower lipid content than did their respective controls. This corn sterol effect is particularly noteworthy in view of the lack of difference in plasma cholesterol as well as in aortic cholesterol level (see next paragraph) between the sterol treatments and their respective controls.

The aortic cholesterol content for the abdominal segments shows differences due to diet for the 3 groups which had been given either whole egg powder or egg oil. These 3 groups have generally higher cholesterol levels than their respective control groups or the group that had been given egg powder for the first 12 weeks only (Table I). It should be noted that the cholesterol content of the sterol-supplemented groups was not appreciably lowered despite the reduced total fat content of these groups compared with their respective controls. These results point to a continuing process involving cholesterol deposition, irrespective of the type of dietary treatment.

Atherosclerosis measurements. Table I gives the average visual scores of the abdominal aortic section for the birds killed at 12 and at 20 months and weight-area ratios for the birds killed at 20 months. Addition of corn oil sterols to the egg powder diet appeared responsible for a reduced severity of atherosclerosis, as was evidenced by a significantly lower abdominal visual score ($P < 0.05$) and by the lower weight-area ratio.

In contrast with the relatively large amount of sterol (1.3%) which Peterson *et al.*(1) had used in their early studies, in this experiment the inclusion of as little as 0.05% also seemed effective in retarding atherogenesis during a long-term feeding trial. This is in agreement with our previous observations made over a shorter experimental period(4). The sterol excretion results (Table II) did not indicate an increased cholesterol excretion due to the supplementation with corn sterols. We would therefore suggest that the beneficial effect of the corn sterol results from a mechanism other than interference with cholesterol absorption alone. This is strengthened by the observation that total lipid deposition (but not cholesterol deposition) in the abdominal aorta was considerably retarded in the corn-sterol fed groups. Evidence for the appreciable absorption of plant sterols for man and the rat have been cited by Reiner *et al.*(5).

The atherogenic effects of whole egg powder appear to reside primarily in the egg oil, since addition of this material resulted in a severity of atherosclerosis similar to that experienced with the egg powder.

The early exposure of one group of birds to the stress of the egg powder diet did not leave lasting effects, and these birds had low visual scores and the lowest weight-area ratio at the end of the 20-month experiment.

While the atherogenic properties of the egg diets (which supplied 0.5% cholesterol) are clear, the inexorable development of spontaneous atherosclerosis, as illustrated by the scores for the control groups, should not be minimized or overlooked. In contrast with the retarding effect of corn sterols on the induced or accelerated type of atherogenesis, no such beneficial effect was observed in relation to the spontaneous type exhibited by the control group.

Summary. Leghorn cockerels were fed for 20 months on diets containing either no supplement, whole egg powder, egg oil equivalent to that contained by egg powder or these diets in combination with corn sterols, the latter added in amounts equivalent to that supplied by 5% corn oil. An additional group was given egg powder for 12 weeks and then placed on the control ration for the remainder of the experimental period. The inclusion of corn oil sterol retarded the atherogenesis that was accentuated by the addition of egg powder or egg oil. Total fat deposition in the abdominal section of the aorta was reduced in the groups fed the sterols. The brief exposure to egg powder did not leave lasting effects on those animals that were changed over to the control diet. Egg oil appeared to be similar in its atherogenicity to egg powder. Sterol excretion patterns suggested that the beneficial effect of the corn sterols was not due to interference with cholesterol absorption alone.

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Effects of Copper Ion on Oxygen Uptake by Planktonic Cyprids of the Barnacle, *Balanus amphitrite niveus.** (28383)

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Copper ion is absorbed by planktonic cyprids of *Balanus amphitrite niveus* mainly through the surface epithelium of the thorax (1), and is excreted into the lumen of the gut in certain regions by the lining epithelium. Concentration and storage of copper in specialized intracellular loci, in the cypris, suggest a role for this ion in normal development. Because it appeared to be involved in metabolism and because of its widespread use in antifouling coatings, the effect of various concentrations of copper ion on oxygen uptake by barnacle cyprids was evaluated.

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Materials and methods. Isotonic solutions containing 0.5, 1, 5, 10, 20, and 50 mg of copper ion per liter were made by adding appropriate volumes of a standard 500 mg Cu^{++}/l solution to Millipore-filtered Gulf Stream sea water. Solutions of 100, 200, 500, and 1,000 mg/l were made by dissolving cupric acetate directly in Gulf Stream water. At higher copper concentrations it was necessary to lower the pH slightly to dissolve sufficient copper acetate. The higher concentrations are well above those ever present in nature. Individual animals were studied in Kirk differential capillary respirometers(2). Oxygen consumption of individual animals was determined at 23°-25°C during a one-hour period. Values for 10 animals which survived this treatment and did not attach to the vessel wall during the period