certain conditions. Cytochrome c reduced by $Na_2S_2O_4$ does not appear to stabilize lysozyme activity to heat. Protection of lysozyme activity by oxidized Cytochrome c is afforded from 28°C to 60°C in solutions buffered at pH 5.0 to 90 minutes. Excellent protection is afforded also to aqueous, unbuffered solutions at 100°C for incubation of 10 minutes. The optimal molecular ratio for exertion of beneficial effects upon lysozyme appears to be about 10 molecules of Cytochrome c to 1 of lysozyme. Under certain conditions Cytochrome c preparations appear to exert a slight stimulation of initial velocity of lysis by lysozyme.

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Effect of Beta-Aminopropionitrile and Cholesterol on Lipids and Aortic S^{35}-Sulphated Mucopolysaccharides in Cockerels. (25548)

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When fed to animals and birds, Lathyrus factor beta-aminopropionitrile (BAPN) results in severe osteoporosis, skeletal deformities, dissecting aneurysms of aorta and various internal hemorrhages(1,2). The toxic action of BAPN is related to defective metabolism of connective tissue and lesions in lathyrism are due to abnormal formation or destruction of mucopolysaccharides of ground substance(3,4). There seems to exist an analogy between vascular lesion of connective tissue noted in lathyrism and some changes in vascular ground substance observed in animals with experimental atherosclerosis. Both histological and biochemical data seem to indicate, that alteration in mucopolysaccharides of connective tissue is involved in entrance of lipids into the arterial wall(6,7,8,9). Because of possible analogy between vascular lesions in experimental atherosclerosis and those noted in lathyrism, we studied the effect of BAPN and cholesterol, added separately or in combination to cockerels' diet, on some lipids and mucopolysaccharides of the aorta.

Materials and methods. Cockerels of White-Rock Broiler strain used for this study were obtained from a commercial hatchery when 8 days old and kept, in groups of 8 to 10, in thermostatic brooders. Experimental diets were supplied from 16 days of age. The BAPN-containing diet was prepared by drying a 1% aqueous solution of synthetic beta-aminopropionitrile fumarate (Abbott Laboratories) on a weighed amount of commercial chicken starter. The concentration of 0.025% of BAPN in the diet was used. The cholesterol diet contained 2% of cholesterol and 5% of olive oil. The following 4 diets were given to 4 groups of birds during a 4 week period: 1. Basal diet (normal control); 2. BAPN diet; 3. BAPN + cholesterol diet; 4. Cholesterol diet. 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

TABLE I. Average Weight of Body and Organs (in g) in Cockerels Fed Various Diets during 4 Weeks.

	Control diet	BAPN diet	BAPN + cholesterol diet	Cholesterol diet
No. of birds	26	21	19	28
Initial B.W.	166	178	157	175
Final "	950	826	768	804
Liver	22	21	26	27
Aorta	.33	.34	.31	.31
Femur	4.94	4.85	4.79	4.56

dose was dissolved in 5 ml of distilled water together with 40 mg of sodium sulphate (10, 11). The cockerels were anaesthetized, 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. Bone was studied to compare S^{35} uptake of the aorta with that of a tissue (bone) having specifically high sulphate-fixing capacity(10,11). Majority of aortas were studied for S^{35}-radioactive mucopolysaccharides following the method described previously(12,13). The specificity of S^{35} uptake method for study of certain mucopolysaccharides in tissue is well known. In some aortas lipid content was determined. The bones were dissolved by the nitric-perchloric acid wet-digestion procedure(14). 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 Tracerlab Superscaler S.C. 18A was used for readings. Results were expressed in counts per minute (c.p.m.). Total cholesterol was determined following the method of Abell *et al.*(15). For study of lipid phosphate the samples were extracted with alcohol-ether and analysis carried out by the method of Zilversmit and Davis(16).

Results. All cockerels used in this experiment showed hock deformities and crooked toes of varying degrees of severity(2,11). Of cockerels receiving BAPN diet alone or with cholesterol about 16% died of hemorrhage due to aortic rupture.

Average weight of birds and organs used for biochemical studies is given in Table I. The results of study of lipids in serum, liver and aortas are given in Tables II and III. Cholesterol feeding produced a rise of total cholesterol in all birds. Addition of BAPN to the diet did not result in a marked alteration of lipid pattern in tissues studied. Table IV gives results of measurement of S^{35} uptake by the fraction of aortic tissue considered to contain sulphated mucopolysaccharides. Total radioactivity of femora of birds is also recorded. Increased S^{35} uptake by the sulphated mucopolysaccharides of the aortic wall in cholesterol fed cockerels was observed, and this finding agrees with our previous work (7). Cholesterol diet did not affect radioactivity of bones. Addition of BAPN to control diet or to atherogenic diet did not influence S^{35} uptake by aortas and bones.

Despite the fact that both BAPN and cho-

TABLE II. Serum and Liver Lipids in Cockerels Fed Various Diets during 4 Weeks.

	Control diet	BAPN diet	BAPN + cholesterol diet	Cholesterol diet
No. of birds	26	21	19	28
Serum total cholesterol, mg/100 ml	182 ± 17* (87-235)†	144 ± 11 (101-216)	617 ± 98 (311-1252)	1156 ± 161 (380-2090)
Serum phospholipids, mg/100 ml	271 ± 31 (193-363)	300 ± 17 (200-326)	392 ± 22 (395-520)	423 ± 25 (368-763)
Serum C/P‡	.51 ±.07 (.35-.74)	.42 ±.04 (.26-.58)	1.98 ±.33 (1.09-4.11)	2.81 ±.32 (1.10-3.81)
Liver total cholesterol, mg/100 g	367 ± 11 (324-423)	393 ± 11 (339-479)	4942 ± 136 (2877-7630)	6362 ± 466 (2759-8657)
Liver phospholipids, mg/100 g	2698 ± 72 (2373-3100)	2804 ± 82 (2075-3275)	2942 ± 76 (2450-3200)	2792 ± 89 (1850-3150)
Liver C/P	.14 ±.01 (.11-.18)	.14 ±.006 (.11-.17)	1.70 ±.20 (.97-2.75)	2.36 ±.42 (1.03-4.68)

* Mean ± S.E. † Range. ‡ Total cholesterol/phospholipids ratio.

TABLE III. Lipids of Aorta in Cockerels Fed Various Diets during 4 Week Period.

	Control diet	BAPN diet	BAPN + cholesterol diet	Cholesterol diet
No. of birds	10	11	9	12
Total cholesterol, mg/100 g	194 ± 12* (93-247) †	205 ± 18 (117-233)	372 ± 23 (275-524)	414 ± 50 (264-930)
Phospholipids, mg/100 g	704 ± 48 (465-843)	822 ± 22 (595-1115)	945 ± 30 (765-1075)	877 ± 35 (680-1123)
C/P	.29 ±.02 (.13-.38)	.26 ±.02 (.17-.32)	.40 ±.03 (.30-.55)	.47 ±.04 (.30-.83)

* Mean ± S.E. † Range.

TABLE IV. Uptake of S^{35} by Bone and Radioactivity of S^{35}-Sulphated Mucopolysaccharides of Aortas (S.M.) in Cockerels Fed Various Diets during 4 Week Period.

	Control diet	BAPN diet	BAPN + cholesterol diet	Cholesterol diet
No. of bones	26	21	19	28
S^{35} in bone, cpm/100 mg dry wt	1157 ± 43* (823-1649) †	1150 ± 76 (563-1621)	1265 ± 59 (856-1553)	1185 ± 72 (417-1590)
No. of aortas	16	10	10	16
S^{35} in S.M. fraction of aorta, cpm/100 mg dry wt	723 ± 51 (409-1052)	763 ± 60 (457-1109)	1878 ± 101 (1294-2320)	1564 ± 72 (1180-2034)

* Mean ± S.E. † Range.

lesterol diet may influence collagen metabolism of the vascular wall in birds, there was no marked analogy in the effect of these 2 factors on some aspects of lipid and mucopolysaccharide metabolism, which has been studied under the described experimental conditions.

Summary. 1) Beta - aminopropionitrile (Lathyrus factor) alone, or combined with cholesterol was added to diet of cockerels for 4 weeks. Normal diet or cholesterol rich diet was fed to control birds. BAPN feeding resulted in deformities of extremities and frequent death from aortic rupture. These changes were not influenced by addition of cholesterol to the diet. 2) BAPN had no effect on lipid content in serum, liver or aorta. Alteration in lipid pattern was obtained only with cholesterol rich diet. 3) Parallel to increase of lipids in tissues there was a significant rise of S^{35} uptake by sulphated mucopolysaccharides of the aorta in cholesterol fed cockerels.

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