trophy of ovary after unilateral castration was inhibited in persistent estrous lesioned rat. FSH stores in adenohypophysis were maintained but titers in serum decreased. The results signify that adenohypophysis of persistent estrous lesioned rat loses its capacity to augment secretion of FSH under conditions of enhanced demand (unilateral oophorectomy).

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Comparison of Cholesterol and Estrogen-Induced Atherosclerosis in Cockerels.* (25754)

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Atherosclerosis can be induced in cockerels by administering estrogen(1,2) and by feeding a diet high in cholesterol(3). A comparison of results obtained by these procedures, separately and combined, has been made.

Material and methods. Method used in this laboratory for studying quantitatively the atherogenic response of cockerels to exogenous estrogen has been described(4). Cholesterol induced atherosclerosis was produced in White Leghorn cockerels, 4-5 wks old, by feeding chick growing mash containing cholesterol deposited on the diet from ethyl ether solution. Atherosclerosis was evaluated as % area of the aorta, brachiocephalic, and iliac arteries involved in plaque formation. Other procedures were similar to those described previously(4).

Results. Cholesterol-induced atherosclerosis. Graded levels of cholesterol fed to cockerels for 8 wks resulted in progressively larger values for degree of atherosclerosis, serum cholesterol, and phospholipid (Table I). Feeding .5% cholesterol diet longer than 8 wks did not elevate these values (Table II). Increasing dietary cholesterol to 1% resulted in lower serum cholesterol and phospholipid values in 16 and 20 wks than either 8 or 12 wks. Degree of atherosclerosis reached a peak at 12 wks which was twice as great as either dietary cholesterol level in 8 wks. Although increasing the amount of dietary cholesterol to 1% resulted in elevated serum cholesterol and phospholipid values, sensitivity to atherosclerotic vascular involvement was decreased. These data indicate that feeding young cockerels a .5% cholesterol diet for 8 wks is optimal for inducing hypercholesterolemia and plaque formation in the major arteries. This method for producing cholesterol-induced atherosclerosis in cockerels has been adopted for studying the disease in this laboratory.

Cholesterol-estrogen induced atherosclerosis. Comparison of results obtained with cholesterol and estrogen as atherogenic agents, separately and combined, during 8 wks was made (Table III). Although serum cholesterol, phospholipid, and polysaccharides in-

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creased with larger doses of estrogen, % of aorta, brachiocephalics, and iliacs involved in plaque formation was greater at the 5 mg dose of estrogen than when smaller or larger amounts were injected. Feeding cholesterol diet to estrogen-treated birds augmented hy-

TABLE I. Dose Response of Cockerels to Dietary Cholesterol—8 Week Period.

Cholesterol added to diet (%)	No. cockerels	Atherosclerosis (% area)	Blood serum values: Cholesterol Total (mg %)	Cholesterol % free/total	Phospholipid (mg %)
.0 (controls)	24*	.03	113	27.1	225
.25	4	.9	186	27.2	151
.5	24*	17.9	391	28.3	249
1.0	12†	22.9	668	28.8	326
2.0	6	33.5	1189	27.8	406

* In 4 groups. † In 2 groups.

TABLE II.* Time and Dose Response of Cockerels to Dietary Cholesterol.

Exp. period (wk)	Cholesterol added to diet (%)	Atherosclerosis (% area)	Blood serum values: Cholesterol Total (mg %)	Cholesterol % free/total	Phospholipid (mg %)
8	.0	.1	121	26.1	190
8	.5	16.8	303	28.4	172
8	1.0	18.5	851	27.9	346
12	.0	1.6	99	28.2	169
"	.5	18.0	353	28.2	216
"	1.0	38.9	722	28.5	309
16	.0	.2	98	25.1	164
"	.5	19.4	243	25.8	148
"	1.0	28.9	330	23.5	179
20	.0	1.2	94	25.9	174
"	.5	11.4	312	24.9	213
"	1.0	25.7	379	24.8	229

* Each group consisted of 5 or 7 cockerels excepting 1 group of 4.

TABLE III. Dose Response of Cockerels to ECP, with and without Cholesterol Added to the Diet—8 Week Period.

ECP,* mg/bird (IM)	Cholesterol added to diet (%)	Atherosclerosis (% area)	Blood serum values: Cholesterol Total (mg %)	Cholesterol % free/total	Phospholipid (mg %)	Total polysaccharides (mg %)
Controls						
.0†	.0	.02	100	21.3	174	72
.0‡	.5	16.2	227	26.5	162	76
1.0	.0	.3	109	22.0	208	74
1.0	.5	12.0	273	30.8	214	81
5.0	.0	22.8	383	58.1	1206	117
5.0	.5	19.5	839	37.3	1243	120
7.5	.0	9.2	746	65.3	1986	163
7.5	.5	22.2	1280	42.2	1968	171
10.0	.0	10.2	995	78.1	4423	181
10.0	.5	24.0	1260	49.5	2954	161

* Reg. U. S. Pat. Off., Upjohn Co. brand of Estradiol Cyclopentylpropionate dissolved in cottonseed oil.

† Nine cockerels in 2 groups.

‡ Eleven cockerels in 2 groups.

Other groups consisted of 5 or 6 cockerels.

Dosage equivalent administered to each bird on a 10-day basis.

TABLE IV.* Response of Cockerels to ECP and Dietary Cholesterol—1 Week Period.

ECP, mg/bird (IM)	Cholesterol added to diet (%)	Atherosclerosis (0 to 4+)	Blood serum values: Cholesterol Total (mg %)	Cholesterol % free/total	Lipoprotein, mg % cholesterol in: Alpha	Beta
.00	.0	.0	166	24.5	84	100
.00	.5	.0	372	27.0	53	339
3.75	.0	2.2	343	31.9	11	368
3.75	.5	2.2	623	23.5	2	660

* Each group consisted of 5 or 6 cockerels.

percholesterolemia, but serum phospholipid and polysaccharide values were unchanged except in birds injected with 10 mg estrogen, when lower values were observed. Influence on plaque formation of feeding cholesterol to estrogen-treated birds resulted in relatively small increases beyond the 5 mg dosage.

Combining .5% dietary cholesterol with estrogen treatment at the 5 mg level over an 8 wks period provides another useful method for studying atherosclerosis in cockerels, since there is a progressive increment in plaque formation and hypercholesterolemia, as well as serum phospholipids and polysaccharides in the cholesterol fed birds between the 1 mg and 7.5 mg doses of estrogen, indicating that this is a sensitive area of response.

Using a modification of this method for a 1 wk experiment, birds were fed .5% cholesterol diet and injected with 3.75 mg of estrogen (Table IV). Under these conditions it is necessary to use an alternate method for evaluating atherosclerotic plaque formation (4). Although the cholesterol diet alone caused hypercholesterolemia and induced alpha and beta lipoprotein patterns characteristic of atherosclerosis, no plaques were formed during the 1 wk period. This is in contrast to the 8 wk experiment described above in which plaques were associated with similar serum cholesterol values. It is of considerable interest also that cockerels injected with estrogen and those fed cholesterol diet had comparable hypercholesterolemia, but plaques were found only in the hormone-treated birds. Combining dietary cholesterol with estrogen treatment resulted in 2-fold increases in serum cholesterol and beta lipoprotein; however, the degree of plaque formation was not altered.

Summary. Method is described for inducing atherosclerosis in cockerels by feeding chick growing mash containing .5% commercial cholesterol. Procedures for inducing atherosclerosis in birds by exogenous estrogen have been published(4). Experimental procedures are presented also for studying atherosclerosis induced in cockerels by combining estrogen and cholesterol treatment. Comparison of artery and blood serum changes associated with this disease as induced by these methods shows that a critical level of estrogen causes more rapid plaque formation than cholesterol, and that degree of hypercholesterolemia does not determine extent of atheromatosis.

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