roethylene-extracted soybean oil meal/day/100 lb to young calves for 10 days induced aplastic anemia and caused death 14-25 days after feeding of the toxic material had ceased. DL-batyl alcohol injected by various routes after feeding of the toxic agent had ceased, but before appearance of clinical symptoms, failed to prevent development of aplastic anemia.

1. Evans, W. C., Evans, I. A., Edwards, C. M., Thomas, A. J., *Biochem. J.*, 1957, v65, 6P.

2. Naftalin, J. M., Cushnie, G. H., *J. Comp. Path. Therap.*, 1956, v66, 354.

3. Pritchard, W. R., Mattson, W. E., Sautter, J. H., Schultze, M. O., *Am. J. Vet. Res.*, 1956, v17, 437.

4. Schultze, M. O., Seto, T. A., Mizuno, N. S., Bates, F. W., Perman, V., Sautter, J. H., *Proc. 6th Congress, Intern. Soc. Hematol.*, Grune and Stratton, N. Y., 1958, p794.

5. McKinney, L. L., Weakley, F. B., Eldridge, A. C., Campbell, R. E., Cowan, J. C., Picken, J. C., Jr., Biester, H. E., *J. Am. Chem. Soc.*, 1957, v79, 3932.

6. Edlund, T., *Nature*, 1954, v174, 1102.

7. Brohult, A., Holmberg, J., *ibid.*, 1954, v174, 1102.

8. Perman, V., Rehfeld, C. E., Sautter, J. H., Schultze, M. O., *J. Agric. Food Chem.*, 1956, v4, 959.

9. Gradwohl, R. B. H., *Clinical Laboratory Methods and Diagnosis*, C. V. Mosby Co., St. Louis, 1948.

10. Ford, S. G., Marvel, C. S., in Blatt, A. H., Editor, *Organic Syntheses*, 1943, Coll. Vol. II, 372, John Wiley and Sons, N. Y.

11. Marvel, C. S., Sekera, V. C., in Horning, E. C., Editor, *Organic Syntheses*, 1955, Coll. vIII, 366, *ibid.*

12. Renoll, M., Newman, M. S., *ibid.*, Coll. vIII, 502.

13. Baer, E., Rubin, L. J., Fischer, H. O. L., *J. Biol. Chem.*, 1944, v155, 447.

14. Baer, E., Fischer, H. O. L., *ibid.*, 1947, v170, 337.

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Influence of Cobaltous Chloride on Aortic Atheromatosis and Plasma Lipid Pattern in Cholesterol-Fed Chickens. (24080)

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Several investigators have studied the influence of various metallic elements on cholesterol metabolism and experimental atheromatosis in animals(1-8). The action of cobaltous chloride is of particular interest because of its damaging effect on pancreatic α-cells (9). Caren and Carbo(8) found that cobaltous chloride given intravenously causes an increase in plasma cholesterol concentration of rabbits lasting 1 or 2 weeks.

The present paper reports the influence of cobaltous chloride, given in the diet and by subcutaneous injection, on aortic atheromatosis and plasma lipid pattern in cholesterol-fed chickens.

Methods used have been described in detail previously(10). Eight-week-old White Leghorn cockerels were set out in groups of 10 in wire batteries and fed a diet containing 2% cholesterol and 5% cottonseed oil until they were 16 weeks old. Experiments were then terminated. Food was withheld overnight, 4 ml of blood were drawn from alar vein and mixed with citrate solution, the birds were decapitated, and thoracic aortas and brachiocephalic arteries were removed and graded for degree of atheromatosis, using a scale of 0 to 4. Plasma aliquots were analyzed for total cholesterol(11) and lipid phosphorus(12,13), and for cholesterol in lipoprotein fractions separated by Cohn's Method 10(14,15).

Results. $CoCl_2 \cdot 6H_2O$ was given initially in the diet at concentration of 0.5%. It was toxic and accordingly was fed only for the second half of the 8-week experimental period. The cobalt-fed birds in 5 experiments (Table I) had less atheromatosis and lower blood cholesterol concentrations than did the controls. There was no effect on lipid phosphorus concentrations. The treated birds

TABLE I. Lesion Scores, Plasma Lipid Concentrations, and Weight Changes in Cholesterol-Fed Cockerels Given $CoCl_2 \cdot 6H_2O$ in the Diet for 8 Weeks.

Dose, %	Lesions: Incidence	Lesions: Avg score	Cholesterol: Total (mg/100 ml)	Cholesterol: α-Lipoprotein (mg/100 ml)	Cholesterol: β-Lipoprotein (mg/100 ml)	α/(α+β)	Lipid P, mg/100 ml	C/PL*	Wt gain, g
.5†	24/45	.88	323	54	247	.20	6.35	2.03	342
0	38/47	1.52	525	46	456	.12	6.35	3.18	955
P	.005	<.01	<.001	<.05	<.001	<.001	1	<.001	<.001
0	6/10	1.4	362	34	311	.11	6.22	2.24	1011
.001	7/10	1.25	368	28	322	.12	5.77	2.29	1118
.01	7/10	1.3	281	36	237	.15	5.22	2.08	1116
.05	4/10	.6	293	34	241	.16	4.88	2.46	1000
.25	2/ 4‡	1.0	370	54	289	.20	6.95	2.03	150
.5 †	5/10	.8	257	42	210	.18	5.43	1.81	332
.05	11/30	.42	265	33	217	.16	5.22	2.07	916
0	19/30	1.17	344	35	292	.13	5.66	2.34	990
P	.04	.002	.06	.2	.07	.09	.2	.1	.05

* C/PL = Cholesterol/phospholipid ratio. † $CoCl_2 \cdot 6H_2O$ fed at 0.5% for only 4 wk.
‡ 6 out of 10 died.

generally lost weight during 4 weeks of cobalt feeding, with the result that their over-all weight gain during the 8-week period was only 35% of that of controls. Next, cobaltous chloride was fed in various concentrations in the diet to determine greatest dose that would not reduce weight gain. Four groups of birds were given 0.001%, 0.01%, 0.05%, or 0.25% $CoCl_2 \cdot 6H_2O$ for the entire 8-week period. A fifth group was given 0.5% $CoCl_2 \cdot 6H_2O$ for the second 4 weeks only, and a sixth group, given no cobalt, served as controls. As shown in Table I, small amounts of cobalt caused weight gain, and large amounts caused weight loss and death. Birds fed $CoCl_2 \cdot 6H_2O$ at 0.05% of the diet gained about the same weight as controls, but had less atheromatosis and lower plasma cholesterol concentrations. $CoCl_2 \cdot 6H_2O$ was given at 0.05% in the diet in 2 further experiments. The results of all 3 tests at this level are summarized in Table I. Treatment consistently reduced arterial involvement and lowered plasma cholesterol concentrations. In the second and third experiments the treated groups gained less weight than controls, but the data do not show any tendency within treatments for smaller weight gain to be associated with lower lesion score.

Organ weights. Table II presents the weights of the pituitaries, adrenals, thyroids, and testes, and erythrocyte counts and blood hemoglobin concentrations at the end of one of the experiments with 0.05% $CoCl_2 \cdot 6H_2O$ in the diet. Cobalt-fed birds had smaller pituitaries and testes and somewhat lower erythrocyte counts than controls. The differences in average hemoglobin concentration and thyroid weight were not large enough to be significant. There was no apparent effect on the adrenals.

Examination of pancreas. Pieces taken from 10 birds fed 0.05% $CoCl_2 \cdot 6H_2O$ in the diet for 8 weeks were fixed in Bouin's solution. Sections from head, body, and tail, stained with hematoxylin-eosin and by the method of Gomori(16), showed no evidence of damage to the α-cells. Additional birds were then given cobalt chloride intravenously in single and multiple doses in lethal and sub-lethal

TABLE II. Organ Weights, Erythrocyte Counts, and Blood Hemoglobin in Cholesterol-Fed Cockerels Given 0.05% $CoCl_2 \cdot 6H_2O$ in the Diet for 8 Weeks.

Treatment	Pituitary, mg	Adrenal, mg	Thyroid, mg	Testes, g	RBC × 10^6	Hb, g/100 ml
Cobalt (10)	8.19	174	116	2.73	2.90	9.63
Controls (10)	9.63	177	133	7.71	3.23	10.03
P	.03	.8	.4	.004	.1	.4

TABLE III. Radioiodine Uptake in Cholesterol-Fed Birds Given $CoCl_2 \cdot 6H_2O$, 0.05% in the Diet.

Treatment	Thyroid wt, mg	Thyroid activity, c/m/mg	Blood activity, c/m/ml	Thyroid / Blood
Cobalt (10)	128	802	26.1	35.1
Controls (10)	151	790	24.9	33.4
P	.1	.2	.8	.7

amounts. The single doses ranged from 0.012 mg cobalt chloride to 3.0 mg cobalt chloride. Single doses above this range resulted in immediate death from respiratory failure. Several chicks from each group were sacrificed at 5 hours, 1 day, 3 days, 15 days, and 30 days. One group of 3 chicks was given 4 daily doses of 3 mg cobalt chloride each, intravenously and sacrificed on the 5th day. Again, there was no evidence of damage to the pancreas. As positive controls, 4 guinea pigs were given 3 daily doses of 30 mg/kg cobalt chloride each intravenously and sacrificed 6 or 24 hours after the last dose. Sections of pancreas from these guinea pigs, as well as some normal control animals, were stained simultaneously with those of the chickens. Alpha cell damage was present in the dosed guinea pigs.

Radioiodine uptake test. Damage to the thyroid after oral dosing with cobalt has been observed in man(17). For this reason radioiodine uptake tests were done here on 10 cockerels fed 0.05% $CoCl_2 \cdot 6H_2O$ for 8 weeks and on 10 controls. Each bird was given 1 μc of I^{131} intravenously. Five hours later 4 ml of blood were taken by venipuncture, the birds were decapitated, and the thyroids weighed, placed in Bouin's solution, and counted in a well-type scintillation counter. The results are presented in Table III. The dosed birds again had somewhat smaller thyroids than the controls, and their specific radioactivity was a little higher. The iodine uptakes, as judged by ratio of activity in thyroid to that in blood, were about the same in both groups.

Subcutaneous injection of $CoCl_2 \cdot 6H_2O$ in 3 experiments 5 times a week in doses from 0.005 γ to 25 mg (Table IV) increased both lesion scores and plasma cholesterol concentrations. At highest dose, which killed half the birds, lesion score was reduced, but here again plasma cholesterol was elevated. Inasmuch as birds eat about 80 g of food daily, the 40 mg ingested each day as 0.05% of the diet considerably exceeds the highest tolerated subcutaneous dose.

Summary and conclusions. (1) Feeding of cobaltous chloride to cholesterol-fed chickens reduced the incidence and severity of their aortic lesions and caused blood cholesterol concentrations to be lower than in controls.

TABLE IV. Lesion Scores, Plasma Lipid Concentrations, and Weight Changes in Cholesterol-Fed Cockerels Given Different Amounts of $CoCl_2 \cdot 6H_2O$ by Subcutaneous Injection for 8 Weeks.

Dose	Lesions: Incidence	Lesions: Avg score	Cholesterol: Total (mg/100 ml)	Cholesterol: α-Lipoprotein (mg/100 ml)	Cholesterol: β-Lipoprotein (mg/100 ml)	$\alpha/(\alpha+\beta)$	Lipid P, mg/100 ml	C/PL*	Wt gain, g
γ									
0	6/ 9	1.15	371	39	294	.10	3.58	4.54	813
.005	8/10	1.65	475	42	418	.11	3.70	5.06	948
.05	5/10	1.00	307	34	272	.15	2.83	4.12	996
.5	7/10	1.8	619	47	544	.10	4.30	5.63	855
5	8/10	1.75	404	41	343	.14	3.06	5.25	874
50	9/10	1.3	350	46	297	.15	3.48	4.02	838
0	4/10	.75	291	32	246	.14	4.23	2.57	962
50	6/ 9	1.55	423	37	357	.11	5.63	2.84	853
150	6/ 9	1.17	303	55	233	.20	5.48	2.30	880
1000	8/10	1.45	319	23	273	.10	4.66	2.63	924
mg									
0	5/ 9	.67	222	29	189	.14	4.62	1.96	838
1	6/ 9	1.29	356	31	324	.10	6.39	2.10	912
10	7/10	1.4	386	43	335	.14	7.07	2.13	727
25	0/ 5†	0	451	30	415	.08	6.75	2.67	220

* C/PL = Cholesterol/phospholipid ratio. † 5 out of 10 died.

The amounts of cholesterol in β-lipoprotein were similarly reduced and $\frac{\alpha}{\alpha+\beta}$ ratios were increased. There was no consistent effect on lipid phosphorus concentrations. Cobalt in the diet did not seem to influence activity of the thyroid. When given either in the diet or intravenously, cobalt did not change the structure of the pancreatic α-cells. (2) Subcutaneous injection of cobaltous chloride generally increased the incidence and severity of aortic lesions and concentration of cholesterol in blood. These findings are similar to those of Caren and Carbo in the rabbit(8). (3) The influence of cobalt in the diet on lipid pattern and aortic lesions was opposite to that of parenterally administered cobalt. It did not produce other effects indicative of previously reported specific systemic actions. Siperstein, Nichols, and Chaikoff(1) concluded that blood-cholesterol-lowering effect of ferric chloride given in the diet was due to precipitation of bile acids, resulting in decreased cholesterol absorption. It is concluded here that the effect of cobalt in the diet of cholesterol-fed chickens similarly is due to a local action in the intestinal tract.

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1. Siperstein, M. D., Nichols, C. W., Jr., Chaikoff, I. L., *Science,* 1953, v111, 386.

2. Curran, G. L., *J. Biol. Chem.,* 1954, v210, 765.

3. Curran, G. L., Costello, R. L., *J. Exp. Med.,* 1956, v103, 49.

4. Mountain, J. T., Stockell, F. R., Jr., Stokinger, H. E., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 582.

5. Eades, C. H., Jr., Gallo, D. G., *Fed. Proc.,* 1957, v16, 176.

6. Vitale, J. J., White, P. L., Kakamura, M., Hegsted, D. M., Zamcheck, N., Hellerstein, E. E., *J. Exp. Med.,* 1957, v106, 757.

7. Hellerstein, E. E., Vitale, J. J., White, P. L., Hegsted, D. M., Zamcheck, N., Nakamura, M., *ibid.,* 1957, v106, 767.

8. Caren, M., Carbo, L., *J. Clin. Endocrin. and Metab.,* 1956, v16, 507.

9. VanCampenhout, E., Cornelis, G., *Comp. Rend. Soc. Biol.,* 1951, v145, 933.

10. Tennent, D. M., Siegel, H., Kuron, G. W., Ott, W. H., Mushett, C. W., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 679.

11. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., *J. Biol. Chem.,* 1952, v195, 357.

12. King, E. J., *Microanalysis in Medical Biochemistry,* London, J. and A. Churchill, 1951, p67.

13. Zilversmit, D. E., Davis, A. K., *J. Lab. Clin. Med.,* 1950, v35, 155.

14. Cohn, E. J., *et al., J. Am. Chem. Soc.,* 1950, v72, 465.

15. Lever, W. F., *et al., J. Clin. Invest.,* 1951, v30, 99.

16. Gomori, G., *Am. J. Path.,* 1941, v17, 395.

17. Kriss, J. P., Carnes, W. H., Gross, R. T., *J.A.M.A.,* 1955, v157, 117.

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Abnormal Fat Absorption in Tumor-Bearing Rats.* (24081)

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A number of workers have shown that loss of body weight accompanying tumor growth is associated with depletion of total body lipids(1,2). This loss in body weight, however, is not due to anorexia alone, since force-feeding will not overcome depletion of carcass lipid previously described(3). Despite this lipid loss, a progressive, marked hyperlipemia is often associated with tumor bearing in rats when tumor weight exceeds 10% of total body weight(4). Studies of intestinal absorption in cancerous animals have never been described, although absorption may play a very signifi-

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