erpine, on the other hand, a tranquilizer belonging to the group of autonomic suppressants, has no effect on SP of the brain of dogs (11). Our results showed that the amount of SP remains unchanged in hares subjected to fear for 14 days. No attempt was made to investigate the amount of SP in brains after shorter periods of time. That the amount of SP in brains of hares given SP intravenously is increased, indicates that this substance is able to pass the blood brain barrier. Presence of SP in the reticular formation(8,12,13) and especially the high amounts present in substantia nigra (which represents the condensation area of the reticular substance(14)), indicates that SP may represent an active principle closely connected with the function of nerve cells.

The marked tranquilizing effect of SP in hares lends support to the view that this substance belongs to the class of biological regulators of the neuro-endocrine system or that it performs the function of a physiological tranquilizer.

Summary. Wild hares have 20 to 23 units of Substance P/g of brain. This amount does not change when animals are kept 14 days in cages in a state of fear, although hyperthyrosis develops during this time. When Substance P is given intravenously its amount in the brain increases. Wild hares given Substance P intravenously behave like tame rabbits.

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Quantitative Study of Estrogen-Induced Atherosclerosis in Cockerels.* (24918)

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Hyperlipemic atheromatosis has been produced in chickens by subcutaneous implantation of diethylstilbestrol(1). Starting with 3 mos old cockerels, aortic lesions developed in 6 to 7 mos which resembled more closely those occurring spontaneously in birds than the cholesterol-induced type. The 3 types of lesions were fundamentally similar and appeared to differ mainly in concentration of various lipid components(2). Use of 6 wk old birds decreased the possibility that the observed lesions were of spontaneous origin(3). These observations suggested the possibility of developing a quantitative method for study of atherosclerosis. This has been accomplished through time and dose response studies of the atherogenic effect of estrogenic material on young cockerels. Study of the sequence of artery and blood serum changes was made concomitantly.

Material and methods. Approximately 4

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wk old White Leghorn cockerels were used. Each hatch was organized into experimental and control groups. Because of the number required, more than one series of groups is represented in each Table. Therefore, control data are shown in each Table as means of mean values for all control groups with respective standard deviations. A commercial chick growing mash was fed *ad lib.*† Experimental periods were 1 to 56 days. On the first day of a period of 10 days or less, a single dose of estradiol cyclopentylpropionate dissolved in cottonseed oil (ECP)‡ was administered intramuscularly. At the end of experimental period, the birds were rendered unconscious by electrocution and exsanguinated from the right cardiac ventricle. The aorta and other large arteries, including brachiocephalics and iliacs, were dissected to determine grossly the degree of atherosclerosis. Determination of total and free cholesterol, phospholipid phosphorus, total polysaccharides, and alpha and beta lipoproteins was done on pooled serum samples. Degree of atherosclerosis is expressed as 0 to 4 plus for the 7 day or shorter experimental periods. The "% area" of surface of large arteries examined, which showed gross atherosclerosis, was used for periods of longer duration. Total and free cholesterol were determined by our modification of the Sperry and Webb method(4). Phospholipid phosphorus results were obtained by the phosphate procedure of Fiske and SubbaRow(5). The anthrone method of Graff, Greenspan, Lehman, and Holechek(6) with modification of color development procedure was used for determination of total polysaccharides. Alpha and beta lipoprotein determinations were made by paper electrophoresis, using our modification of the method of Langan, Durrum, and Jencks(7). The modified procedure involved use of a larger (.075 ml) serum sample for a higher degree of accuracy, especially in measurement of alpha lipoprotein; the ferric chloride-sulfuric acid cholesterol reagent was prepared fresh daily; paper strips were pre-extracted with mixture of chloroform and methanol; 4 concentrations of cholesterol standard were employed in preparation of a reference curve.

Results. During 56 days of estrogen administration, a small amount of artery involvement occurred with 2.5 mg dose (Table I). Feminizing effects evidenced by relative comb size occurred with .1 mg dose. Extensive plaque formation accompanied by pronounced blood serum changes characteristic of the disease occurred with doses of 5 mg and larger.

The minimum period to produce a high degree of atherosclerosis was investigated next. Although major changes in all measurements were observed at the 5 mg level in the 56-day experiment, dose was increased to 10 mg to insure development of definite atherosclerotic lesions during shorter periods. The effects of 10 mg dose with variable time periods was evaluated. The cockerels used in the groups, representing first and last 7 wks of experiment were from 2 different sources. Therefore, the means of control group-mean values with standard deviations are shown separately for 1 through 7 day, and 8 through 56 day periods (Table II).

Blood serum changes occurred progressively during first 5 days after treatment. However, rate of change was different for individual serum components. This is demonstrated in free to total cholesterol, and cholesterol to phospholipid ratios. In 2 days free cholesterol and phospholipid values had increased so rapidly that the respective ratios reached values which remained almost constant during remainder of time studied. These rapid changes also appear to coincide with appearance of gross atherosclerotic change in the arteries.

All values represent a high degree of change characteristic of atherosclerosis as early as 7 days after administration of ECP, indicating this to be the period of choice for a quantitative method. However, the 10 mg dose appeared too large to provide sufficiently sensitive conditions. A more desirable dose was determined by experiments in which time was constant at 7 days, with dose of ECP as the variable (Table III).

It is of particular interest that atherosclero-

† Little Brothers Chick Growing Mash, Little Brothers, Kalamazoo.

‡ Registered trademark, The Upjohn Co.

TABLE I.* Dose Response of Cockerels to ECP—56 Day Period.

ECP dosage equivalent, mg/bird/10 days (IM)	Atherosclerosis (% area)	Blood serum values: Cholesterol Total (mg %)	Cholesterol % free/total	Phospholipid (mg %)	Cholesterol/Phospholipid	Lipoprotein (mg %) Cholesterol in: Alpha	Lipoprotein (mg %) Cholesterol in: Beta	Total polysaccharides (mg %)
Controls								
.0	.0	106 ± 8	22 ± 1.6	188 ± 23	.57 ± .04	78 ± 7	42 ± 2	74 ± 8
ECP treated								
.0037	.0	99	23	166	.60	79	40	72
.0074	.0	92	21	166	.55	69	37	77
.0370	.0	97	22	179	.54	70	34	72
.1	.0	112	21	221	.51	—	—	82
.5	.0	98	24	213	.46	74	37	75
1.0	.0	86	24	166	.52	73	36	70
2.5	.1	86	23	205	.42	67	46	69
5.0	22.8	383	58	1206	.32	2.4	710	117
7.5	9.2	746	65	1986	.38	—	—	163
10.0	10.3	883	61	2678	.33	1	966	145

* Control group consisted of 60 cockerels; each treated group consisted of 6 or 7 cockerels.

sis occurred 7 days after treatment with ECP at doses which did not cause changes in serum cholesterol, beta lipoproteins, and total polysaccharides. At these lower levels there were small but probably insignificant increases in serum phospholipids. It is also noteworthy that the feminizing effect was observed in all groups.

Attention is called to high degree of atherosclerosis which resulted from 1.25 mg dose. This also is of special interest since the relatively severe artery involvement is accompanied by only minor changes in serum cholesterol, phospholipids, and total polysaccharides. The alpha and beta lipoprotein changes appear to be more extensive.

The quantitative nature of the response to these large and unphysiologic amounts of ECP is further demonstrated by greater blood serum changes associated with severe athero-

TABLE II.† Time Response of Cockerels to ECP.

Exp. period (days)	Atherosclerosis (% area)	Blood serum values: Cholesterol Total (mg %)	Cholesterol % free/total	Phospholipid (mg %)	Cholesterol/Phospholipid	Lipoprotein (mg %) Cholesterol in: Alpha	Lipoprotein (mg %) Cholesterol in: Beta	Total polysaccharides (mg %)
Controls								
1– 7	.00	96 ± 4	19 ± .9	157 ± 9	.62 ± .05	84 ± 2	24 ± 5	64 ± 1.3
8–56	.03	113 ± 9	23 ± 1	200 ± 24	.57 ± .04	86 ± 13	47 ± 7	69 ± 7
ECP treated								
1	.00	148	39	406	.36	22	153	70
2	Suggestive	270	60	962	.28	10	306	94
3	Diffuse	447	67	1711	.26	7	528	121
5	*	662	68	2496	.27	5	728	121
7	*	616	67	2413	.25	3	640	132
8	14.9	711	64	2423	.29	13	1093	142
15	18.6	814	69	2756	.30	0	955	149
22	12.1	932	68	2938	.32	0	1085	186
29	7.9	836	66	2938	.28	4	975	155
43	19.7	632	57	2010	.31	0	725	141
56	10.3	883	61	2678	.33	1	966	145

* Positive—not measurable.

† Control group consisted of 56 cockerels; each experimental group consisted of 6 cockerels. Dose 10 mg at beginning of period. Equivalent dosage each 10 days.

TABLE III.† Dose Response of Cockerels to ECP—7 Day Period.

ECP dosage equivalent, mg/bird (IM)	Atherosclerosis (plus value)	Blood serum values: Cholesterol Total (mg %)	Cholesterol % free/total	Phospholipid (mg %)	Cholesterol/Phospholipid	Lipoprotein (mg %) Cholesterol in: Alpha	Lipoprotein (mg %) Cholesterol in: Beta	Total polysaccharides (mg %)
Controls								
.0	.0	111 ± 6	22 ± 2	167 ± 19	.67 ± .07	89 ± 3	37 ± 7	68 ± 5
ECP treated								
.156	.2	105	25	192	.55	90	38	78
.312	.6	113	23	205	.55	100	30	84
.625	.4	103	22	192	.54	81	47	75
1.250	2.2	127	24	229	.55	50	112	84
2.5	2.8	216	30	390	.55	17	233	92
5.0	2.4	423	58	1095	.39	—	—	113
7.5	2.0	578	66	1560	.37	2	664	135
10.0	*	616	67	2413	.26	3	640	132

* Positive—not measurable.

† Control group consisted of 39 cockerels; each experimental group consisted of 5 or 6 cockerels.

sclerosis at 2.5 mg level. Maximum artery involvement appears to have been reached at this dose level; however, additional increases in amount of ECP resulted in increasingly greater serum changes.

These results (Tables I and III) also suggest that atheroma, present 7 days after initial treatment, regressed as treatment was continued through 56 days Even the lowest dosage level (.156 mg) resulted in atherosclerotic change 7 days after treatment (Table III), while no atherosclerosis was observed at doses of 1 mg or less after 56 days (Table I).

It is of major interest that intramuscular administration of 2.5 mg of ECP to approximately 4 wk old cockerels at beginning of 7 day period are experimental conditions that constitute a practical quantitative method for study of atherosclerosis.

Summary. 1) Results of dose and time response studies in quantitation of atherogenic effect of pharmacologic amounts of estrogen on young cockerels have been used in formulating a method for the study of atherosclerosis. 2) The method is based upon response of immature birds to 2.5 mg of estradiol cyclopentylpropionate dissolved in cottonseed oil (ECP) administered intramuscularly at beginning of 7 day experiment. 3) Results suggest that feminizing effect and some degree of atherosclerosis result from ECP dosage levels lower than those required to produce hypercholesterolemia and other blood serum changes associated with atherosclerosis. There are indications that atheroma may regress with time.

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