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Influence of Crystalline Elastase on Experimental Atherosclerosis in the Chicken

Elastase is a pancreatic enzyme that has been studied and described by Baló and Banga (*1*). Lansing (*2*) and Carter (*3*) have determined that it comes from islet tissue and specifically from the α-cells. Preparation of crystalline elastase from beef pancreas has been reported by Banga (*4*) and from pork pancreas by Lewis, Williams, and Brink (*5*).

It is thought that there may be a connection between elastase and arteriosclerosis. Baló and Banga (*6*) noted that men suffering from arteriosclerosis had less elastase in the pancreas than did healthy individuals, and Lansing (*7*) gave elastase by mouth to cholesterol-fed rabbits and found that it retarded the development of atheromatosis.

We report here the influence of crystalline porcine elastase (*8*), given orally and parenterally, and of trypsin, included as a control for the proteolytic action, on atheromatosis and plasma lipid pattern in cholesterol-fed chickens. Two preparations of crystalline material containing 130 and 134 elastase units per milligram were tested in separate experiments 24 weeks apart. The procedures are described in more detail elsewhere (*9*).

For each test, six groups of 8-week-old White Leghorn cockerels, raised on starter ration, were set out on diet containing 2 percent USP cholesterol and 5 percent cottonseed oil and treated as follows: (i) no further treatment; (ii) diet fortified with 57 mg of crystallized trypsin (Worthington) per kilogram; (iii) diet fortified with 57 mg of elastase per kilogram; (iv) given 0.2 ml of saline intramuscularly five times per week; (v) given 4 mg of crystallized trypsin intramuscularly five times per week; (vi) given 4 mg of elastase intramuscularly five times per week.

After 8 weeks of treatment, the birds were fasted overnight, bled, and sacrificed. Four milliliters of blood was drawn from the alar vein of each bird and mixed with 0.7 ml of solution of citric acid, sodium citrate, and dextrose (ACD solution) (*10*). The prepared plasma samples were analyzed for total cholesterol (*11*) and lipid phosphorus (*12*) and for cholesterol in α- and β-lipoprotein after fractionation by Cohn's method 10 (*10, 13, 14*). The thoracic aortas and brachiocephalic arteries were removed and examined for degree of atheromatosis by two independent observers. A score of 1 was assigned for thin, scattered plaques; a score of 2 for either light, uniform deposit or heavy, scattered plaques; a score of 3 for heavy, uniform deposit; and a score of 4 for extremely heavy and lumpy deposit.

The results are shown in Table 1. Lipid concentrations in ACD plasma should be multiplied by 1.3 to obtain corresponding serum values.

Food consumption data showed that the average enzyme intakes by birds on the dietary regimens in the first experiment were 4.9 mg of trypsin per day and 4.4 mg of elastase per day. In the second experiment, the intakes were 4.5 mg of trypsin per day and 4.3 mg of elastase per day. Samples of different lots of diet were analyzed by U. J. Lewis, who found that there was no loss of enzyme activity in them before they were consumed.

The two experiments differed in the severity and incidence of atheromatosis. The injected birds seemed to have higher α-lipoprotein cholesterol and a higher $\alpha/(\alpha+\beta)$ ratio than those on dietary regimens.

Crystalline elastase, given either in the diet or by intramuscular injection, did not reduce either incidence or severity of atheromatosis in cholesterol-fed chickens. The elastase-treated birds gained less weight on the average, and had slightly more α-lipoprotein cholesterol ($p < 0.05$) than their companions. The lipid patterns were not otherwise influenced in a direction that would be considered beneficial in man.

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Table 1. Lesion scores, plasma cholesterol concentrations, and distributions between lipoproteins, plasma lipid-phosphorus concentrations, ratios of cholesterol to phospholipid (C/PL), and weight changes in control and treated cockerels.

Substance	Lesions		Cholesterol (mg/ml)				Lipid P (μg/ml)	C/PL	Wt. gain (g)
	Incidence	Avg. score	Total	α-Lipoprotein	β-Lipoprotein	$\frac{\alpha}{\alpha+\beta}$			
Experiment 1, Enzyme in the diet									
None	5/10	0.9	3.56	0.27	3.29	0.12	55.0	2.21	939
Trypsin	4/10	0.6	2.32	0.26	2.02	0.13	40.4	2.27	1042
Elastase	8/10	1.55	4.40	0.37	3.89	0.11	55.4	3.00	927
Experiment 1, Intramuscular injection									
Saline	4/10	0.45	2.14	0.33	1.83	0.16	43.4	1.94	984
Trypsin	3/7	1.0	3.33	0.35	2.78	0.14	54.7	2.37	896
Elastase	3/10	0.5	4.28	0.65	3.34	0.16	63.3	2.50	775
Experiment 2, Enzyme in the diet									
None	7/10	1.7	4.75	0.37	4.39	0.10	60.3	2.99	1007
Trypsin	8/10	1.5	3.31	0.33	2.98	0.11	54.7	2.37	1007
Elastase	9/10	1.95	4.54	0.39	4.16	0.09	62.3	2.85	966
Experiment 2, Intramuscular injection									
Saline	8/10	1.7	4.68	0.41	4.17	0.12	63.6	2.99	957
Trypsin	7/9	1.45	4.41	0.46	3.88	0.13	64.5	2.51	918
Elastase	8/9	1.9	4.66	0.52	4.09	0.12	64.6	2.66	891

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