

from 57% to 73% of the inoculated animals, after a latency varying from $2\frac{1}{2}$ to $4\frac{1}{2}$ months. 3. Either localized thymic lymphosarcomas, or generalized lymphatic leukemia, involving also spleen, liver, and mesenteric lymph nodes, developed in the inoculated animals. A few rats developed acute stem-cell leukemia.

1. Gross, L., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 767.

2. ———, *Acta Haemat.*, 1960, v23, 259.

3. ———, PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 64.

4. Graffi, A., Gimmy, J., *Naturwissenschaften*, 1957, v44, 518.

5. Graffi, A., *Progr. Exp. Tumor Res.*, Karger Publ., 1960, v1, 112.

6. Moloney, J., *Nat. Cancer Inst. Monograph No.* 4, 1960, 7.

7. Eddy, B. E., Stewart, S. E., Berkeley, W., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 848.

8. Gross, L., *ibid.*, 1953, v83, 414.

9. Eddy, B. E., Stewart, S. E., Young, R., Mider, G. B., *J. Nat. Cancer Inst.*, 1958, v20, 747.

10. Eddy, B. E., Stewart, S. E., Stanton, M. F., Marcotte, J. M., *ibid.*, 1959, v22, 161.

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Serum Free Cholesterol and Atheroma in Young Cockerels. (26513)

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Partial conversion of free to combined cholesterol upon incubation of normal human serum has been reported(1). Thirty-one to 50% decrease in free cholesterol occurred when serum from 20 normal patients was incubated(2). Incubation of serum from most patients with active coronary atherosclerosis resulted in less than normal conversion(3). These observations suggested the value of studying the relationship between serum free cholesterol not combined upon incubation and development of atheroma in normal and atherosclerotic young cockerels.

Material and methods. Approximately 4 wk old White Leghorn cockerels were used. Normal birds were fed a commercial chick growing mash *ad lib.** Atherosclerosis was induced by feeding the basal diet containing .5% cholesterol deposited from ethyl ether solution. Atherosclerotic condition was evaluated as % area of the aorta, brachiocephalic, and iliac arteries involved in plaque formation. Pooled blood serum from each group was incubated 30 hr at 37-38°C. Other methods employed have been described(4).

Results and discussion. Extent of artery involvement and serum cholesterol data be-

fore and after incubation were determined in 3 series of groups of birds, representing normal, mild spontaneous, and severe cholesterol-induced atherosclerosis respectively (Tables I, II, II). Total cholesterol values did not change appreciably upon incubation. Last column in each table shows the % original free cholesterol remaining after serum incubation.

Of particular interest is the incubation increase of % uncombined free cholesterol from normal (17 to 45) through mild spontaneous (45 to 56) and severe cholesterol-induced atherosclerosis (57 to 87%). The range, 17 to 45%, for normal birds suggests an increasing tendency toward atherosclerotic development although plaques were not grossly observable (Table I). Corresponding results, 45 to 56%, associated with the first appearance of plaques as mild spontaneous atherosclerosis in birds on normal diet support this suggestion (Table II).

Plaques were always found when more original free cholesterol remained than was combined upon incubation. They were not found when less than 45% remained. Approximately 57% marked the beginning of severe atherosclerosis. This narrow range for mild spontaneous artery involvement represents a

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

TABLE I. Eight Groups of Cockerels Fed Normal Diet—8 Weeks Experimental Period—*Without* Atherosclerotic Plaque Development.

Series 1 (38 birds)		Blood serum cholesterol				
Atherosclerotic plaques (% area)	Not incubated		Incubated 30 hr at 37 to 38°C			% original free remain- ing after incubation
	Total (mg %)	Free (mg %)	Free to com- bined form (mg %)	Free remaining (mg %)		
.0	104	27.2	22.5	4.7	17.3	
.0	106	27.4	19.3	8.1	29.6	
.0	100	26.6	18.0	8.6	32.3	
.0	104	26.2	16.8	9.4	35.9	
.0	94	24.5	15.1	9.4	38.4	
.0	103	26.9	16.1	10.7	39.8	
.0	104	26.0	15.0	11.9	44.2	
.0	98	24.4	13.5	10.9	44.7	
Mean values	.0	102	26.2	17.0	9.2	35.3
Range	—	94-106	24.4-27.4	22.5-13.5	4.7-11.9	17.3-44.7

relative balance between amounts of original free cholesterol combined and not combined upon incubation.

An outstanding incubation difference is the large increase above normal for uncombined free cholesterol in the severely atherosclerotic series. Values for the normal, mild spontaneous, and atherosclerotic series are 9, 14, and 67 mean mg% respectively. Original free cholesterol combined upon incubation shows small differences for the three series (17, 13, and 23 mean mg %). Corresponding approximate ratios of uncombined to combined upon incubation for the 3 series in the order named are .5, 1, and 3.

Statistical. Small increases in serum cholesterol mean values for normal compared with mild spontaneous series before incubation are not statistically significant. In contrast, all corresponding probability values

comparing normal with severe atherosclerotic, or the latter with mild spontaneous series, and in all possible interseries comparisons of extent of arterial plaques were highly significant, with $P < .001$. Probability values comparing normal with mild spontaneous series relative to (a) mg % original free cholesterol combined, (b) mg % and (c) % of original remaining after incubation are $> .02$, $> .001$, and $< .01$ respectively. Corresponding sets of values for comparisons of normal and mild spontaneous with severe series are .05, $< .001$, $< .01$, and $< .02$, $< .001$, $> .001$.

Summary. Relationship between serum free cholesterol not combined upon incubation and atheroma development has been studied using sera from normal and cholesterol-fed young cockerels. Original free cholesterol uncombined after incubation of serum from normal, mild spontaneous, and severely

TABLE II. Five Groups of Cockerels Fed Normal Diet—8 to 31 Weeks Experimental Periods—*With Low Degrees of Spontaneous* Atherosclerotic Plaque Development.

Series 2 (31 birds)		Blood serum cholesterol				
Atherosclerotic plaques (% area)	Not incubated		Incubated 30 hr at 37 to 38°C			% original free remain- ing after incubation
	Total (mg %)	Free (mg %)	Free to com- bined form (mg %)	Free remaining (mg %)		
.16	100	26.5	14.5	12.0	45.3	
.20	123	29.7	15.8	13.9	46.8	
.14	119	28.6	13.9	14.7	51.4	
.20	105	26.2	11.6	14.6	55.7	
.02	94	22.8	10.1	12.7	55.7	
Mean values	.14	108	26.8	13.2	13.6	51.0
Range	.02-.20	94-123	22.8-29.7	15.8-10.1	12.0-14.7	45.3-55.7

TABLE III. Eight Groups of Cockerels Fed .5% Cholesterol Atherogenic Diet—8 Weeks Experimental Period—With Severe Atherosclerotic Plaque Development.

Series 3 (57 birds)		Blood serum cholesterol				
Atherosclerotic plaques (% area)	Not incubated		Incubated 30 hr at 37 to 38°C			% original free remain- ing after incubation
	Total (mg %)	Free (mg %)	Free to com- bined form (mg %)	Free remaining (mg %)		
14.1	219	59.5	25.8	33.7	56.6	
20.1	297	76.1	26.5	49.6	65.2	
19.6	416	104.6	35.1	69.5	66.4	
10.8	325	82.0	24.6	57.4	70.0	
26.8	314	84.5	21.0	63.5	75.1	
18.0	456	121.7	23.7	98.0	80.5	
19.1	396	102.1	14.7	87.4	85.6	
16.6	328	90.2	11.8	78.4	86.9	
Mean values	18.1	344	90.1	22.9	67.2	73.3
Range	10.8-26.8	219-456	59.5-121.7	35.1-11.8	33.7-98.0	56.6-86.9

atherosclerotic birds is 9, 14, and 67 mean mg % respectively. Amounts combined upon incubation show smaller differences (17, 13, and 23 mean mg %) for the 3 series. Corresponding ranges of per cent original free cholesterol not combined are 17 to 45 for normal, 45 to 56 for mild spontaneous, and 57 to 87% for severe cholesterol-induced atherosclerosis. Results of this study indicate that there is a relationship between serum free cholesterol not combined upon incubation and development of atheroma in young cockerels.

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1. Sperry, W. M., *J. Biol. Chem.*, 1935, v111, 467.
2. Turner, K. B., McCormack, G. H., Jr., Richards, A., *J. Clin. Invest.*, 1953, v32, 801.
3. Morrison, L. M., Wolfson, E., Berlin, P., *Circulation*, 1950, v2, 479.
4. Caldwell, C. T., Suydam, D. E., *Proc. Soc. Exp. Biol. and Med.*, 1959, v101, 299.

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Mitochondrial α -Glycerophosphate Dehydrogenase Activity of Juxtaglomerular Cells in Experimental Hypertension and Adrenal Insufficiency. (26514)

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It has been established in both man and experimental animals that the juxtaglomerular (JG) cells are related to formation of extractable renin in the kidney, that they respond to alterations of sodium metabolism and, possibly, participate in mechanisms maintaining systemic arterial pressure. The physiological state of these cells, which form part of the preglomerular arterioles, is usually assessed by estimating the amount of stainable cytoplasmic granules(1), which are believed to be secretory in nature and may contain renin

(2). The type of metabolic pathway operating in JG cells is unknown.

Using a histochemical technic, mitochondrial α -glycerophosphate dehydrogenase (GPD, Meyerhof-Green enzyme) can be demonstrated to be particularly active in the JG cells. This activity changes in the same direction as renin content in both experimental hypertension and adrenal insufficiency and seems to indicate one of the major metabolic steps which govern the functional state of the juxtaglomerular complex.