17067. Fractionation of Serum Cholesterol of Chickens.*

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In a recent publication[1] we reported that only a small percentage of the total cholesterol was extracted from lyophilized normal human serum by chloroform at about 5°C. The fraction thus extracted was designated as the "readily extractable cholesterol", and extraction was found to be complete in 3 hours. This fraction is very high in the serum of nephrotic patients and hypercholesterolemic rabbits, comprising in some cases apparently all of the serum cholesterol.[1] Since atherosclerosis is a common occurrence in both of these instances, it was felt that further studies on the possible significance of this fraction would be of value.

Various authors[2-8] have shown that spontaneous arteriosclerotic lesions are common in chickens; lesions being evident in some by the time they reach 6 months of age. In the hen these lesions are found in both the elastic and muscular aorta, while they are normally confined to the muscular aorta in the rooster.[6] Since these lesions can develop while the cholesterol concentrations are less than 200 mg per 100 cc of serum,[5] it is evident that the *concentration* of cholesterol in the plasma can hardly be the chief etiological factor in the development of the arterial lesions. The purpose of the present investigation was to determine the concentration of the "readily extractable cholesterol" fraction and neutral fat of the serum of old and young chickens with the hope of obtaining some information which might help to explain atherosclerosis in the presence of relatively low concentrations of total serum cholesterol.

Experimental. Chickens of both sexes and of 3 age groups were studied. One group was 3 years of age or older, another from about 1 to 2 years and the third from 8-10 weeks old. The diet of the older birds is believed to have been mostly scraps plus some commercial feed. The young chickens received primarily starter mash. In most cases the animals were sacrificed the day they were received in the laboratory, but in a few cases, they were kept in an animal room and fed commercial laying mash for a few days prior to sacrificing. Blood for analyses was obtained by beheading the birds and collecting the blood in a beaker. One-half cc samples of the serum were lyophilized as described previously.[1] The dried serum was extracted with cold chloroform at a temperature of about 5°C for definite periods of time. The bottles were shaken every 10 to 15 minutes for the first 3 hours and then occasionally for the remainder of the extraction period. One or 2 bottles were filtered in the cold at different periods of time and the cholesterol content of the extract determined. The results shown in Tables I and II are typical of those obtained. For comparison, a number of results obtained on normal human sera are shown in Table III. The fraction designated as "neutral fat plus cholesterol" consists of neutral fat, cholesterol and cholesterol esters. The method employed for its determination has been described previously.[1]

Discussion. It will be seen from the experimental results that the "readily extractable" fraction is quite high in the serum of old hens; the older hens behaving in this re-

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6 Dauber, D. V., *Arch. Path.*, 1944, **38**, 46.

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TABLE I.
Extraction of Cholesterol from Lyophilized Serum of Female Chickens.

Number	Age	Cholesterol extracted in 3 hr, mg %	24 hr, mg %	72 hr, mg %	Total Chol., mg %	Neutral fat + Chol., mg %
1	>3 yrs.	225	222	—	234	1248
2	" "	152	168	—	162	1207
3	" "	158	171	—	169	1355
4	About 3 yrs.	200	196	—	216	2259
5	" "	115	126	—	157	1487
6	" "	83	82	—	115	847
7	" "	34	71	—	275	419
8	About 2 yrs.	123	136	—	183	969
9	" "	17	18	—	207	309
10	" "	4	5	—	120	226
11	About 10 wks.	8	12	18	129	773
12	" "	7	11	17	132	333
13	" "	16	100	110	129	232
14	" "	11	8	11	132	387

TABLE II.
Extraction of Cholesterol from Lyophilized Serum of Male Chickens.

Number	Age	Cholesterol extracted in 3 hr, mg %	24 hr, mg %	72 hr, mg %	Total Chol., mg %	Neutral fat + Chol., mg %
1	>3 yrs.	7	9	9	117	234
2	" "	11	13	17	136	281
3	" "	13	67	126	138	235
4	" "	8	7	6	128	310
5	About 3 yrs.	7	8	10	145	202
6	About 2 yrs.	7	9	12	94	250
7	" "	17	72	145	202	437
8	About 1 yr.	7	6	7	139	303
9	" "	8	6	12	119	249
10	About 10 wks.	9	11	28	144	258
11	" "	16	16	32	147	258
12	" "	15	110	107	141	303
13	" "	8	12	18	129	773
14	" "	11	11	65	113	387

gard very similar to the nephrotic patient, or the hypercholesterolemic rabbit.[1] Roosters, both old and young, as well as young female chickens, showed a low value for this fraction, but in some cases the amount extracted increased with increased extraction time. We have obtained a similar increased extraction with increased extraction time in a few apparently normal human subjects as well as in a considerable number of patients. The reason for this deviation from the average normal is unknown, but preliminary experimental work with roosters indicates that it is probably dependent upon dietary composition.

It will be noted that old hens showed, on the whole, a much higher level of serum fat than did old roosters or young chickens. There also appears to be some correlation between the percentage of the cholesterol present in the "readily extractable" form and the neutral fat content of the serum, especially in the case of the old hens. Dauber and Katz[7] have emphasized the close similarity between the lesions produced by the administration of cholesterol to roosters and the spontaneous lesions in old birds, described by Fox.[8] They also have pointed out their dissimilarity from those which usually occur in roosters. The possible significance of the concentration of the "readily extractable" cholesterol as an etiological factor in the development of the lesions found in old hens and cholesterol-fed

TABLE III.
Extraction of Cholesterol of Lyophilized Normal Human Serum.

Subject	Cholesterol extracted in 3 hr, mg%	24 hr, mg %	27 hr, mg %	96 hr, mg %	Total Chol., mg %	Neutral fat + Chol., mg %
A.L.F.	20	20	25	—	227	428
D.F.M.	30	31	35	—	322	511
C.J.R.	28	25	27	—	270	428
A.F.	22	22	20	—	226	404
D.F.	22	29	28	—	240	594
A.B.	30	22	28	—	286	392
K.N.	20	26	21	23	215	533
G.W.J.	22	27	27	38	272	551
R.B.	34	38	37	35	272	735
G.C.	37	37	42	43	331	705
E.R.	27	42	55	—	267	627
L.S.	36	76	102	—	233	722
M.D.	24	24	32	—	210	365

chickens is being investigated.

Although the data which have been presented are not sufficient to enable one to draw conclusions as to the physical state in which cholesterol exists in serum, they do indicate that it probably exists in at least three states not related to the usual classification of free and ester cholesterol. These are (a) a fraction not extracted by chloroform under the conditions used; (b) a fraction which is readily extractable by chloroform, a 3 hour extraction period being adequate for its complete extraction; and (c) a fraction which is slowly extractable. We believe that (a) possibly represents cholesterol which is held firmly by the serum proteins, while (b) possibly represents cholesterol which is free in the serum or which is associated with other lipid materials such as fats and phospholipids. Our experimental evidence indicates that all the fractions contain both free and ester cholesterol.

Summary. A large percentage of the cholesterol of lyophilized serum from old hens is present in the "readily extractable" fraction. The concentration of this fraction in the serum of roosters and young chickens is quite low but, in some cases, the amount extracted increases with increased extraction time. There appears to be a correlation between the percentage of the total cholesterol present in the "readily extractable" fraction and the neutral fat content of the serum of old hens.

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17068. Acetaldehyde Disappearance and Acetoin Formation *in vitro*.*

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The presence of a diphosphothiamin enzyme in animal tissues capable of forming acetoin from pyruvate, and the utilization of acetaldehyde as a substrate in this reaction, has been described.[1] A limited study of this reaction, in brain homogenate was reported previously.[2] The present study was devoted to a comparison of the abilities of various tissues to meta-

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